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Poster

PSTR146: Neurobehavioral Disorders in ADHD and Dyslexia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR146.01/A1

Topic: A.07. Developmental Disorders

Support: NSF 1640909

Title: Longitudinal study of motor statistics' biometrics of attention-deficit/hyperactivity disorder (ADHD)

Authors: C. MCKEEVER[1], K. DOCTOR[2], ***J. JOSE**[1];
[1]Indiana Univ., Bloomington, IN; [2]Universiity of Massachusetts, Amherst, Bloomington, IN

Abstract: Neurodivergent disorders (NDD) are encountered in a significant number of young children in the U.S. One leading NDD condition is attention-deficit/hyperactivity disorder (ADHD). 5-8% of children are diagnosed with ADHD, and the effects often last into adulthood. Individuals with ADHD can show problems of inattention, lack of staying focused, show excess movements including fidgeting or tapping, not all at the same time. Children with ADHD tend to also show persistent motor skill impairment. The prevalence of motor problems in children with ADHD may reach high levels. We have been pursuing the hypothesis that important cognitive information is contained in human's movements, if we look at them at millisecond time scales, away from naked eye detection. We use wrist mounted Bluetooth sensors that can capture motion with high frequency definition (~120Hz) (www.xsens.com.) We studied ADHD participants carrying out a reaching protocol to targets that appear periodically on a laptop touch screen. The kinematic measurements provide the information we used to extract the statistical properties of the motor millisecond fluctuations characteristic of each ADHD participant. Recently, we have identified two statistical motor biometrics in NDD participants, quantitatively characterizing their kinematic statistical millisecond time fluctuations (Doctor et al 2024). Here we looked at those biometrical changes as a function of time after a participant has taken ADHD medication. The changes in the biometrics represent changes on the probability distribution functions of the kinematic fluctuations as a function of time. These longitudinal changes may be connected to the usual changes' participants may have without treatments as well as the effectiveness of medical therapies, the latter of significant importance to providers. These biometrics can also provide a quantitative screening tool about the nature of the ADHD condition of each participant. Our preliminary results already identify changes in the corresponding participant's jerk biometrics. We will present detailed statistical analyses of the jerk, plus results for other kinematic variables.

Disclosures: C. McKeever: None. **K. Doctor:** None. **J. Jose:** None.

Poster

PSTR146: Neurobehavioral Disorders in ADHD and Dyslexia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR146.02/A2

Topic: A.07. Developmental Disorders

Support: HIM2021-022 SSA 1728

Title: Chronic treatment with atomoxetine and methylphenidate induced neuronal alterations, oxidative stress and inflammation in the brain of young rats

Authors: *J. CORONA, M. BEJARANO-CORIA, D. BECIEZ-FLORES;
Lab. of Neurosciences, Hosp. Infantil de Mexico Federico Gomez, Mexico City, Mexico

Abstract: Methylphenidate (MPH) and atomoxetine (ATX) are at present used as a treatment for attention-deficit/hyperactivity disorder (ADHD). However, MPH has been shown to produce changes in neurotransmission, DNA alterations and damage to the brain *in vivo*; ATX affects several signal transduction pathways and increases oxidative stress, mitochondrial damage and cell death *in vitro*. The pathophysiology of ADHD is not entirely known, but oxidative stress and neuroinflammation have emerged as a causal hypothesis. In this study, we aim to determine whether chronic treatment with MPH and ATX affects neuronal damage, oxidative stress and inflammation in the hippocampus and striatum of young rats. We used Wistar rats (PD23), which were injected intraperitoneally with MPH (10 mg/kg) and ATX (3 mg/kg) once a day for 28 consecutive days; Brain sections were taken from the hippocampus and striatum, which were stained with cresyl violet and the measurement of superoxide by dihydroethidium and GFAP immunostaining. We found that chronic MPH treatment in young rats decreased the number of neurons in both the striatum and the CA3 region of the hippocampus. Also, chronic ATX treatment in young rats decreases the number of neurons in the striatum and the CA1 and CA3 regions of the hippocampus. Furthermore, we found that chronic treatment with MPH and ATX increased oxidative stress in the hippocampus and striatum, as seen by elevated superoxide production indicated by dihydroethidium fluorescent signals in both brain areas. Additionally, in the regions mentioned earlier, an increase in the number of astrocytes labelled with GFAP was observed with both treatments. We conclude that the chronic treatments with ATX and MPH produced differential neuronal alterations which would trigger oxidative stress and consequently an increase in the neuroinflammation process in both the striatum and the hippocampus.

Disclosures: J. Corona: None. **M. Bejarano-Coria:** None. **D. Beciez-Flores:** None.

Poster

PSTR146: Neurobehavioral Disorders in ADHD and Dyslexia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR146.03/A3

Topic: A.07. Developmental Disorders

Support: NIH Grant R03NS109836
University of Dayton (UD) Graduate School and UD Office for Graduate Affairs through the Graduate Student Summer Fellowship (GSSF) Program

Title: Investigating the Novel Role of Phospholamban (PLN) in the Brain

Authors: B. KLOCKE, ***P. M. PITYCHOUTIS**;
Dept. of Biol., Univ. of Dayton, Dayton, OH

Abstract: Phospholamban (PLN) is a small phosphoprotein that acts as a negative regulator of the sarco/endoplasmic (SR/ER) reticulum calcium (Ca^{2+}) ATPase 2 (SERCA2), an integral Ca^{2+}-handling protein pump involved in maintaining intracellular Ca^{2+} homeostasis. PLN function has been characterized in cardiomyocytes and the role of the PLN/SERCA2 pathway is well established in cardiovascular physiology. In our recently published study, we reported a novel role for PLN in the thalamic reticular nucleus (TRN) of the adult mouse brain[1]. The TRN comprises a thin sheet of gamma-aminobutyric acid (GABA)-ergic neurons that surrounds the thalamus and is involved in the regulation of critical behaviors including locomotor activity, sleep regulation, and executive functioning. By employing constitutive and novel conditional knockout mouse models we provided first evidence that genetic *Pln* deletion in the GABAergic TRN neurons results in hyperactivity, impulsivity and sleep alterations in adult mice[1]. In the context of the current study, we employed high-performance liquid chromatography (HPLC) with coulometric detection to assess how loss of PLN function in mice (N=8-10/genotype/sex) may affect the monoamine neurotransmitters dopamine (DA), noradrenaline (NA), and serotonin (5-HT), as well as their metabolites and the amino acid neurotransmitters glutamate, aspartate, and GABA, in distinct brain regions implicated in the pathophysiology of brain disorders (i.e., prefrontal cortex, hypothalamus, hippocampus, striatum). Overall, our data show that loss of PLN function results in brain region-specific noradrenergic alterations and further contribute to understanding the role of this novel Ca^{2+}-handling player in brain physiology and pathophysiology.
References:[1] Klocke B, *et al.,* A Novel Role for Phospholamban in the Thalamic Reticular Nucleus (2024). *Sci Rep.* 14(1):6376.

Disclosures: B. Klocke: None. **P.M. Pitychoutis:** None.

Poster

PSTR146: Neurobehavioral Disorders in ADHD and Dyslexia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR146.04/A4

Topic: A.07. Developmental Disorders

Support: Korean Government, NRF-2022R1I1A3063177

Title: Prenatal treatment of corticosterone disrupts the dopaminergic regulation in synaptic functions of prefrontal cortex by interfering with neurodevelopmental signaling in rats.

Authors: *S.-C. JUNG[1], O.-B. KWON[2];
[1]Jeju Natl. Univ., 102 Jejudaehak-ro, Jeju-si, Korea, Republic of; [2]DGMIF, Daegu, Korea, Republic of

Abstract: Prenatal treatment of corticosterone disrupts the dopaminergic regulation in synaptic functions of prefrontal cortex by interfering with neurodevelopmental signaling in rats.
Sung-Cherl Jung[1,2], Amarsanaa Khulan[1], Badarch Michidmaa[1], Eun-A Ko[1], Oh-Bin Kwon[3], [1]Department of Physiology, College of Medicine, [2]Institute of Medical Science, Jeju National University, Jeju, 63243, [3]New Drug Development Center, Kmedihub, Daegu, 41061, Republic of Korea
We previously reported that rat pups (Corti.Pups) born from rat mothers that were repetitively injected with corticosterone (s.c., 20 mg/kg/day, 21 days) during pregnancy, exhibited ADHD-like behaviors, delay of cognitive functions, the deficit of long-term potentiation and impaired synaptic development in the hippocampal CA1 neurons. In this study, we examined alterations in dopaminergic regulation at glutamatergic synapses within the prefrontal cortex (PFC) of Corti.Pups, which are presumably linked to the pathogenesis of ADHD. In results acquired from PFCs within postnatal 1 to 21 days, ELISA analysis verified that levels of BDNF and cAMP were significantly lower in the prefrontal cortex (PFC) of Corti.Pups compared to the control group (Nor.Pups). Levels of mTOR, PKA, and PSD95 were also found to be lower in Corti.Pups, suggesting that crucial factors for neurodevelopmental signaling and synaptic development were compromised in PFCs of Corti.Pups. Furthermore, a slight increase in D1R and a significant decrease in D2R expression were noted in the PFC neurons of Corti.Pups compared to Nor.Pups. This variation in dopaminergic receptor expression led to different synaptic potential responses to dopamine treatment between two groups in electrophysiological experiments. Specifically, the amplitude of excitatory postsynaptic currents in the PFC of Corti.Pups was slightly but significantly enhanced by dopamine treatment, unlike in Nor.Pups. This strongly suggests that PFC neurons in Corti.Pups exhibit increased sensitivity to dopamine.Our results clearly provide evidence that prenatal exposure to high cortisol disrupts the neurodevelopment of PFC neurons via downregulating dopaminergic and PKA-mediated signaling cascades, possibly triggering the pathogenesis of neuropsychiatric disorders such as ADHD (Korean Government Research Fund, Grant No. NRF-2022R1I1A3063177).

Disclosures: S. Jung: None. **O. Kwon:** None.

Poster

PSTR146: Neurobehavioral Disorders in ADHD and Dyslexia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR146.05/A5

Topic: A.07. Developmental Disorders

Support: Japan Society for the Promotion of Science (JSPS) KAKENHI grant number 21H05042 22H00986, 23H03883

Title: Elucidating the role of a non-clustered protocadherin gene in ADHD on dopaminergic and visual behavior systems using zebrafish

Authors: *S. R. SUZUKI[1], R. KIMURA[2], Y. LI[3], T. NISHIMURA[4], S. MAEGAWA[5], M. HAGIWARA[3];
[1]Kyoto Univ. Hosp., Kyoto City, Kyoto, Japan; [2]Child Develop., United Grad. Sch. of Child Develop., Osaka Univ., Suita, Osaka, Japan; [3]Grad. Sch. of Med., Kyoto Univ., Kyoto, Japan; [4]Kyoto Univ., Kyoto, Japan; [5]Grad. Sch. of Infomatics, Kyoto Univ., Kyoto, Japan

Abstract: Attention deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder with a largely unknown pathology. Previous research suggests the contribution of the dopaminergic system to the condition. A genome-wide association study indicates the potential link between the disorder and some genes involved in the development of the nervous system. Non-clustered protocadherin genes play a pivotal role in neural development in vertebrates, and some of them are associated with neurodevelopmental disorders, including ADHD. In order to explore the potential role of a non-clustered protocadherin gene in ADHD pathophysiology, we induced a disruptive mutation in a non-clustered protocadherin gene in zebrafish. Mutant zebrafish presented altered responses to light in both the larval and juvenile stages. The dopamine levels in the juvenile brains were lower, and the number of tyrosine hydroxylase-positive cells was larger in mutant fish. Transcriptome analyses of RNA extracted from larval heads revealed that the differentially expressed genes in mutant fish were enriched in genes related to synaptic signaling and visual perception. Our research shows that the dysfunction of the non-clustered protocadherin gene results in an alteration in the dopaminergic system and transcriptome in the developing visual and neural systems, possibly leading to an aberrant behavioral response to light. The study highlights the link between ADHD and the dopaminergic system and also sheds light on the potential role of visual perception in ADHD pathophysiology.

Disclosures: S.R. Suzuki: None. **R. Kimura:** None. **Y. Li:** None. **T. Nishimura:** None. **S. Maegawa:** None. **M. Hagiwara:** None.

Poster

PSTR146: Neurobehavioral Disorders in ADHD and Dyslexia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR146.06/A6

Topic: A.07. Developmental Disorders

Support: CAPES
CNPQ
FAPERJ

Title: Alteration in equilibrative nucleoside transporter type 1 (ENT1) levels in synaptic and sub-synaptic fractions of three brain regions in an animal model of ADHD

Authors: *S. C. VALLADÃO[1], A. DOS SANTOS-RODRIGUES[2], P. PANDOLFO[1,3]; [1]Grad. Program in Biomed. Sciences: Physiol. and Pharmacol. - Biomed. Inst., Federal Fluminense Univ., Niteroi, Brazil; [2]Grad. Program in Neurosciences - Inst. of Biol., Federal Fluminense Univ., Niteroi, Brazil; [3]Graduate Program in Neurosciences - Institute of Biology, Federal Fluminense University, Niteroi, Brazil

Abstract: Background: Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental condition mainly caused by dopaminergic imbalance in critical brain regions. Yet, adenosinergic signaling is known to have implications in the dopaminergic system and can normalize the dopaminergic imbalance seen in ADHD. Extracellular adenosine levels are crucial for its signaling and can be regulated by nucleoside transporters, such as the equilibrative nucleoside transporter type 1 (ENT1). Our study aims to investigate ENT1 levels in the frontocortical, striatal, and hippocampal regions of a validated animal model of ADHD and explore the impact of the ENT1 inhibition in behavioral tasks and adenosinergic/dopaminergic markers in this model. Methods: All procedures were approved by the Institution's Ethics Committee on Animal Use under the protocol #7023070523. The brain frontal cortex, striatum, and hippocampus from 75-days-old male and female Wistar Kyoto (WKY) and Spontaneously Hypertensive Rats (SHR) were collected and processed for synaptosomal and total membrane fractions. After western blotting, membranes were incubated with an anti-ENT1 primary antibody. As loading protein control, anti-α-tubulin primary antibody was used. ImageJ software was used for quantification and GraphPad Prism, for statistical analysis (two-way ANOVA and Tukey's post hoc comparison). Results: In synaptosomes (n=4-5), there was no significant difference in cortical ENT1 levels when comparing strain ($p=0.669$), sex ($p=0.595$) nor their interaction ($p=0.570$), as well as in striatal ENT1 levels (strain, $p=0.354$; sex, $p=0.963$; interaction, $p=0.824$). Hippocampal ENT1 levels, however, were higher in female SHRs ($p=0.004$) when compared to male SHR or male WKY, with an effect of sex ($p=0.004$), but not strain ($p=0.112$) nor interaction ($p=0.350$). In total membranes (n=4-5), cortical ENT1 levels showed no difference between strain ($p=0.555$), sex ($p=0.994$), or interaction ($p=0.451$), as well as in hippocampal levels (strain, $p=0.866$; sex, $p=0.177$; interaction $p=0.335$). Lastly, striatal levels were also higher in female SHRs when compared to males ($p=0.043$), but there was not an effect of strain ($p=0.090$) or interaction ($p=0.149$). Conclusions: Based on our study, higher levels of ENT1 in striatal synaptosomes and hippocampal total membranes, especially in females, may contribute to ADHD symptoms. Further research is necessary to determine whether ENT1 levels might play a significant role in the behavioral variations between younger male and female individuals.

Disclosures: S.C. Valladão: None. **A. dos Santos-Rodrigues:** None. **P. Pandolfo:** None.

Poster

PSTR146: Neurobehavioral Disorders in ADHD and Dyslexia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR146.07/A7

Topic: A.07. Developmental Disorders

Title: Compensatory Mechanism of cortical gray matter in Children with ADHD: insights from voxel-based cortical thickness analysis

Authors: E. GARCÍA[1], B. MACIAS[2], *S. HIDALGO-TOBON[3], D. ALVAREZ-AMADO[4], B. ROMERO BAIZABAL[5], **B. DE CELIS ALONSO**[7], J. GARCIA BERISTAIN[6]; [1]PHYSICS - Med. Physics, UNIVERSIDAD AUTONOMA METROPOLITANA IZTALAPALA, mexico, Mexico; [2]uam iztapalapa, México, Mexico; [3]UAM-Hospital Infantil de Mexico Federico Gomez, Ciudad DE Mexico, Mexico; [4]Hosp. Infantil de Mexico Federico Gomez, DF, Mexico; [5]Hosp. Infantil de Mexico Federico Gomez, MEXICO, Mexico; [6]Hosp. Infantil de Mexico Federico Gomez, Mexico City, Mexico; [7]Fac. of Physical and Mathematical Sci., Benemerita Univ. Autonoma De Puebla, Puebla, Mexico

Abstract: Magnetic resonance imaging (MRI) and voxel-based morphometry (VBM) are non-invasive quantitative imaging techniques for the diagnosis and treatment of nervous system disorders. Attention deficit hyperactivity disorder (ADHD) indicates a neurobehavioral condition that usually begins in childhood and MRI studies coincide in abnormalities in brain structure. Structural analysis-based volumetry to quantify volumetric changes in brain tissue and structures. The volumetric sequence was performed using a 3T SIEMENS Skyra scanner using an eight-channel receive-only head coil at the Hospital Infantil de México Federico Gómez to patients diagnosed with ADHD (n=17, age 5 to 15 years, 16 men). A volumetric T1-weighted 3D gradient echo MRI sequence was used, TR/TE parameters = 2200/2.45 ns, flip angle = 8°, acquisition matrix = 256 x 256, slice thickness = 1 mm. SPM12-CAT Software was used for volumetry and sequence analysis of brain imaging data. Patients with ADHD compare with control patients without a diagnosis of ADHD or other adjacent neurological disorder, given the results of a t test for independent samples in the CAT-12 software, only a value of $p<0.05$ was obtained. The VBM results of gray matter volume showed reduction in the left thalamus, right hippocampal, left amygdala, left hippocampus, left and right cerebellum_6, right lingual and right putamen in the patients (controls>ADHD) in t-test two tails. In addition to an increase in cortical thickness (0-5mm) in the superior frontal gyrus and a decrease in the inferior temporal gyrus and the occipital lobe. These results indicate abnormalities in pediatric patients with ADHD. Previous studies have shown a delay in maturation of cortical thickness and surface area in frontal regions. The findings of the present investigation could be explained as a compensatory mechanism of the cortical gray matter in the aforementioned areas. Longitudinal studies with a larger number of patients should confirm these results.

Disclosures: E. García: None. **B. Macias:** None. **S. Hidalgo-Tobon:** None. **D. Alvarez-Amado:** None. **B. Romero baizabal:** None. **B. De Celis Alonso:** None. **J. Garcia Beristain:** None.

Poster

PSTR146: Neurobehavioral Disorders in ADHD and Dyslexia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR146.08/A8

Topic: A.07. Developmental Disorders

Title: Deep Learning Applications for Dimensional ADHD in Children using Task fMRI during Reward Processing Tasks

Authors: *S. CHOI, E. LEE, H. WANG, J. SEO, S.-B. HONG, J. CHA;
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Advances in neuroimaging and machine learning offer new avenues for understanding and diagnosing Attention-Deficit/Hyperactivity Disorder (ADHD), yet the nuanced differentiation of ADHD symptoms through these technologies remains underexplored. This study employs deep learning methodologies to predict ADHD in children using task-based functional magnetic resonance imaging (fMRI) contrast maps from the Monetary Incentive Delay (MID) task, aimed at assessing reward processing. Data from the Adolescent Brain Cognitive Development (ABCD) study were used, with ADHD status determined by the parent-reported Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS). Participants were categorized into control (1-3 symptoms) and full syndrome ADHD (6-9 symptoms) groups, focusing on two specific neural contrasts: reward anticipation vs. neutral anticipation, and reward feedback vs. neutral feedback. To address sample size imbalances, downsampling was implemented, resulting in balanced groups for analysis: 740 participants for inattention (IA)-reward anticipation, 749 for IA-reward feedback, 409 for hyperactivity/impulsivity (HI)-reward anticipation, and 428 for HI-reward feedback.The deep learning approach, employing a 3D-CNN, demonstrated better performance in predi cting ADHD from task-based fMRI data compared to the baseline SVM model. For IA sympto ms, the 3D-CNN with reward anticipation achieved an accuracy of 0.52 and an Area Under the Curve (AUC) of 0.59, matching the SVM's accuracy but surpassing its AUC of 0.57. Nota bly, for HI symptoms, the 3D-CNN significantly outperformed the SVM, achieving an accurac y of 0.59 and an AUC of 0.64, compared to the SVM's accuracy of 0.45 and AUC of 0.56. The 3D-CNN models with reward feedback (IA: Accuracy = 0.59, AUC = 0.59; HI: Accuracy = 0.56, AUC = 0.61) also outperformed the SVM models (IA: Accuracy = 0.53, AUC = 0.58; HI: Accuracy = 0.50, AUC = 0.56). These results indicate that the 3D-CNN model provides a more robust prediction of hyperactivity/impulsivity symptoms than inattention when analyzed through fMRI data from the MID task. These findings suggest that neural correlates for hyperactivity/impulsivity may be more distinct and detectable in reward-processing tasks than those for inattention. The enhanced performance of the 3D-CNN over the SVM model highlights the potential of deep learning to improve neuroimaging-based diagnostics in ADHD, especially for hyperactivity/impulsivity symptoms. This research underscores the promise of deep learning approaches in refining diagnostic methodologies and contributing to more personalized treatment strategies for ADHD.

Disclosures: S. Choi: None. **E. Lee:** None. **H. Wang:** None. **J. Seo:** None. **S. Hong:** None. **J. Cha:** None.

Poster

PSTR146: Neurobehavioral Disorders in ADHD and Dyslexia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR146.09/A9

Topic: A.07. Developmental Disorders

Support: U01-KOMP 210370-0523
U01-VCU 310158-0422

Title: Epistatic mutations in Ssc4d and Kpna3 regulate age dependent hyperactivity in mice

Authors: *Y. M. H. BARAKAT[1,2], J. BEIERLE[1], T. SPROULE[1], S. P. DEATS[3], M. SANTOS[1], V. KUMAR[4,2], J. S. TAKAHASHI[5];
[1]Jackson Lab., Bar Harbor, ME; [2]Tufts University, Boston, MA; [3]JAX aging Ctr., Jackson Lab., Bar Harbor, ME; [4]Neurosci., Jackson Lab., Bar Harbor, ME; [5]Chair, Dept. of Neurosci., UT Southwestern Med. Cen, Howard Hughes Med. Inst., Dallas, TX

Abstract: The NCHS estimates that 8.56% of children in the United States are diagnosed with developmental disorders. It is therefore critical to understand the neurobiological mediators of developmental disorders to improve patient outcomes. Through ENU mutagenesis our lab has generated a mutant mouse line (Crichton ENU) that exhibits age dependent hyperactivity starting at 6 and plateauing at 20 weeks of age. Quantitative trait loci analysis (QTL) identified 2 genome wide significant QTLs and identified missense mutations within *Ssc4d* and *Kpna3* as causal. Both mutations are needed to express the hyperkinetic phenotype, demonstrating a surprising epistatic relationship between the understudied scavenger receptor *Ssc4d* and nuclear import factor *Kpna3*. This was confirmed by generating a $Ssc4d^{mut/mut}$ and $Kpna3^{mut/mut}$ CRISPR mouse line (Crichton CRISPR) which had a similar pattern of hyperactivity. To interrogate the mechanism of this epistasis, we examined protein stability, protein binding, and RNA expression in the brain. We observe no destabilization in the mutant proteins but analysis of differential KPNA3 binding partners revealed a general loss of canonical binding partners important for its nuclear import capabilities. We also investigated transcriptomic differences between young (4-week-old) and adult (20-week-old) Crichton and wildtype mice in the prefrontal cortex (PFC), hippocampus, and choroid plexus using bulk RNAseq. *Ssc4d* is highly expressed in the ChP, where differentially expressed gene (DEG) analysis and gene ontology enrichment analysis revealed decreases in cell junction genes in old mutants. DEG analysis revealed cilia pathways being impacted in Crichton CRISPR. Finally, PFC DEG analysis highlighted myelination and oligodendrocyte differentiation downregulation in the young mutants. These results reveal a broad set of developmental changes that will enable us to interrogate this epistatic relationship on a mechanistic level. Additional future directions will involve examining choroid plexus structurally and functionally and using viral tools to examine the role of SSC4D in the brain. By pursuing these gene expression changes it will lead us to closer to understanding how *Kpna3* and *Ssc4d* interact to promote motor control development.

Disclosures: Y.M.H. Barakat: A. Employment/Salary (full or part-time):; Tufts University, Jackson Laboratory. **J. Beierle:** A. Employment/Salary (full or part-time):; Jackson Laboratory. **T. Sproule:** A. Employment/Salary (full or part-time):; Jackson Laboratory. **S.P. Deats:** A. Employment/Salary (full or part-time):; Jackson Laboratory. **M. santos:** A. Employment/Salary

(full or part-time):; Jackson Laboratory. **V. Kumar:** A. Employment/Salary (full or part-time):; Jackson Laboratory, Tufts University. **J.S. Takahashi:** None.

Poster

PSTR146: Neurobehavioral Disorders in ADHD and Dyslexia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR146.10/A10

Topic: A.07. Developmental Disorders

Support: NIH NICHD R15HD087937
Alan & Wendy Pesky Foundation Research Grant
Humboldt Foundation

Title: Saccadic Detection in Virtual Gaming for Dyslexia Classification in Children

Authors: Y.-C. YU[1], H. SHYNTASSOV[2], P. A. KAUSHIK[1], ***L. GABEL**[3];
[1]Electrical and Computer Engin., Lafayette Col., Easton, PA; [2]Electrical and Computer Engin., Lafayette Col., Conshohocken, PA; [3]Neurosci., Lafayette Col., Easton, PA

Abstract: Reading disabilities (RDs) affect 5-17% of the population and account for approximately 80% of all learning disabilities. If learning disabilities remain untreated, a child may experience long-term social and emotional problems, which may influence future success in all aspects of their lives. Early identification and intervention will help close the gap between typically developing readers and children with reading difficulties in acquiring reading skills. Studies have shown that identifying children with dyslexia at young age is crucial to provide effective intervention to improve learning outcomes We have previously demonstrated that performance on a virtual maze learning task is associated the reading ability in young children (5-13 years of age), regardless of orthographic depth of the native language. This relationship is further strengthened by genetic risk for RD. Furthermore, poor performance on this task in developing readers (kindergarten) appears to predict future reading ability in second grade. across language orthographies. Applying biological and performance variables (e.g., biological sex, time to complete the task, errors committed, and deviation from the true path) into a machine learning algorithm resulted in a classification accuracy (i.e., typical vs. atypical readers) up to 80%. In order to better understand user intent as the participant traverses the mazes, we examine eye movements using an eye tracker. Eye tracking provides a mean of assessing where a participant is looking when performing a task on the computer. In this study, we investigated whether eye movement in regions of interest near correct/incorrect turns in the maze would enhance classification accuracy. To assess the impact of integrating eye movement data on the classifier's accuracy, it is essential to synchronize the eye movement data with the cursor movement data recorded during the virtual maze testing. A signal processing algorithm, including image segmentation, image and text data synchronization, saccade detection, and event alignment, was developed to assess the correlation between the participants' eye-gazing and the cursor movements during maze-solving tasks. This algorithm was tested with the data from 13

participants while solving the virtual Hebb-Williams Maze tasks. The identified saccadic events exhibit a strong correlation with participants' erroneous decisions during maze-solving, indicating their potential as an additional variable for improving accuracy in dyslexia classification within machine learning algorithms.

Disclosures: Y. Yu: None. **H. Shyntassov:** None. **P.A. Kaushik:** None. **L. Gabel:** None.

Poster

PSTR146: Neurobehavioral Disorders in ADHD and Dyslexia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR146.11/A11

Topic: A.07. Developmental Disorders

Title: Familial history of dyslexia is associated with functional brain differences in children: a meta-analysis of fMRI studies

Authors: *S. LAWSON[1], J. MORREL[2], T. GUPTA[1], A. J. KRAFNICK[3], T. M. EVANS[1]; [1]Univ. of Virginia, CHARLOTTESVILLE, VA; [2]USC, Los Angeles, CA; [3]Psychology, Dominican Univ., River Forest, IL

Abstract: Objectives: Dyslexia is a highly heritable and prevalent neurocognitive condition, affecting approximately 20% of the population. Structural and functional MRI studies have suggested that individuals with a family history of dyslexia manifest with disruptions to a core network of left lateralized, language related brain regions. While several studies have investigated the neurobiological origin of these brain differences, findings remain somewhat inconsistent. This meta-analysis seeks to synthesize functional brain differences in pre- and emerging-readers with a familial history of dyslexia (FHD+) relative to those without a family history of dyslexia (FHD-). Methods: A systematic literature search of PubMed, SCOPUS, and Web of Science was conducted in February 2024 to identify primary research studies using whole-brain, functional MRI (fMRI) during reading and language tasks in 4-11-year-olds with and without a family history of dyslexia. Using the PRISMA Framework, two raters independently screened 1,448 studies for eligibility. Using the meta-analytic tool GingerALE 3.0.2, ten studies were analyzed using a voxel-level threshold of $p<0.001$ (uncorrected) and a cluster threshold $\geq$ 150 mm3. Results: Results of the FHD- > FHD+ contrast identified significant clusters ($p<0.001$) in primary visual cortex [18, -86, 8], caudate [16, 8, 16], superior temporal sulcus [52, -16, -16], primary auditory cortex [-34, -30, 20], left cerebellum [-26, -78, -28], and primary motor cortex [54, -6, 20]. Results of the FHD+ > FHD- contrast identified a significant cluster ($p<0.001$) in dorsolateral prefrontal cortex [38, 32, 22]. Discussion: We conducted a systematic search of the literature and then performed a quantitative meta-analysis of research studies (n = 10) examining functional brain differences in young children with and without a family history of dyslexia. Results of this meta-analysis suggest that pre- and emerging-readers with a familial history of dyslexia exhibit decreased activation in brain regions responsible for sensorimotor processing, and increased levels of brain activity in dorsolateral

prefrontal cortex during reading and language tasks relative to their FHD- peers. These findings suggest that reduced engagement of brain regions responsible for sensorimotor integration and compensation via executive control processes may be key features of reading and language differences in children with a family history of dyslexia.

Disclosures: S. Lawson: None. **J. Morrel:** None. **T. Gupta:** None. **A.J. Krafnick:** None. **T.M. Evans:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.01/A12

Topic: A.09. Adolescent Development

Support: NIH R21 DA055105

Title: Influences of exposure to methamphetamine during adolescence on dopamine receptor expression in parvalbumin interneurons of the mPFC in intact and ovariectomized female rats

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Abstract: During the adolescent period, initiation of methamphetamine (METH) use has been associated with greater long-term consequences which could be due, in part, to ongoing development of the prefrontal cortex which in the rat continues up to postnatal day (P) 60. Previous work our laboratories found that exposure to METH during specific windows of adolescence resulted in sex-specific alterations to parvalbumin (PV) interneurons, a GABAergic interneuron known to mature during adolescence. Interestingly, we observed fewer PV cells in females exposed to METH from P40-P48, but increased PV cell number in females exposed from P30-38 and P60-68 in the medial prefrontal cortex (mPFC). We hypothesize the reversal of direction may be due to female pubertal onset, which occurs at P35-38 in our colony, since our laboratory has shown pubertal onset to be a major catalyst of adolescent female neural maturation and plasticity within the rat mPFC.
To causally test the potential interaction between METH and female pubertal onset, we performed pre-pubertal ovariectomies (OVX) at P20, followed by METH or saline exposure from P30-38, P40-48, or P60-68. 24-hours following exposure, the mPFC was collected and an in-situ hybridization assay was used to label dopamine receptors, *drd1* and *drd2*, as well as PV, *pvalb*. Preliminary analysis suggests dense dopamine receptor expression within neurons expressing PV, layer specificity has been noted with drd1 expression being most prominent in

layers II/III while drd2 expression is most prominent in the deeper layers V/VI. Ongoing data analysis will include layer-specific analysis of expression of dopamine receptors within PV neurons following METH exposure. The results of this analysis will provide a subcellular basis for age- and sex- specific changes noted previously while also directly linking female pubertal onset as a contributor to age- and sex-specific findings.

Disclosures: A.S. Brinks: None. **E.M. Hoffman:** None. **L.K. Carrica:** None. **J.M. Gulley:** None. **J.M. Juraska:** None.

Poster

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Topic: A.09. Adolescent Development

Support: NIH R21 DA055105

Title: Impact of noncontingent methamphetamine exposure during adolescence on the number of parvalbumin interneurons in the mPFC of intact and ovariectomized female rats

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Abstract: Adolescence is a period of heightened susceptibility to negative outcomes of substance use, which is possibly attributable to the prolonged development of the medial prefrontal cortex. Additionally, progression to problematic use is more dramatic in women, whose average onset of dependence occurs at earlier ages. Previous work in our lab, Brinks et al., 2024, has shown that exposure to methamphetamine (METH) at timepoints surrounding puberty leads to varied outcomes in parvalbumin (PV) number in females: exposure during early adolescence (P30-38) and early adulthood (P60-68) show increases in PV number and intensity, whereas late adolescence (P40-48) shows the opposite effect. Our lab has shown that changes neuron number, perineuronal net number, and dendritic pruning show fluctuations around pubertal onset, and these variations in maturation may mediate the age-dependent vulnerability of females in response to METH. We aim to determine whether female pubertal onset mediates the role of structural changes of the mPFC after methamphetamine exposure in females. Female Sprague Dawley rats were ovariectomized before puberty or sham operated at P20 and were then exposed to 3 mg/kg METH i.p. at P30-38, P40-48 or P60-68. The mPFC was collected 24 hours after the final injection. In-situ hybridization and automated quantification identifying parvalbumin will be used to evaluate parvalbumin expression and intensity. Preliminary results

(n = 2) suggest similar age-specific effects of METH on PV-expressing cell density as observed in our previous work. Results also suggest pre-pubertal ovariectomy increases the density of cells expressing PV. Ovariectomy of female Sprague Dawley rats prior to puberty will provide a better understanding of the biological mechanism underlying the heightened sensitivity of adolescent females toward persistent methamphetamine use in a timing-dependent fashion.

Disclosures: E.M. Hoffman: None. **A.S. Brinks:** None. **X. Yin:** None. **L.K. Carrica:** None. **J.M. Gulley:** None. **J.M. Juraska:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: F32DA060685
State of Washington Initiative Measure No. 502

Title: Adolescent vaporized cannabis-induced medial prefrontal cortex dysfunction in adult rats: Cognitive flexibility, parvalbumin interneurons, and perineuronal nets

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Abstract: Cannabis use has significantly increased in recent years, particularly in adolescents, as the illicit drug becomes legal in certain states. This is alarming as the long-term neurobehavioral consequences of adolescent cannabis use remain poorly understood. Recently, we reported that vaporized cannabis in adolescence can lead to long-lasting alterations of the medial prefrontal cortex (mPFC), specifically, impaired mPFC-dependent strategy shifting in a cognitive flexibility task. Parvalbumin interneurons (PV) mediate cognitive flexibility as their inhibitory function tightly regulates mPFC output neurons. Perineuronal nets (PNNs) support PV interneuron function, and their disruption results in reduced firing rate and intrinsic excitability of PV interneurons. Notably, microglia can breakdown perineuronal nets, and we recently reported increased mPFC microglia activation after vaporized cannabis in adolescence. Thus, we hypothesized that vaporized cannabis during adolescence leads to long-lasting mPFC dysfunction by activated microglia breaking down PNNs around PV interneurons, which reduces PV function, thereby leading to the cognitive flexibility deficit. We sought to test this hypothesis by exposing adolescent (postnatal day [P]35-55) Sprague-Dawley rats of both sexes to 3 weeks of daily 1-h non-contingent vaporized cannabis extract or vehicle sessions. Two weeks later (P70), cognitive flexibility was tested, after which, brains were collected to stain for mPFC PV interneurons and PNNs. Male rats exposed to vaporized cannabis during adolescence took more trials to learn to shift strategies and made more perseverative and regressive errors compared to

their vehicle-exposed counterparts, suggesting difficulty in learning to inhibit the previous strategy and maintain this inhibition. Preliminary findings suggest that adolescent vaporized cannabis exposure reduced the percentage of PV interneurons surrounded by PNNs only in male rats, consistent with our behavioral results. These effects were not present in females. The current findings partially support the hypothesis that adolescent cannabis vapor impairs cognitive flexibility in adulthood by reducing PNNs around PV interneurons as we only observed this effect in male rats. We previously reported mPFC dysfunction in females, but not males, following adolescent response-contingent cannabis vapor. Our previous work and these current findings underscore the importance of the contingency of adolescent vapor administration in determining the nature of sex differences in long-term cannabis-induced mPFC dysfunction.

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Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.04/A15

Topic: A.09. Adolescent Development

Support: NIH DA 055105

Title: Female rats exposed to methamphetamine during adolescence show altered drug-taking behaviors later in life compared to those exposed in early adulthood

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Abstract: Research has shown that individuals who begin using drugs like methamphetamine (METH) during adolescence are more likely to develop problematic drug use behaviors compared to adult-onset users. Work using rodent models has suggested unique drug-taking behaviors in adolescents, combined with the impact of drug exposure on a still developing brain, may contribute to the heightened vulnerability of adolescents. Recently, we showed that in female rats, METH exposure during early adolescence or young adulthood increased the number of parvalbumin-expressing (PV) interneurons in the medial prefrontal cortex (mPFC), but decreased PV number in late adolescence with no effect at any age in males. Here, we investigated the effects of prior METH exposure in adolescence or adulthood on the development of i.v. METH self-administration behavior later in life. Female Sprague-Dawley rats were exposed once daily to either 3.0 mg/kg METH or saline from P40-P48 (LA) or P70-P78 (adult) and were then allowed to self-administer 0.1 mg/kg (i.v.) METH three weeks later (P70 or P100). Animals were tested under short-access (ShA), progressive ratio (PR), long-

access (LgA), and cue-induced relapse (CIR) paradigms. We found that during ShA conditions, METH pre-exposed rats had lower intake of the drug regardless of their age at pre-exposure. Under LgA conditions, rats exposed to METH during LA rapidly escalated their intake whereas adult-exposed rats exhibited stable intake patterns. Rats exposed to METH during LA were also the only animals to significantly increase their responding for METH during PR testing; however, we did not see any group differences in the CIR test. We are currently analyzing brain tissue from these animals to determine whether changes to dopamine clearance mechanisms and PV expression within the mPFC are associated with the group differences in drug-taking behavior we observed. Our results to date suggest METH pre-exposure impacts adolescent and adult females differently, and the long-lasting consequences of drug exposure during adolescence may make an individual more vulnerable to developing problematic drug taking behaviors as adults.

Disclosures: L. Carrica: None. **A.S. Brinks:** None. **J.M. Juraska:** None. **J.M. Gulley:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Program #/Poster #: PSTR147.05/A16

Topic: A.09. Adolescent Development

Support: NIH R21 DA055105 A

Title: Effects of escalating dose exposure to methamphetamine on withdrawal-associated hedonic and motivational anhedonia in adolescent- and adult-aged rats

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Abstract: Individuals with stimulant use disorder often experience a challenging pattern of relapse, driven in part by a desire to alleviate withdrawal symptoms. Understanding the potential impact of age and gender/sex on withdrawal effects could enhance treatment approaches and relapse prevention. Here, we investigated methamphetamine (METH) withdrawal-induced anhedonia in adolescent and adult rats of both sexes using behavioral tasks that assess consummatory and motivational aspects of anhedonia through, respectively, hedonic (pleasure-related) and motivational (goal-directed) responses. We hypothesized that METH withdrawal would result in varied patterns of anhedonia, with adolescent-exposed and female rats expressing elevated hedonic and motivational anhedonia. Male and female Sprague-Dawley rats were injected with saline or an escalating dose of METH (0.5-5.0 mg/kg, i.p.) during adolescence (35-44 days old) or adulthood (75-84 days old) and then assessed on either a sucrose preference test (SPT) or sucrose-reinforced instrumental task under a progressive ratio (PR) schedule of reinforcement. Our findings from the SPT (n = 10-12 rats/group) revealed no group differences

in sucrose preference, suggesting withdrawal from escalating dose METH exposure did not induce hedonic anhedonia. This result was not consistent with our *a priori* hypothesis and was somewhat surprising in light of previous studies in rats and mice showing exposure to METH and other stimulants can induce hedonic anhedonia. Our assessment of motivational anhedonia in rats performing the instrumental behavior task is ongoing, but our preliminary results (n = 6 rats/group) suggest that METH-exposed adolescent females and adults of both sexes reached lower PR breakpoints compared to their saline-treated controls. Male adolescents exposed to METH reached higher PR breakpoints compared to their saline controls, suggesting enhanced motivation for sucrose in these rats. Taken together, these findings highlight distinct differences in the disruption of hedonic processing following METH exposure that depend on age and sex. Further research should explore the neurobiological mechanisms that underlie these differences, with particular emphasis on brain-derived neurotrophic factor signaling given its known downregulation in the hippocampus during METH withdrawal.

Disclosures: C.D. Strumberger: None. **J. Caruana:** None. **I.S. Anstee:** None. **J.M. Gulley:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: NIH Grant P50AA017823
NIH Grant T32AA025606

Title: Adolescent Intermittent Ethanol Exposure Produces Sex-Specific Increases in Vascular Complexity that Correlate with Altered HIF-1a Signaling.

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Abstract: Vascular dysfunction is commonly implicated in the pathology of alcohol use disorders. Acute alcohol has been shown to increase vascular endothelial growth factor (VEGF) signaling as well as increase neuroinflammatory cytokines. Both contribute to angiogenesis, particularly under hypoxic conditions. While acute alcohol is known to produce liver hypoxia and alter liver angiogenesis through hypoxia inducible factor (HIF)-1α signaling, little is known about the impact of adolescent intermittent ethanol exposure (AIE) on cerebral angiogenesis and the HIF-1α pathway. Thus, we evaluated whether AIE was capable of producing long-term changes in cortical vascular structure that correlated with changes in HIF-1α gene expression and downstream targets. In experiment 1, male and female rats received 4.0 g/kg intragastric ethanol

exposures (3 days on, 2 days off) or were left undisturbed across adolescence (P30-P50, total of 12 ethanol exposures). Afterwards, all rats remained unmanipulated until P80 at which point they received intracardiac injection of 1,1-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) to label the vasculature. The whole dorsal and ventral surface of these brains was then imaged using fluorescent microscopy and vascular analysis was performed. Traditional vascular metrics as well as local connected fractal dimension analysis revealed that only male rats with a history of AIE showed increased total vascular length, reduced lacunarity, and an increase in high complexity vessels on the dorsal surface maps relative to vehicle and unmanipulated controls. To probe mechanistic contributors to these changes, experiment 2 utilized male and female rats that again received either AIE or vehicle exposures from P30-P50 using the procedure described above. These rats were allowed to develop undisturbed until P70 at which point they received either 2.5 g/kg intraperitoneal ethanol or saline challenge. Changes in mRNA expression of angiopoietin (ANG) 1, ANG2, TIE2 receptor, VEGFa, and HIF-1α was then assessed in the cingulate cortex and HPC using RT-PCR. Male rats with a history of AIE that received adult saline challenge showed significant increases in HIF-1α, ANG1, and ANG2. We hypothesize that adolescence is a time of increased vascular change that, in conjunction with AIE, leads to dysregulation of HIF-1α signaling and pathological angiogenesis. These changes likely mirror vascular remodeling associated with alcohol liver disease and may have significant implications for cortical dysfunction and the pathogenesis of vascular-related diseases such as vascular dementia.

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Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Program #/Poster #: PSTR147.07/A18

Topic: A.09. Adolescent Development

Title: Changes in circadian rhythm and 24h activity patterns after fluoxetine exposure in adolescent rats

Authors: *M. GONZALES[1], L. GONZALEZ[2], A. MORA[5], S. GOMEZ[6], K. GUERRERO LEON[7], C. CUETO[8], A. CABRERA[9], L. R. AMODEO[3], J. VALENCIA[4];
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Abstract: Fluoxetine (Prozac) is one of the most prescribed SSRIs for adolescents diagnosed with generalized anxiety and major depressive disorder. Rates of SSRI prescriptions rose with

the COVID pandemic and have exponentially increased over the past few years leading to concerns with overprescribing in youth. While SSRIs have been shown to be effective in adolescents, the potential acute withdrawal symptoms are concerning and difficult to assess in clinical trials. The present study assesses the effects of fluoxetine in a non-diseased adolescent rat during treatment and after abrupt discontinuation to determine changes in activity rhythms throughout a 24h period. To this end, male and female rats were administered fluoxetine (0 or 10 mg/kg) once a day beginning in adolescence on postnatal day (P)40 to P53. Twenty-four hour activity was assessed at various timepoints during and after discontinuation from fluoxetine. Rats were evaluated for changes in circadian rhythm and the quality/quantity of activity and inactivity (rest) during the 24h period using non-invasive activity monitors secured to the rats via individually sized jacket. Rats were also assessed for sucrose preference, open field, and spontaneous alternation. Fluoxetine exposure in adolescence lead to reductions in activity during the light and dark phases. This also led to changes in the pattern of activity with less inactive episodes, but longer mean duration. Circadian measures were also impact after exposure and throughout acute discontinuation, but were no longer evident after 10 days of withdrawal. Overall, these results suggesting that while adolescent fluoxetine exposure can potentially reduce overall activity, the quality of inactive (rest) episodes improved which may be contributing to its therapeutic efficacy. However, these effects on activity are transient and do not persist after prolonged discontinuation.

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Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: NIH Grant DA045175

Title: The effects of moderate alcohol and THC co-use in adolescence on AKT-GSK3ß signaling in adulthood

Authors: *L. SHI[1], L. CARRICA[1], C. Y. CHOI[1], N.-C. LIANG[1,2], J. M. GULLEY[1,2,3]; [1]Psychology, [2]Neurosci. Program, [3]Carl R. Woese Inst. for Genomic Biol., Univ. of Illinois, Urbana-Champaign, Champaign, IL

Abstract: The combined use of alcohol and cannabis among adolescents and young adults is prevalent, and heavy co-use has been linked to distinct cognitive and metabolic changes in humans. The molecular mechanisms underlying these changes remain unclear, but a potential target of interest is a protein kinase in the downstream signaling pathway of cannabinoid

receptors that is important for normal cognitive functions and glucose metabolism: glycogen synthase kinase 3 (GSK3). Here, we use a rat model of moderate co-use of ethanol and delta-9-tetrahydrocannbinol (THC) to examine long-term effects of drug exposure on the GSK3 signaling pathway. Peri-adolescent male and female Long Evans rats (postnatal day (P) 30 to 47, *n*=6-9 per group) voluntarily consumed 10% ethanol sweetened with 0.1% saccharin, cookies laced with THC (3-10 mg/kg), or both. On P114, which was ~3 days after rats completed ~50 days of behavioral testing, rats were given a challenge injection of 5 mg/kg THC (i.p.) or its vehicle and sacrificed 30 min later for brain extraction. We subsequently measured expression of total and phosphorylated GSK3ß (the main isoform in the brain) and its regulator protein kinase B (AKT) in the prefrontal cortex (PFC) and mediobasal hypothalamus (MBH) using Western blot techniques. We hypothesized that drug co-use would reduce the function of this pathway under baseline conditions but increase its sensitivity to cannabinoid receptor activation induced by THC challenge. Our results revealed that THC challenge increased phosphorylated AKT and GSK3ß compared to vehicle challenge, while no treatment group differences in phosphorylation levels of AKT and GSK3ß were detected. Treatment group or challenge type had no effect on total protein levels of AKT or GSK3ß. These findings do not support our hypothesis that moderate co-use of ethanol and THC would alter the function of the AKT-GSK3ß pathway. Because the current study assessed activation nearly two months after drug use, further investigation is needed to determine if adaptations were reversed following protracted abstinence. Taken with our previous study that used the same exposure paradigm and found no differences in working memory or glucose clearance measured in early adulthood, the current findings suggest that the effects of moderate co-use of alcohol and THC are less robust than those that have been demonstrated following heavy co-use.

Disclosures: L. Shi: None. **L. Carrica:** None. **C.Y. Choi:** None. **N. Liang:** None. **J.M. Gulley:** None.

Poster

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Topic: A.09. Adolescent Development

Support: TRDRP T30FT0967 to VL
TRDRP T34IP8023 to VL
NIDA DA051831 to CDF

Title: Investigating the effects of prenatal nicotine and THC exposure on cognitive function and placental development in a rat model

Authors: *V. LALLAI[1], C. D. FOWLER[2];
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Abstract: The increasing prevalence of concurrent nicotine and THC use among pregnant women is influenced by the widespread availability of e-cigarettes marketed as a 'safer' alternative and the growing accessibility of THC-infused edibles. Nicotine and cannabinoids affect dopaminergic signaling through distinct receptors within overlapping cellular groups, suggesting potential diverse consequences from their combined consumption. This complex interplay during prenatal development raises questions about their cumulative impact on embryonic growth, neurodevelopment, and overall well-being. To mirror human usage patterns, female Wistar rats underwent pre-exposure before mating and were then administered daily doses of nicotine vape and oral THC throughout pregnancy. Maternal blood samples were collected to quantify nicotine and cannabinoid metabolites, ensuring accurate assessment of drug exposure. Offspring from the first cohort underwent cognitive behavioral tests during adolescence and drug intake assessments during adulthood. For the second cohort, placental tissue and fetal brains were collected on gestational day (GD) 18 for RT-q-PCR analysis of mRNA expression levels of nicotinic subunits, cannabinoid receptors (nAChRs and CBR), dopamine receptors and transporters (D3 and DAT), and sex-specific markers. We first confirmed the presence of cotinine, nicotine's principal metabolite, and THC in maternal blood, placenta, and fetal brain. Our initial findings demonstrate differential effects of prenatal exposure to e-cigarette nicotine vape and/or edible THC on cognitive function, with varying impacts within male and female groups. Additionally, our results reveal a specific distribution of nAChRs across various placental regions, with fetal sex-dependent influences on these expression patterns following drug exposure. RNA scope analysis further revealed a distinct distribution of CBR, primarily localized within the junctional zone and decidua, with notable absence in the labyrinth zone. This research unveils the intricate relationship between maternal e-cigarette usage, drug exposure, and developmental outcomes in rats, offering potential insights to enhance public health strategies and interventions for the benefit of future generations.

Disclosures: V. Lallai: None. **C.D. Fowler:** None.

Poster

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Topic: A.09. Adolescent Development

Support: NIH Grant AOD24006-001-00000; MOA-AI-21002-01

Title: Identifying epimutation inheritance following acute maternal organophosphorus nerve agent (OPNA) exposure

Authors: *S. COE[1,2], O. BELLEK[1,2];
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Abstract: The field of epigenetics has allowed for the exploration of environmental influences on non-genotoxic gene expression, influences including exposure to both organophosphorus nerve agents (OPNAs) and acetylcholinesterase (AChE) reactivators. Previous studies have identified brain-specific epimutations in offspring, via DNA methylation, following maternal OPNA and reactivator exposure in a humanized mouse model. While OPNA- and reactivator-induced multigenerational epimutations have been observed previously in mice, the possibility and subsequent genotypic comparability of transgenerational epimutations has not yet been explored. As an improved model of human organophosphorus nerve agent (OPNA) exposure and treatment, C57BL/6J mice were genetically modified to knockout (C57BL/6-*Ces1*$^{tm1.1Loc}$/J; Es1 KO) serum carboxylesterase (CaE) and knock in(C57BL/6-*AChE*$^{tm1.1Loc}$/J; AChE KI) the AChE human enzyme homolog. The resulting KIKO mouse strain eliminated the respective limitations of species-specific OPNA resistance and AChE interactions. While researchers are aware of the adverse effects of OPNA exposure, it is vital to identify if these effects can influence the health of future generations. By utilizing both WT and KIKO mice, knowledge of comprehensive epigenetic effects and molecular mechanisms could be applied to both broad scale mouse models as well as human health research. In this study, pregnant WT and KIKO mice (P0) will be assigned to one of three exposure/treatment combinations (vehicle/vehicle, vehicle/reactivator, or OPNA/reactivator) on embryonic day 14 (E14). These dams will then progress through pregnancy and weaning, at which point tissue will be collected for further analysis. The offspring of exposed animals(F1) will be randomly assigned as breeders (females only) or for tissue collection at key stages in their lifecycle. DNA methylation, quantitative polymerase chain reaction (qPCR), and matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) may be used to analyze a variety of key tissues. F1 breeders from each condition will be utilized to produce two subsequent generations from which tissue will be collected for analysis. We anticipate observable epimutations will be stable across multiple generations from both genotypes, thus confirming the transgenerational effects of OPNA and reactivator exposure.

Disclosures: S. Coe: None. **O. Bellek:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.11/

Topic: A.09. Adolescent Development

Support: Fundamental Research Grant Scheme (FRGS/1/2018/SKK08/UITM/02/9)

Title: Prenatal bisphenol A exposure induced changes in the microRNA expression for regulating NMDA receptor subunits in the male rat hippocampus

Authors: *N. M. NAYAN, R. SIRAN;
Physiol., Fac. of Med., Universiti Teknologi MARA, Sungai Buloh, Malaysia

Abstract: Bisphenol A (BPA) is one of the most widely used chemicals in the production of plastic and epoxy resin. BPA exposure during pregnancy is a particular concern since this compound can cross the placenta barrier, leading to disruptions in fetal development. Prenatal BPA exposure has been shown to influence learning and memory function via dysregulation of NMDA receptor subunits in the hippocampus. However, it is presently unknown whether the impairment is potentially associated with modifications of microRNA (miRNA) expression. This study was done to investigate the effect of prenatal BPA exposure on the expression of miR-19a and miR-539 in regulating NMDA receptor subunits in the male rat hippocampus as well as its neurobehavioral outcome. The effect was also observed on the level of estrogen receptor (Er) in the mother placenta. Pregnant Sprague Dawley rats were orally exposed to 5 mg/kg/day of BPA from pregnancy day 1 until day 2. Whereas, the control mother was without BPA. The mothers were monitored daily until gestation day 21 (GD21) for either a cesarean section or spontaneous delivery. At GD21, the mother placenta was collected to determine the levels of Erα and Erβ. The hippocampus of male offspring was dissected and collected when reaching GD21, postnatal days 7, 14, 21 (PND7, 14 and 21) and adolescent day 35 (AD35). At each stage of age, the expression of miR-19a, miR-539, GRIN2A and GRIN2B were determined by qRT-PCR. The levels of GluN2A and GluN2B were estimated by Western Blot. The male rats were assessed with step-down passive avoidance and Morris water maze test when reaching AD35. The findings showed that prenatal BPA exposure significantly increased the levels of Erα and Erβ in the placenta. In the hippocampus, the decrement of miR-19a and miR-539 expression in the BPA-treated group significantly reduced the expression of GRIN2A and GRIN2B at all stages of age. The levels of GluN2A and GluN2B in the BPA-treated group are also significantly reduced when reaching AD35. Consequently, rats from the BPA-treated group show impairments in fear and spatial learning and memory compared to the control rats. The findings indicate that higher Er levels in the placenta may increase the transfer rate of BPA, thereby increasing the fetus's exposure to BPA. The BPA-induced miRNA decrement negatively influenced the regulation of GRIN2A and GRIN2B in translating GluN2A and GluN2B in the male rat hippocampus. In conclusion, BPA-induced miRNA decrement is suggested to be the primary factor that disrupts the development of NMDA receptor subunits, subsequently leading to learning and memory impairment.

Disclosures: N.M. Nayan: None. **R. Siran:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.12/A22

Topic: A.09. Adolescent Development

Support: NSERC

Title: Investigating the effects of combined oral contraceptives on biomarkers of neuronal growth, synaptogenesis and on cognition in adult and pubertal CD-1 mice

Authors: *S. KHELOUI[1], A. HINTERBERGER[2], J. LIANG[3], N. ISMAIL[4];
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Abstract: Combined oral contraceptives (COCs) are used daily by over 150 million adolescent and adult females. Findings suggest that COCs may alter brain structure and function, but that these effects may depend on factors like age of onset, COC formula, and androgenicity of the progestin. However, the mechanisms underlying the effects of COC on brain structure and function remain unknown. The current study investigated the effects of two COCs formulations usage on cognition, the expression of biomarkers of neural growth and synaptogenesis in pubertal and adult CD-1 mice. At 5 or 10 weeks of age, female mice were daily administered 240µL of either a combination of ethinyl estradiol/levonorgestrel (EE/LNG) or ethinyl estradiol/drospirenone (EE/DRSP) through oral gavage for seven weeks. Adult naturally cycling females and male controls were administered 240µL of sterile water via oral gavage daily for seven weeks. Estrus cycle acyclicity was confirmed through vaginal lavage. Cognitive function was assessed using the Barnes maze, social recognition, and novel object recognition tests. Mice were euthanized, and brain samples were collected. BDNF and postsynaptic density protein (PSD-95) expressions were examined through immunohistochemistry to assess neural growth and synaptogenesis in the hippocampus (HIPP) and the medial prefrontal cortex (mPFC). Results revealed an increase in chemo investigation time and altered spatial acquisition patterns, as indicated by increased errors, distance, latency for pubertal groups. In addition, the EE/LNG pubertal group displayed increased BDNF expression in the HIPP CA1 region and decreased PSD-95 expression in the mPFC infralimbic area region. The EE/LNG pubertal group showed higher expression for BDNF in the CA3 and PSD-95 in the CA1 regions of the HIPP. EE/LNG adult mice had increased BDNF expression in the dorsal anterior cingulate cortex and prelimbic regions of the mPFC. These results suggest that COC use, especially during puberty, alters brain structure by impacting neural growth, synaptogenesis and induces cognitive changes, but these effects depend on the COC formula. Understanding the impacts of COC use is pivotal for informed clinical decisions aimed at supporting women's reproductive health.

Disclosures: S. Kheloui: None. **A. Hinterberger:** None. **J. Liang:** None. **N. Ismail:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.13/A23

Topic: A.09. Adolescent Development

Support: NSERC Grant 2020-04302
MITACS Accelerate

Title: Identification of the model of chronic stress that causes the greatest activation of the NLRP3 inflammasome pathway and induces anxiety- and depression-like behaviours in CD-1 mice

Authors: *M. DWORSKY-FRIED[1], U. H. IQBAL[2], N. ISMAIL[1,3,4];
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Abstract: Puberty is a critical period of development that is susceptible to stress exposure, which can induce enduring anxiety, depression, and neuroinflammation. The NLRP3 inflammasome, a crucial component of the innate immune system, detects various signals, such as cellular stress. In periods of stress, activating the NLRP3 inflammasome pathway in microglia triggers a cascade of events, including the production of pro-inflammatory cytokines. In instances of chronic stress, over-activated microglia can render individuals more vulnerable to psychiatric disorders in ways that differ between sexes. Although many chronic stress models are well-validated, the extent to which they influence the NLRP3 inflammasome remains unclear. Therefore, this study aimed to elucidate the relationship between pubertal chronic stress and the activation of the NLRP3 inflammasome. By investigating the influence of various chronic stress models on NLRP3 activation and subsequent anxiety- and depression-like behaviours, we sought to identify pathways for potential therapeutic interventions in psychiatric disorders. During the pubertal stress-sensitive period (at six weeks of age), male and female CD-1 mice were exposed to either seven days of chronic sleep deprivation, 14 days of chronic unpredictable stress (CUS), 28 days of CUS, or remained unstressed. All mice went through behaviour assays, namely, the elevated-plus maze (EPM), the open field test (OFT), and the forced swim test (FST). The mice were euthanized the following day, and blood was collected for multiplex bead-based Luminex immunoassay to analyze concentrations of pro-inflammatory cytokines interleukin-17A (IL-17A), interleukin-22 (IL-22), and interleukin-1B. Sleep-disrupted mice displayed significantly greater depression-like behaviour in the FST compared to control and other stressed groups. The 28-day CUS group showed heightened anxiety-like behaviour in the EPM, while the 14-day CUS group exhibited the highest anxiety-like behaviour in the OFT compared to other groups. Additionally, plasma cytokine quantification revealed that the 28-day CUS group exhibited the highest concentration of IL-17A compared to all other groups, whereas levels of IL-22 were comparable across the stressed groups. These findings demonstrate distinct behavioural and inflammatory responses to different stress paradigms, emphasizing the complex relationship between pubertal stress, neuroinflammation, and psychiatric symptoms. These findings highlight the importance of targeted interventions against the NLRP3 inflammasome pathway in managing stress-related neuropsychiatric disorders.

Disclosures: M. Dworsky-Fried: None. **U.H. Iqbal:** None. **N. Ismail:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.14/A24

Topic: A.09. Adolescent Development

Support: NSERC Grant RGPIN-2020-04302 to N.I.

Title: Pubertal chronic sleep disruption increases blood-brain barrier permeability in a time, region, and sex-specific manner in CD-1 mice

Authors: *A. E. HINTERBERGER[1], P. ESPOSITO[1], L. CAPPELLETTI[1], L. WANG[1], N. ISMAIL[1,2];
[1]Psychology, NISE Lab., Univ. of Ottawa, Ottawa, ON, Canada; [2]LIFE Research Institute, University of Ottawa, Ottawa, ON, Canada

Abstract: Many adolescents experience changes in their circadian rhythm during the peripubertal period, resulting in delayed and inadequate sleep. This change may have negative consequences within the brain, as puberty and adolescence are periods of extensive neuronal maturation. Insufficient or inadequate sleep during puberty and adolescence has been associated with increased depression-like behavior. To understand the mechanism underlying this effect, our lab has developed a mouse model in which we showed that pubertal chronic sleep disruption (CSD) induces depression-like behavior in male and female CD-1 mice and increases cFOS activation in the prelimbic cortex of pubertal females. One possibility for these effects could be that pubertal CSD alters the brain and behavior by increasing BBB permeability. Thus, this study was designed to investigate whether pubertal CSD increases BBB permeability in the hypothalamus, hippocampus, prefrontal cortex, and whole brains of male and female CD-1 mice. At 6 weeks of age (during the stress-sensitive peripubertal period), mice underwent either CSD through gentle handling for the first four hours of their rest phase for seven consecutive days, or were left undisturbed. Then, mice were euthanized at either 24- , 72-, or 168-hours following the last sleep disruption. BBB permeability was examined in the whole brain or in specific brain regions (prefrontal cortex, hippocampus, and hypothalamus) by injecting the mice with radiolabeled 14C-sucrose, and determining the mean disintegrations per minute (DPM) per brain sample (g) divided by the DPM per μL of serum in the corresponding sample (μL/g). Two-way ANOVAs revealed that at 72 hours post-CSD, sleep disrupted females had higher BBB permeability in the prefrontal cortex and hippocampus than non-sleep disrupted females and sleep-disrupted males. In the hypothalamus only, sleep-disrupted mice had higher BBB permeability 168 hours post-CSD compared to non-sleep disrupted mice, regardless of sex. There was no significant difference between groups in the whole brain analyses. These findings demonstrate that CSD increases BBB permeability, but in a sex-, time-, and brain region-dependent manner. Thus, pubertal CSD may induce depression-like behavior by increasing BBB permeability, particularly in females. These findings add to our understanding of factors and mechanisms involved in the onset of mental illness during puberty and adolescence.

Disclosures: A.E. Hinterberger: None. **P. Esposito:** None. **L. Cappelletti:** None. **L. Wang:** None. **N. Ismail:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Program #/Poster #: PSTR147.15/A25

Topic: A.09. Adolescent Development

Support: NSERC (grant # 2020-04302)

Title: Enduring Effects of Pubertal Antimicrobials and Lipopolysaccharide Treatments on Cellular Mechanisms Associated with Neurodegeneration in Male and Female CD1 Mice

Authors: *E. DUBÉ-ZINATELLI[1,2], P. ESPOSITO[3], L. CAPPELLETTI[3], N. ISMAIL[3]; [1]Psychology, Univ. of Ottawa, Ottawa, ON, Canada; [2]School of Psychology, University of Ottawa, Ottawa, ON, Canada; [3]Sch. of Psychology, Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Neurodegenerative disorders are one of the leading causes of mortality and morbidity worldwide. While the etiology of neurodegenerative disorders remains unknown, alterations to the gut microbiome during puberty may increase susceptibility to neurodegeneration later in life. Recent research demonstrated that pubertal antimicrobial (AMNS) and lipopolysaccharide (LPS) treatments reduce gut microbial diversity and induce sex-specific changes in acute cellular mechanisms associated with neurodegeneration; however, the enduring effects of these treatments remain unknown. Therefore, the objective of this study was to investigate the enduring effects of pubertal LPS and AMNS treatments on biomarkers (sigma-1 receptor; S1R and glial-derived neurotrophic factor alpha 1; GFRA1) associated with neurodegeneration. At five weeks of age, male and female CD-1 mice received broad-spectrum antimicrobials or water through oral gavage twice daily for seven days. Mice received an intraperitoneal injection of either saline or LPS at 6 weeks of age. At 12 weeks, mice were euthanized, and brain samples were collected for immunohistochemical analysis of the medial prefrontal cortex, hippocampus, and motor cortex. The results indicated that pubertal AMNS and LPS treatments reduced the expression of S1R and GFRA1 in the hippocampus, particularly leading to decreased S1R expression in the CA1 and CA2 regions and reduced expression of both GFRA1 and S1R in the dentate gyrus. Additionally, sex-specific effects of AMNS and LPS treatments were observed in the motor cortex, where S1R expression was lower in the M2 region, and GFRA1 expression was reduced in the M1 region of male mice. These findings suggest that AMNS and LPS treatments during puberty lead to enduring deficits in cell survival and neurogenesis and that males may have increased susceptibility to motor neuron degeneration compared to females. This insight contributes to our comprehension of how alterations to the gut microbiome during puberty can increase susceptibility to neurodegeneration later in life in a sex-dependent manner.

Disclosures: E. Dubé-Zinatelli: None. **P. Esposito:** None. **L. Cappelletti:** None. **N. Ismail:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.16/A26

Topic: A.09. Adolescent Development

Support: CGS-M NSERC
PGS-D NSERC
Mitacs Accelerate Grant

Title: Age and sex differences in brain glucose and lactate metabolism following lipopolysaccharide administration and Probiotic intervention

Authors: *S. K. GOSTLIN[1,2], J. LIANG[1], U. IQBAL[4], E. MAYOTTE[1,2], E. DUBÉ-ZINATELLI[1,2], L. CAPPELLETTI[1,2], R. KRNEL[1,2], E. ERENBEN[1,2], C. MESSIER[2], N. ISMAIL[1,2,3];
[1]NISE Lab., [2]Sch. of Psychology, Fac. of Social Sci., [3]LIFE Res. Inst., Univ. of Ottawa, Ottawa, ON, Canada; [4]Rosell Inst. for Microbiome and Probiotics, Montreal, QC, Canada

Abstract: Fatigue is a common consequence of both systemic infection and chronic stress, and recent theories posit that disrupted brain metabolism could be a mechanism underlying this effect. Exposure to an immune challenge, like the administration of lipopolysaccharide (LPS) in rodents, is a common method of eliciting an immune response and sickness behaviours that mirror those seen in humans. It is unclear, however, if immune activation and sickness behaviours following infection are accompanied by fluctuations in the brain's main metabolic substrate, glucose, and its alternate energy source, lactate, and whether they can be mitigated by probiotic consumption. It is also unclear whether these fluctuations, if any, are different across age (puberty to adulthood) and sex (male and female). To address these knowledge gaps, male and female CD-1 mice received a bilateral cannulation surgery at 4 weeks or 8 weeks of age. To allow for the *in vivo* measurement of glucose and lactate via biosensing electrodes in the hippocampus. Following recovery, mice received either a probiotic formula or water for one week starting at either 5 weeks of age (pubertal group) or at 9 weeks of age (adult group). At 6 weeks (pubertal group) or 10 weeks (adult group) of age, all mice received either a saline or LPS injection and *in vivo* glucose and lactate measurements in the dorsal hippocampus began, continuing for 48 hours. Recordings did not show robust sex differences in baseline glucose and lactate concentrations, but cerebral lactate levels were lower in adult, compared to pubertal, male and female control groups. LPS-treated adult mice showed consistently higher glucose concentrations across time than their saline-treated counterparts, but this effect was reversed in the pubertal groups. Additionally, probiotics appear to have a protective effect against LPS-induced increases in lactate concentration across sexes in adulthood. To our knowledge, this study is the first to demonstrate the ability of probiotics to compensate for the disrupted hippocampal lactate levels that follow sickness and exposure to an immune challenge. These findings contribute to the growing body of research into infection-induced metabolic disruptions in the brain and is particularly relevant for our understanding of the long-term impact of infection, such as that seen in Long COVID.

Disclosures: S.K. Gostlin: None. **J. Liang:** None. **U. Iqbal:** None. **E. Mayotte:** None. **E. Dubé-Zinatelli:** None. **L. Cappelletti:** None. **R. Krnel:** None. **E. Erenben:** None. **C. Messier:** None. **N. Ismail:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.17/A27

Topic: F.03. Stress and the Brain

Title: Pubertal differences in the expression of excitatory amino acid receptor subunits in the paraventricular nucleus of male and female rats: relevance to stress reactivity

Authors: *R. ROMEO[1], C. PARKIN[1], K. G. BATH[2];
[1]Barnard Col., New York, NY; [2]Psychiatry, Columbia Univ., New York, NY

Abstract: Adolescence is a crucial developmental period that is marked by physiological, psychological, and social changes. However, it is also the time during which many stress-related vulnerabilities emerge, such as mood disorders. There are noted differences in stress-induced hypothalamic-pituitary-adrenal axis (HPA) responses before and after pubertal development, which may be contributing to these vulnerabilities. For instance, previous studies reveal that prepubertal animals show higher and more prolonged stress-induced hormonal responses compared to adult animals. This appears to be in part due to greater stress-induced activation in the paraventricular nucleus of the hypothalamus (PVN) in prepubertal compared to adult animals. However, the neural mechanisms that mediate this heightened reactivity of the PVN remain unknown. To address this gap, using qRT-PCR we examined the expression of the excitatory amino acid receptor subunits *Grin1*, *Grin2a*, *Grin2b*, *Gria1*, *Gria2*, *Grik1* and *Grik2* in the PVN before and after a stressor in prepubertal (30 days of age) and adult (70 days of age) male and female rats (n= 6 per age and time point). Our findings show that there is little association between pubertal changes in hormonal stress reactivity and the expression of these subunits in the PVN. In fact, many of the differences we found were main effects of age, with adult males and females exhibiting higher expression levels of these subunits compared to prepubertal males and females. Thus, these data suggest that mechanisms other than an upregulation of excitatory receptors in the PVN are responsible for mediating the alterations in hormonal stress reactivity that occur with pubertal maturation.

Disclosures: R. Romeo: None. **C. Parkin:** None. **K.G. Bath:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.18/A28

Topic: F.03. Stress and the Brain

Support: Intramural Cibersam Grant SAM21PI04/2024
Ministry of Science, Innovation and Universities Grant PID2021-127497OB-I00

Title: Effects of ultraprocessed food on rat depressive-like behavior

Authors: F. PILAR-CUELLAR[1], D. LORENO[1], E. NOSKOVA[1], L. QUIJANO SÁRRAGA[1], J. SENSERRICH GUERRERO[1], O. CONTRERAS RODRÍGUEZ[2], ***A. ADELL**[3];
[1]IBBTEC-University of Cantabria, Santander, Spain; [2]Univ. Autonoma de Barcelona, Bellaterra, Spain; [3]IBBTEC, Consejo Superior de Investigaciones Cientifícas, Santander, Spain

Abstract: Major Depressive Disorder (MDD) is a debilitating mental health condition highly diffused among adolescents and young adults with important differences between genders. Growing evidence suggests a correlation between MDD and increasing consumption of ultraprocessed food (UPF) during adolescence. Industrial food intake influences the peripheral tryptophan (Trp) availability and triggers inflammatory responses known to indirectly alter Trp metabolism and potentially shift to kynurenine (Kyn) pathway, leading to variations in other Kyn-based neuroactive metabolites. Therefore, it is possible that a reduction in brain serotonin (5-hydroxytryptamine, 5-HT) together with an elevation of Kyn metabolites could be associated with brain dysfunctions and mental illness. The precise changes in 5-HT, Trp, and Kyn in response to UPF consumption remain largely unknown. Addressing this knowledge gap is crucial to understanding the potential effects of UPF intake on emotional and cognitive functions during adolescence. In the present work we have studied the behavioral and neurochemical aspects of the administration of a six-week UPF diet with or without a four-week chronic stress exposure (chronic restraint stress, CRS) on male and female Long-Evans rats. Differences in the concentration of monoamine neurotransmitters in the prefrontal cortex (PFC) and hippocampus (Hp) have been evaluated through ultra high-performance liquid chromatography (HPLC). The intake of UPF produced a significant increase in the consumption of fat and sugar. Behavioral results showed additive effects of UPF diet and CRS in increasing immobility in the forced swim test (FST), both in males and females. In females, but not in males, UPF diet increased the latency to eat in the novelty-suppressed feeding test (NSFT), both in non-stressed and CRS rats. HPLC analysis showed a significant reduction of Trp in the PFC and Hp elicited by the administration of the UPF diet. The ratio 5-HT/Trp was also lower in female rats compared to males, which may confer a higher vulnerability to depressive-like behavior. We also found that UPF diet further decreased the ratio Kyn/5-HT in female rats.
In summary, our results showed that the UPF diet administered during adolescence may have a detrimental effect on female rats possibly due to the alteration of Trp metabolism. Thus, the UPF-induced increased intake of fat and sugar may result in an increased vulnerability of female Long-Evans rats to depressive-like behavior.

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Poster

PSTR147: Developmental Mechanisms of Vulnerability

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.19/A29

Topic: F.03. Stress and the Brain

Support: R21DA052815

Title: Locus coeruleus-specific viral manipulation of delta opioid receptor: effects on stress-induced physiological and behavioral changes

Authors: *J. TKACZYNSKI, J. PARK, D. J. CHANDLER;
Dept. of Neurosci., Virtua Hlth. Col. of Med. & Life Sci. of Rowan Univ., Stratford, NJ

Abstract: The stress system is engaged in response to aversive stimuli to regulate homeostasis and promote survival. The noradrenergic locus coeruleus (LC) is part of this system, with stress promoting LC hyperactivity and increased norepinephrine release to downstream regions. Previous work from our laboratory has shown that acute stress (15 minutes of combined restraint and predator odor) leads to a downregulation of δ opioid receptor (DOR) in the LC of adolescent male rats, and that LC-specific overexpression of the DOR is able to block the effects of stress on anxiety-like behaviors and LC spontaneous firing rate. Therefore, we have begun investigating the effects of LC-specific knockdown of DOR in a variety of behavioral tasks [open field test, elevated plus maze, defensive shock probe burying task (DSPB)] and electrophysiological tests in order to further investigate the role of this receptor in stress-responsive behavior. Preliminary results show a significant effect of DOR expression on rearing time in the DSPB independent of stress, with knockdown animals spending more time rearing and overexpressing animals spending less time rearing when compared to mCherry controls. We hypothesize that this change in rearing indicates differences in how animals cope with the shock, with knockdown animals showing a higher active coping response in the form of escape behavior. Functional DOR knockdown was confirmed electrophysiologically through bath application of the selective DOR agonist TAN-67, which reduced LC firing rate in the mCherry control group, but not in the DOR knockdown animals. Collectively, these findings provide further evidence to suggest that LC-specific DOR expression is important in the stress response.

Disclosures: J. Tkaczynski: None. **J. Park:** None. **D.J. Chandler:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.20/A30

Topic: F.03. Stress and the Brain

Support: Huck Institutes of Life Sciences
Department of Biobehavioral Health at Penn State University

Title: The effect of increasing house dust mite exposure to cause allergic asthma lung inflammation on behavioral, neurological, and growth comorbidities in BALB/cJ mice

Authors: *S. VELU[1], R. SAGAR[1], I. MARTINI BERNARDI[1], L. MERESSI[1], S. A. CAVIGELLI[2];
[1]Pennsylvania State Univ., University Park, PA; [2]Biobehavioral Hlth., Penn State Univ., University Park, PA

Abstract: Children and adolescents with asthma often suffer from different psychological and neurological co-morbidities such as anxiety, depression, social/cognitive difficulties, and growth impairment. A causal relationship between asthma and these outcomes has been investigated using two main allergens in rodent models: house dust mite (HDM) extract and ovalbumin (OVA). Even though HDM is most like allergens that trigger asthma in humans, it is used less frequently than OVA. In the current study, we conducted a dose-response study to identify the effect of increasing HDM exposure for allergic asthma induction on behavioral and biological symptoms often co-morbid with asthma. A total of 75 BALB/cJ mice were bred and divided into four treatment groups: saline control, low HDM, mid HDM, or high HDM doses that were repeatedly administered throughout development. After saline/HDM was administered 3 times/week for 7 weeks, mice underwent a series of behavioral tests to measure anxiety (elevated plus maze, light dark test), depression (sucrose preference test), social behavior (novel social partner test), and cognitive function (object location memory, object recognition memory). Body weight was monitored throughout development and blood and tissue samples were collected from adults to quantify asthma-like symptoms (e.g. airway inflammation), glucocorticoid secretion (which is often altered in asthma patients), and gene expression in brain areas associated with different behavioral outcomes. We present results on behavioral, glucocorticoid, growth, and brain gene expression outcomes to determine if and what dose of HDM is effective in stimulating known asthma comorbidities in a mouse model of developmental allergic asthma. Research was funded by the Huck Institutes of Life Sciences and the Department of Biobehavioral Health at Penn State University.

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Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.21/A31

Topic: A.09. Adolescent Development

Title: Adolescent intermittent stress causes lasting proinflammatory innate immune activation and neurodegeneration associated with increased alcohol drinking that persists into adulthood

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Abstract: Adolescence is a conserved neurodevelopmental period of neurotransmitter system maturation and increased hippocampal neurogenesis encompassing cognitive and emotive maturation that marks the transition from the prepubescent juvenile period to independence and adulthood. Chronic stress is common during adolescence, and we hypothesize that chronic intermittent adolescent stress exposure negatively affects adolescent brain maturation leading to lasting changes to adult neurobiology. Using a preclinical Wistar rat model of adolescent intermittent restraint stress (AIRS; 4 hr restraint stress, 2-days on/2-days off from postnatal day [P]25 to P55), we discovered that AIRS reduces late adolescent (P56) hippocampal neurogenesis, basal forebrain cholinergic neuron (i.e., ChAT, TrkA), and raphe nucleus serotonergic neuron (i.e., 5-HT, TPH2) populations that persist into adulthood (P80). Adolescent intermittent restraint stress increases expression of microglial Iba-1 and upregulates proinflammatory HMGB1-TLR4 signaling cascades consistent with persistent microglial activation and lasting induction of innate immune signaling. Adult AIRS-treated demonstrate increased alcohol preference and alcohol drinking on the two-bottle alcohol task in adulthood. Taken together, AIRS treatment persistently alters hippocampal neurogenesis, and cholinergic and serotonergic neuron populations that persist into adulthood. Further, persistent microglial activation and upregulation of innate immune signaling molecules may contribute to the observed neurodegeneration. Targeting microglial activation and/or persistent innate immune activation could represent a potential therapeutic target for treatment of developmental stress-induced neuropathology.

Disclosures: R. Vetreno: None. **L. Qin:** None. **F.T. Crews:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.22/A32

Topic: A.09. Adolescent Development

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Natural Sciences and Engineering Research Council of Canada (RGPIN-2017-06344)
The Brain and Behavior Research Foundation (formerly NARSAD; Young Investigator Award 26016)

Title: Investigating the effects of early-life stress on brain-wide patterns of neuronal activity and adolescent fear learning, anxiety- and depression-like behavior

Authors: *A. PATEL[1], A. ARYA[2], A. MAMBOU[2], A. CANELLA[2], H. PREMACHANDRAN[2], A. KHLAIFIA[2], M. MATTHIESEN[3], F. VIOLI[2], M. ARRUDA-CARVALHO[2];
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Abstract: Early life stress (ELS) caused by adverse experiences, including parental neglect and deprivation, is postulated to alter normal developmental processes during infancy, a critical period for brain development. ELS has been associated with an increased prevalence of adolescent depression and persistent neurobehavioral changes similar to those observed in psychiatric disorders. The dynamics of ELS activity-dependent brain-wide neuronal recruitment in adolescent mouse behaviour and brain maturation have not been well characterized. Here, we used full-litter or split-litter maternal deprivation (MD), an established rodent model of ELS, to stress $Fos^{2A\text{-}iCreER}$ (TRAP2) × Ai9(RCL-tdT) mice on postnatal day (PND) 11 for 24 hours. The pups were injected with tamoxifen (4-OHT) one hour following the onset of MD to genetically label stress-activated neuronal populations brain-wide. At the end of MD, the mice were returned to the home cage and left undisturbed until behavioural testing. A battery of behavioural tests were used to assess fear learning (auditory fear), anxiety- (elevated plus maze and open field test), and depression-related behaviours (tail suspension test and forced swim test) during adolescence (PND 35-40). Our results demonstrate that deprived mice acquired auditory fear training faster and exhibited greater freezing time during fear training and retrieval. Similarly, deprived mice exhibited impaired fear extinction learning and extinction retrieval in adolescence. Furthermore, deprived mice exhibited an increase in anxiety-like behaviour in the open field test, but not elevated plus maze test. Interestingly, in contrast with full-litter-deprived mice, split-litter-deprived mice did not exhibit a change in depression-like behaviour in either the forced swim test or tail suspension test. Preliminary immunohistochemical analyses revealed distinct brain-wide neuron labelling patterns between deprived mice and home-cage controls. Ongoing experiments aim to quantify c-fos expression following each behavioural test to measure preferential reactivation of ELS-associated brain-wide connectome in fear, anxiety- and depression-like behaviour. The findings of this study will highlight the neural correlates underlying increased susceptibility to psychiatric disorders during adolescence following ELS.

Disclosures: A. Patel: None. **A. Arya:** None. **A. Mambou:** None. **A. Canella:** None. **H. Premachandran:** None. **A. khlaifia:** None. **M. Matthiesen:** None. **F. violi:** None. **M. Arruda-Carvalho:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR147.23/A33

Topic: F.03. Stress and the Brain

Support: FRM DEVSTRESS

Title: Long-term impact of social isolation during adolescence: implication of hippocampal neurogenesis

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Abstract: Chronic stress is perceived as the enduring exposure to challenging situations exceeding an individual's resources, leading to cognitive and emotional disturbances. However, it can modulate brain activity and function according to its nature, duration and time window of exposure. Among these, adolescence has been identified as a period of vulnerability to environmental insults and of emergence of mental disorders, but the underlying mechanisms remain unknow. Due to their sensitivity to stress and their role in emotion, dentate granule neurons (DGN) of the hippocampus appear as possible candidates of stress-mediated deficits. Yet, further studies are needed to pinpoint the impact of adolescent stress in the sequential waves of DGN production occurring before (perinatal period), during (adolescence) or after (adulthood) stress exposure, and to analyze their relationship with behavioral disturbances. To address these gaps, we analyzed the behavioral consequences of a 2-weeks social isolation stress during adolescence (postnatal P21 to P35), focusing on anxiety-like and stress reactivity responses in C57/BL6J mice. Furthermore, to identify the DGN population underlying stress-mediated behaviors, we also analyzed its impact on DGN production across life. When tested in adulthood (P80) baseline anxiety assessed in the Open Field Test, Light-Dark box and Elevated Plus Maze was not modified neither in males nor females. However, after exposure to a second stressor (Swim Stress), socially isolated adult mice of both sexes show impaired anxiety responses. To analyze the potential involvement of DGN generated across life, markers of dividing cells EdU and BrdU, were injected at P1 to tag the perinatal period and from P28-P30 to tag the adolescent period, respectively. Mice were sacrificed 90 minutes after exposure to the stress reactivity task to identify the impact of social isolation on both the number of surviving EdU and BrdU cells, as well as their recruitment in the task by means of immediate early genes expression. Ultimately, this will allow us to study the functional implication of cells generated before and during social isolation in mediating the observed behavioral impairments. Our findings suggest that social isolation during adolescence have long-term behavioral consequences in both male and female mice, generating an increased susceptibility to develop anxiety disorders in response to stress. Ongoing analysis of DGN will allow us to understand the role of the different waves of neurogenesis in mediating such behavioral differences.

Disclosures: C. Nabais: None. **M. Koehl:** None. **N.D. Abrous:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Program #/Poster #: PSTR147.24/A34

Topic: A.09. Adolescent Development

Support: NIH grant DA051598
NIH grant DA051977
NIH grant MH129320

Title: Social isolation during adolescence disrupt decision-making trajectories in male and female rats

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Abstract: Title: Social isolation during adolescence disrupt decision-making trajectories in male and female rats
Authors: Marcini Bakis[1], Justin Hill[1], Stefanie Simpson[1], Peroushini Villiamma[2], Kaitlyn LaRocco[2], Melvin Bonilla[1], and Stephanie Groman[1,3]
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Adolescence is a critical neurodevelopmental period associated with robust biobehavioral changes. These age-related changes include improvements in decision-making functions that we have found to predict drug use in adulthood. Neurodevelopmental disruptions that occur during adolescence may, therefore, increase addiction susceptibility in individuals. Social isolation during adolescence leads to decision-making impairments and increased addiction susceptibility in adulthood that we hypothesize to be a consequence of isolation-induced disruptions in select neurodevelopmental mechanisms. To test this hypothesis, we assessed decision-making processes throughout adolescence and adulthood in male and female Long Evans rats that had been socially isolated or grouped housed at different ages. Rats were trained to acquire and reverse three-choice, spatial discrimination problems using a probabilistic reversal-learning (PRL) task and performance was assessed throughout adolescence and into early adulthood. Trial-by-trial choice data was fit with a reinforcement-learning model to obtain an estimate of reward-based decision-making. We found that age-related changes in PRL performance were attenuated in rats that were isolated during adolescence compared to socially housed rats and rats that were only isolated during adulthood. Moreover, our computational approached revealed that the impairment in isolated rats was found to be due to disruptions in reward-based decision-making functions. Ongoing work suggests that social interactions between P50-P65 – when

pruning and myelination are ongoing – may be critical for establishing optimal reward-guided decision-making functions. These data demonstrate that social interactions during adolescence are critical for age-related changes in decision-making and suggest a critical role of adolescent neurodevelopment in the mechanisms underlying addiction susceptibility. Our future studies will narrow in on the biological changes that occur during P50-P65 and determine how these mechanisms are altered by social isolation.

Disclosures: M.V. Bakis: None. **J.K. Hill:** None. **S. Simpson:** None. **P. Villiamma:** None. **K. LaRocco:** None. **M. Bonilla:** None. **S.M. Groman:** None. **A. Thomson:** None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: NIH Grant P50AA017823
NIH Grant R01AA030469
NIH Grant T32AA025606

Title: Selective age differences in glucocorticoid-mediated regulation of neuroimmune signaling pathways in adolescent rats

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Abstract: Adolescence is a period characterized by significant alterations in stress reactivity that includes hypothalamic-pituitary-adrenal (HPA) axis-regulated release of glucocorticoid hormones, such as corticosterone (CORT). Compared to their adult counterparts, adolescent rats display a protracted CORT response to an acute stressor, suggesting that negative feedback regulation of the HPA axis may not be fully mature in adolescents. The glucocorticoid receptor (GR) is a key component of negative feedback suppression of the HPA axis, and its activation through increases in circulating CORT can modulate neuroimmune gene expression. An acute injection of dexamethasone, a GR agonist, produces changes in neuroimmune gene expression via increased IL-6 and IκBα, and decreased IL-1β and TNFα in adult rats. Thus, the first goal of the present study was to examine age-dependent changes in neuroimmune sensitivity to dexamethasone using adolescent (P30-32) and adult (P78-82) Sprague Dawley rats of both sexes (n=8-10 per group; N=76). For Experiment 1, rats were subcutaneously injected with dexamethasone (500 µg/kg) or vehicle (50% PG, 50% saline). Brain tissue and trunk blood were collected 4 hours later to measure CORT and neuroimmune gene expression. Dexamethasone

significantly increased expression of IL-6 and IκBα, and reduced IL-1β and TNFα expression in both the hippocampus and amygdala, thus replicating previous studies. However, the dexamethasone-dependent increases in IL-6 and IκBα gene expression were significantly impaired in adolescents relative to adults in both sexes, suggesting an immaturity of the glucocorticoid-mediated adolescent neuroimmune system. Therefore, Experiment 2 evaluated whether adolescent (P28-32) and adult (P82-91) male rats would show similar differences in dexamethasone-mediated induction of IL-6 and IκBα protein. Rats were subcutaneously injected with differing doses of dexamethasone (0, 50, 250, or 500 µg/kg), then perfused 4 hours later and brains were collected. Immunofluorescence was used to assess changes in IL-6 and IκBα protein, as well as their respective colocalization to astrocytes and endothelial cells. In the dorsal hippocampus, adolescent male rats in the vehicle group displayed significantly higher IκBα compared to their adult vehicle counterparts. However, there were no significant effects of dexamethasone on colocalization of IκBα and tomato lectin or IκBα and GFAP. Together, these findings provide insight into the glucocorticoid-mediated mechanisms by which the adolescent and adult neuroimmune systems differ.

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Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: NIH – Bridges to the Doctorate (T32GM14660)
NIH - GrantUrise 5T34GM136481
NIH - TRDRP SVFSIP T32SR4917

Title: Examining the role of age in NACHO expression in the reward pathway

Authors: *J. VIDES[1], S. POWELL[2], B. MONTESDEOCA[3], K. A. MARQUEZ[4], A. HUSSAIN[5], K. A. RAZAK[6], Y. SHERAFAT[7];
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Abstract: Nicotine addiction has been a prevalent issue worldwide and current therapeutics have only shown to be moderately efficacious. Nicotine's rewarding effect comes from activating the nicotinic receptors in the reward pathway, including the nucleus accumbens, which promotes

various survival behaviors such as eating, drinking, socializing, and sleeping. Although we have a basic understanding of how nicotine signaling works in the nucleus accumbens, we have yet to understand the full scope of nicotinic receptor modulators that affect this process. Furthermore, we do not know how these mechanisms change through development and aging. Of interest, the nicotinic acetylcholine receptor chaperone protein (NACHO) is of key interest as it is necessary for the expression and function of nicotinic receptors, however, how it is involved in nicotine reward and age has yet to be discovered. Nicotine addiction has often been associated with early onset use during adolescence brains, such that if early onset occurs, worse health and psychiatric outcomes will develop later in life. Examining how nicotinic receptors modulators, such as NACHO, change through age will give us better insight as to why adolescents are more susceptible to nicotine addiction compared to older adults. Thus our study examined whether NACHO expression differs in aging brains compared to young brains in mouse models in brain regions involved in nicotine reward. Our early findings suggest that NACHO expression increases with age. These results provide a good foundation to development more efficacious treatment targeted for substance abuse in vulnerable populations.

Disclosures: J. Vides: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research grant NIH. **S. Powell:** None. **B. Montesdeoca:** None. **K.A. Marquez:** None. **A. Hussain:** None. **K.A. Razak:** None. **Y. Sherafat:** A. Employment/Salary (full or part-time):; Assistant professor tenure track.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: AIM-AHEAD and NCATS Training Program - Cohort 2 3OT2OD032581-01S1-483

Title: A neuro-social perspective of understanding the impact of socio-demographic factors on protective and risk mechanisms in treatment for adolescent substance use - A data-driven approach

Authors: *L. ZHANG;
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Abstract: A Neuro-Social Perspective of Understanding the Impact of Socio-Demographic Factors on Protective and Risk Mechanisms in Treatment for Adolescent Substance Use: A Data-Driven Approach
Ling Zhang Northern Arizona University Over the past two decades, substance use and alcohol

have contributed to an increase in the prevalence of addictions and mental depression, especially adolescents exhibit heightened vulnerability to substance use, with pivotal ages at 12, 16, and 20 years marking increased risk of initiation, sensitivity, and addiction, respectively. Therefore, understanding the interplay of socio-demographic factors with neurodevelopmental processes during these stages is crucial for effective early intervention and treatment. This study aims to explore how protective and risk factors for substance use among adolescents vary across different ages and socio-demographic contexts by utilizing data from the OCHIN Community Health Equity Database and AIM-AHEAD Data Bridge. The team will conduct a comprehensive analysis of adolescent substance use patterns. The study will use validated predictive models to identify significant predictors and their interactions with age-related neurodevelopmental changes. The team will specifically examine the key variables related to historical, racial, gender, and socioeconomic factors to determine the risk level of substance use and their impact on the outcome of interventions and treatment. The objective of this study is to guide neuro-social interventions that address functional impairment and responsiveness to the needs of effective secondary prevention and treatment for adolescents substance use disorders at varying risk levels and various developmental stages.

Disclosures: L. Zhang: None.

Poster

PSTR147: Developmental Mechanisms of Vulnerability

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UROP Research and Creative Projects Award
Nu Rho Psi Undergraduate Research Grant
G. Andrew Mickley Undergraduate Research Award

Title: $Pm_{2.5}$ and the brain: how air pollution affects the functional connectivity of the salience network and mental health symptoms in metro detroit youth

Authors: *A. S. JAKUBIEC[1], C. G. ZUNDEL[2], S. ELY[2,3], R. TAMIMI[4], L. GOWATCH[2], C. CARPENTER[2], M. SHAMPINE[2], J. JANDE[4], S. CHANAMOLU[1,2,8], A. BHOGAL[5], H. MARUSAK[6,9,7];
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Abstract: Exposure to air pollution ($PM_{2.5}$) is associated with increased risk of mental health disorders in youth, yet the underlying neurodevelopmental mechanisms remain unclear. This study examined the impact of $PM_{2.5}$ exposure on anxiety and depression symptoms and functional connectivity of the salience network (SN), which plays a pivotal role in orienting attention to emotionally salient stimuli. Fifty youth aged 10-17 (52% female) wore personal air monitors for 7 days and completed surveys assessing anxiety and depression symptomatology. A subset of participants (n=35) underwent functional magnetic resonance imaging to examine within-network SN resting-state functional connectivity (rsFC) and between-network rsFC of the SN and the whole brain. Linear regression models tested associations between average $PM_{2.5}$ and anxiety and depression symptoms using a $p<0.05$ threshold. Linear regressions also tested associations between average $PM_{2.5}$ and rsFC of key SN regions (i.e., anterior cingulate cortex (ACC), insula) using a $p<0.005$, >10 voxel whole-brain threshold. Average past-week $PM_{2.5}$ varied in the sample from 0.58-31.21 μg/m3 (M =10.14 μg/m3). $PM_{2.5}$ was not associated with anxiety or depression symptoms. However, higher $PM_{2.5}$ was associated with lower rsFC between the ACC and right frontal pole (45 voxels, t = -4.75), and between the ACC and the precuneus (40 voxels, t = 4.56) Further, higher $PM_{2.5}$ exposure was associated with higher rsFC between left insula and the left and right supramarginal gyrus (Left: 45 voxels, t = 6.93; Right: 13 voxels, t = 4.10), and between the right insula and the left supramarginal gyrus (23 voxels, t = 4.10). Our findings demonstrate that $PM_{2.5}$ exposure is associated with altered within- and between-network rsFC of the SN. Lower rsFC between the SN and the frontal pole and precuneus in youth with higher $PM_{2.5}$ may indicate a shift towards reduced emotion regulation (frontal pole) and default mode network (precuneus) inhibition. Conversely, higher within-network SN rsFC (insula-supramarginal gyrus) may suggest increased network integration. Future longitudinal studies should examine whether the observed SN alterations predict increased risk of mental health disorders later in development.

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Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.01/A39

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

Title: Transcriptomic Evidence for Homotypic Neuropeptide Signaling Architectures in Mouse Brain

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Abstract: Cell-cell signaling by diffusible neuromodulators (e.g., neuropeptides, monoamines, acetylcholine), is critical to neuronal network function and plasticity. Neuromodulatory network architectures have nonetheless resisted delineation until the recent introduction of molecular genetic approaches. Neuropeptidergic (NP) signaling architectures are now particularly amenable to prediction using gene expression data because both secreted NP ligands and correspondingly selective NP receptors are encoded by diverse and numerous differentially expressed genes: neuropeptide precursor protein (NPP) genes and neuropeptide-selective G-Protein-Coupled Receptors (NP-GPCRs) genes, respectively. Corresponding "cell type" network nodes are also definable today by differential gene expression patterns. To fathom NP network architectures in mouse brain, we have analyzed results from a single-cell RNA-seq transcriptomic study of 4,116,323 cells sampled from 29 regions spanning the entire brain. Of these, 73,347 were sequenced deeply in multi-well plates (SMART-Seq, cortical cells only) and 4,042,976 less deeply in droplets (10X, brain-wide). Our analysis indicates that almost every mouse brain neuron expresses at least one of 61 NPP genes and at least one of 77 NP-GPCR genes. Notably, transcripts found in many individual neurons encode both an NPP and an NP-GPCR conjugate to that same NPP. Prominent examples include co-expression of the NPP *Cck* with the conjugate NP-GPCR *Cckbr* (mean 76%, max 98% of all cells across cortical glutamatergic types), of the NPP *Vip* with a conjugate NP-GPCR (mean 32%, max 74% across all cortical GABAergic types), and of the NPP gene *Penk* with a conjugate NP-GPCR gene in striatum (58% across dorsal GABAergic cells, 51% in ventral). Such co-expression of ligand and receptor immediately suggests the familiar prospect of autocrine (cell-to-self) signaling, but also implies the prospect of "homotypic" (cell-to-same-type) signaling. The biophysics of neuropeptide diffusion and anatomical results further suggest that homotypic neuropeptide peptide signaling must be shaped by cell-cell proximity and well as by gene expression patterns. Traditional findings of cortical column and "somatotopic" organizations, all provide strong hints of cell-cell proximity as major factors governing neuronal network function. Here we offer type-specified NP signaling predictions intended to inspire and enable focused experimental and theoretical studies of possible roles for proximity-shaped homotypic signaling architectures in neuronal network function and plasticity.

Disclosures: S.J. Smith: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aratome, LLC. F. Consulting Fees (e.g., advisory boards); E11.bio. **M.J. Hawrylycz:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.02/A40

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

Support: NIH/NIDA award P30 DA018310

Title: Neuropeptidomic landscape of the mouse locus coeruleus

Authors: *L. J. BECKER[1], E. ROMANOVA[2], J. V. SWEEDLER[2], J. G. MCCALL[1]; [1]Anesthesiol., Washington Univ. in St. Louis, Saint Louis, MO; [2]Univ. Illinois & Beckman Inst., Urbana, IL

Abstract: The locus coeruleus (LC) is a small region of the pons that is phylogenetically conserved in mammals. The LC sends efferent projections to nearly the whole central nervous system and is innervated by a diverse set of brain-wide afferent inputs. This broad efferent network represents the main source of norepinephrine (NE) in the mammalian brain. Consequently, the LC-NE system is involved in the regulation of a wide array of functions such as arousal, behavioral flexibility, pain processing, and stress reactivity. Modulation of these processes is in part mediated by neuropeptide signaling. Indeed, release of neuropeptides such as corticotropin releasing factor or endogenous opioids within the LC is known to mediate stress reactivity and pain processing. Additionally, several neuropeptides are thought to act as co-transmitters along with NE from LC neurons. Gaining insight into the peptidomic content of this brain region could therefore be valuable to better understand its function in health and disease. Neuropeptide detection can be challenging, however, especially in smaller brain regions where peptide content is already limited. Here, we describe a procedure enabling bilateral LC isolation while limiting peptide loss. Leveraging liquid chromatography coupled to tandem mass spectrometry (LC-MS), we then determined the endogenous peptide complement of the mouse LC. A total of >6000 peptides were de novo sequenced and mapped to >800 proteins in the Uniprot mouse database. Signaling proteins comprised ~20 % including 30 neuropeptide prohormones. A quarter of those detected peptides such as neurogranin or members of the cerebellin family have not been previously identified in the LC before. Since the LC-NE system is often thought to be sexually dimorphic, this neuropeptide library will be used to assess sex difference in the future studies. Our results suggest that some neuropeptides in the LC undergo circadian regulation. Notably, we found a decrease in orexin level and a trend for an increase in cholecystokinin level over the light phase of the day. Altogether this work establishes a comprehensive landscape of the peptide content of the mouse LC and demonstrates that this method is sensitive to circadian-driven changes in neuropeptide levels.

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Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

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Program #/Poster #: PSTR148.03/A41

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

Support: NIH Grant 1RF1 NS126061

Title: A silicon nanodialysis probe coupled with mass spectrometry enables localized in vivo sampling for monitoring neurotransmitters and neuropeptides

Authors: *K. LI, W. SHI, Y. DING, A. ARMSTRONG, Y. VLASOV, J. V. SWEEDLER; Univ. of Illinois, Urbana-Champaign, Urbana, IL

Abstract: Neurotransmission is inherently heterogeneous and small-scale. Understanding neuronal function and psychological disorders through *in vivo* neurochemical monitoring relies on precise measurements of chemical concentrations within specific brain regions. However, existing microdialysis probes samples from regions containing tens of thousands of cells thus averaging transmitter levels. Improving the limited spatial resolutions and recovery rates requires new approaches. Our work addresses these challenges using a silicon nanodialysis probe coupled with mass spectrometry (MS) enabling highly localized sampling and sensitive neurochemical detection from small-volume samples. The miniaturized silicon nanodialysis probe features a 3 mm long needle with a cross-section of 75x15 μm^2 and a membrane-free sampling area of 20 x 100 μm^2. Utilizing this probe, we conducted *in vivo* sampling on head-fixed, anesthetized mice, targeting the primary somatosensory cortex at a depth of 500 μm. Sampling occurred over a 3-hour period at a flow rate of 7 nl/min. Subsequently, collected samples were transferred, dried, and reconstituted in diluent for analysis using either ZipChip capillary electrophoresis (CE) MS or nano liquid chromatography-trapped ion mobility spectrometry-time-of-flight (nanoLC-timsTOF) MS. The extracellular concentrations of several important neurotransmitters and metabolites were measured in mice (n = 6). We found that the basal levels of most analytes are consistent with previous studies, while some neurotransmitters show higher concentrations and large discrepancies between samples, such as GABA (225.1 ± 145.3 nM), aspartate (4759.2 ± 1956.1 nM), and dopamine (50.4 ± 14.7 nM). These large variations may result from the small sampling area capturing local variations in neurotransmitter levels. In addition to small molecules, peptides can also be sampled and detected from the small volume samples thanks to the membrane-free design of the probe. In addition, 8 peptides were identified from a 1 μL brain dialysate sample using nanoLC-timsTOF and PEAKS Online database search. Of these, 5 are peptides derived from proteins related to neuroendocrine, including secretogranin-1, secretogranin-2, Pro-opiomelanocortin, and ProSAAS. The probe can also be used for local drug delivery by adding drugs to the perfusate. Our preliminary experiment shows that perfusion of 15 mM 4-aminopyridine (4-AP) results in 2- to 10-fold increased concentrations of select neurotransmitters. These results demonstrate the ability of this platform to monitor concentration gradients of various neurochemicals from precise brain locations.

Disclosures: K. Li: None. **W. Shi:** None. **Y. Ding:** None. **A. Armstrong:** None. **Y. Vlasov:** None. **J.V. Sweedler:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.04/A42

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

Support: NRSA Grant 1F31NS127592-01A1
NIH NINDS R01-NS126247
VA I01-BX004938
5T32NS007466-23
Lacroute Fellowship

Title: Dissecting the effects of exogenous and endogenous enkephalin on synaptic transmission in the mouse hippocampus

Authors: *N. WARIKOO[1], A. ANDERSON[2], C. DONG[3], L. TIAN[4], E. SCHNELL[1];
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Abstract: Exogenous opioid drugs act at opioid receptors to powerfully modulate function throughout the central and peripheral nervous systems. Similarly, endogenous opioid peptides-- produced, packaged and released by neurons-- bind these same opioid receptors, but their functional roles in modulating network excitability are far less understood. Furthermore, the opioid peptidergic system within the hippocampus undergoes dramatic changes during epileptogenesis, underscoring the need to understand how endogenous opioids affect neuronal circuit homeostasis and might contribute to disordered network dynamics. To study the role of the endogenous opioid peptide met-enkephalin (ME) in the hippocampal circuit, we applied ME while performing voltage-clamped single cell recordings from hippocampal granule cells and CA3 pyramidal cells in acutely prepared mouse brain slices, while electrically or optogenetically stimulating afferent fibers. ME inhibited GABA release from inhibitory synapses in the dentate and CA3, but had no effect on glutamatergic transmission in the dentate, suggesting ME differentially modulates inhibitory versus excitatory synapses. Ongoing studies continue to characterize the role of enkephalin-sensitive transmission in the dentate and CA3. Furthermore, we aim to study the effect of evoked opioid release at these synapses. To that end, we have virally expressed the novel opioid biosensor deltaLight in mouse hippocampus and found that it reliably detects exogenous ME. Ongoing studies will attempt to visualize evoked opioid-peptide release from enkephalin-producing neurons to help characterize the effects of enkephalins at key nodes in the hippocampal circuit.

Disclosures: N. Warikoo: None. **A. Anderson:** None. **C. Dong:** None. **L. Tian:** None. **E. Schnell:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.05/A43

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

Title: Rethinking Neuropeptide Processing Enzyme Classification for Regulating Peptide Neurotransmitters

Authors: *K. PHILIBERT[1], X. SHAO[2], C. YANG[3], M. J. GLUCKSMAN[4];
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Abstract: Neuropeptides modulate intercellular communication for metabolic activity, cell differentiation, and growth. Neuropeptide processing enzymes form a nexus in the synapse for regulating signaling through the control of peptide neurotransmitters. The classification of neuropeptide processing enzymes was assigned decades ago when fewer neuropeptides were identified. Today, some names remain misnomers. An example, Neprilysin, was formerly named enkephalinase on the basis of a sole recognized substrate. In the metalloendopeptidase taxonomy, within the M3 family are two closely related neuropeptide processing enzymes that cleave ~9 substrates identically: EC3.4.24.15 (EP24.15; thimet oligopeptidase) and EC3.4.24.16 (EP24.16; neurolysin). These are distinguished solely by a difference in the bond hydrolyzed in neurotensin. Hence, the name neurolysin. We have demonstrated differences in substrate specificity between EP24.16 and EP24.15 in the following peptides beyond neurotensin: **PHOENIXIN:** its function includes modulation of gonadotropin secretion (reproduction), appetite regulation, and stress hormone release and may play a role in pain modulation and cardiovascular function. **RFRP-3/ GnIH**, also known as Gonadotropin inhibitory peptide (GnIH), inhibits gonadotropin-releasing hormone (GnRH) secretion and acts on the pituitary gland to suppress the release of reproductive hormones such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH). **ARG8-VASOPRESSIN (AVP)**: A nonapeptide involved in regulating water retention (to promote water reabsorption, thereby concentrating urine) and blood pressure by virtue of its vasoconstriction properties. AVP is also involved in social behaviors, such as stress response, bonding, and aggression. **COLVELIN**: A neuropeptide (26 amino acids) comprising an activity-dependent neurotrophic factor (ADNF) attached to the N-terminus of a potent Humanin derivative. There are potential neuroprotective properties against damaging excitotoxicity elements by modulating calcium influx and inhibiting glutamate release. In conclusion, the neuropeptide processing enzyme neurolysin is a misnomer based on a single substrate (neurotensin), differentiating the enzyme from thimet oligopeptidase. In general, several processing enzymes can act on the same neuropeptide, and a given neuropeptide can be a substrate for several neuropeptide processing enzymes.

Disclosures: K. Philibert: None. **X. Shao:** None. **C. Yang:** None. **M.J. Glucksman:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.06/A44

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

Support: NSTC 112-2314-B-384 -008 -MY3

Title: Exploring the miRNA and targeted gene involved in induction of neuropathic pain associated with colony stimulating factor 1

Authors: T. CHIU[1], L.-H. CHOW[2], ***P.-H. TAN**[1,3];
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Abstract: Recent research has highlighted the crucial role of microglia in neuropathic pain development, with Colony Stimulating Factor 1 (CSF1) being a significant factor. Guan Z et al. found that nerve damage increases CSF1 production from injured neurons, activating microglial CSF1 receptors and leading to microglial activation and pain onset. However, the specific miRNAs and genes involved in this process remain unexplored. To address this, the expression profiles of miRNAs and mRNA in CSF1- activated microglia were analyzed with microarray and validated to explore the key modulators for neuropathic pain. Primary cultured microglia were exposed to either 100 ng/ml purified CSF1 (Sigma-Aldrich) or PBS for 16 hours at 37℃ after being maintained in serum-free media overnight. Subsequently, CSF1-activated microglia were collected for miRNA and mRNA profiling using Next-Generation Sequencing (NGS). An expression level filter was applied to miRNAs and mRNAs, requiring a p-value less than 0.05 and a 1.5-fold change in either direction. Following an inverse matching integration approach, we identified the top 50 highly expressed and the bottom 60 lowly expressed mRNAs for further investigation of enriched biological functions. Among the highly expressed mRNAs, a robust association with the TNFα pathway was discovered, while the lowly expressed mRNAs exhibited a significant link to Glycosaminoglycans (GAGs) metabolism. Specifically, miR652-5p, 672-5p, and 455-3p were found to target the upregulated genes associated with cell proliferation and the TNFα pathway, whereas the upregulation of miR-222-3p, miR-702-5p, miR-877, and miR-644-2-5p was associated with the downregulation of GAG-related genes. Activated microglia were observed to facilitate the removal of GAGs, contributing to neuropathic pain. Additionally, relatively low expression levels of miRNAs (miR-34a-5p, 34b-5p, 449a-5p) corresponding to the CSF1 receptor (CSF1R) were observed. Treatment with miR-34a was found to mitigate microgliosis. In conclusions, (1) the activation of microgliosis and the TNF alpha pathway were linked to the downregulation of miR-652-5p, miR-672-5p, and miR-455-3p. (2) miRNAs targeting the CSF1 receptor (miR-34a-5p, miR-34b-5p, miR-449a-5p) exhibited decreased expression levels. (3) Reduced GAGs metabolism in microglia cells was associated with the upregulation of miR-222-3p, miR-702-5p, miR-877, and miR-644-2-5p. (4) miR-34a-5p, targeting CSF1R, was validated to attenuate microgliosis and alleviate neuropathic pain. These identified miRNAs hold promise as potential therapeutic targets for future neuropathic pain treatments.

Disclosures: T. Chiu: None. **L. Chow:** None. **P. Tan:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.07/A45

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

Support: NIH Grant R01aa030577

Title: G-csf effects on mesolimbic dopamine function and ethanol interactions

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Abstract: The mesolimbic dopamine (DA) system contributes to reward and reinforcement learning for ethanol (EtOH). The nucleus accumbens (NAc) receives DA input from the ventral tegmental area (VTA) encoding rewarding information. Acute EtOH, like other rewarding substances, increases synaptic DA NAc concentrations. After chronic EtOH exposure, NAc DA signaling is weakened by synaptic adaptations that include reduced DA release, increased reuptake, and activity changes in the presynaptic D2-type auto-receptor. Further research into EtOH-induced neural adaptations that modulate NAc DA release is explored herein, including changes in cholinergic interneurons (CIN), which are known regulators of DA transmission. There is a striking discrepancy between the reported effective concentration of EtOH to evoke increased VTA DA neuron firing *in vivo* vs *ex vivo* in electrophysiological studies, indicating an intermediate step or chemical mediator involved in EtOH's mechanism of action on the mesolimbic system. A potential intermediate includes the peripheral immune system, which could influence dopamine release through cytokine release. Granulocyte colony stimulating factor (G-CSF) is a 25 kD glycoprotein growth factor associated with neutrophil progenitor proliferation, but also known to influence dopamine release and reward behavior. Initial studies in the lab found that G-CSF (10 nM) enhances NAc DA release, as measured by voltammetry. Next, G-CSF was applied while recording CIN firing in NAc brain slices. Opposite to what was expected, G-CSF inhibited CIN firing. This reduction suggests that CINs may have an inhibitory function over DA release, possibly through ongoing activity that desensitizes nicotinic acetylcholine receptors on DA terminals. This finding could also be explained by direct excitatory effects of G-CSF on DA neurons. The study will examine this target and chronic intermittent ethanol induced changes in G-CSF sensitivity and receptor expression.

Disclosures: R.J. Campbell: None. **J. Cecil:** None. **Z. Valentine:** None. **C. Seiter:** None. **J. Woolley:** None. **J.T. Yorgason:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.08/A46

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

Support: R01NS122230 (TLK)
R21AA027460 (TLK)
R01AA019454 (TLK)

Title: Investigating the role of CRF signaling at the Bed Nucleus of the Stria Terminalis undergoing chronic pain process

Authors: *M. XIA[1], H. L. HAUN[2], K. M. BOYT[3], T. L. KASH[4];
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Abstract: Currently, pain is the leading cause of long-term disability in the United States, more than 70 million Americans and 1.5 billion individuals globally are being affected. Opioids have historically been the first choice of drug for treating acute and chronic pain, but using them can lead to overdose, addiction, and dependence. On the basis of accumulating evidence of potential risks to patients, such as overdose and misuse, alternative non-opiate mechanisms are essential to be understood to develop medications for the management of pain.The peptide corticotropin-releasing factor (CRF) regulates stress-related behaviors, has been implied in pain modulation in the central nervous system. The bed nucleus of the stria terminalis (BNST), a CRF-enriched structure, has been shown to significantly impact on the pain transduction especially its affective-motivational components. Rats' studies suggested a suppression of aversive aspects of electrical, visceral, and somatic pain by bilateral lesions of the BNST. The goal of this study was to address CRF neurons' alterations in response to chronic pain at the BNST as a sex-dependent endogenous anti-nociceptive circuit.To determine the role of CRF signaling at BNST undergoing inflammatory pain processing, we combined genetic approach and AAV-mediated site-specific deletion of CRF at the BNST neurons in a Crf-cre mouse line. Behaviorally, deletion of CRF at the BNST caused sex-dependent baseline effect on both mechanical and thermal pain sensitivity, as well as the affective aspects of pain. CFA exposure, as an inflammatory pain model which maintains during opioid administration, showed a Crf-dependent effect in male and female mice. Cre-dependent suppression of vGAT function led to altered thermal hyperalgesia only in males, and sex difference was observed after CFA treatment. Last, to determine the impact of inflammatory pain on the intrinsic properties of BNST-CRF neurons, a reporter mouse line and slice electrophysiology were recruited. Whole-cell recordings were performed and sIPSC frequency of the CRF-neurons were increased by CFA treatment at dBNST, but not vBNST, in a sex-dependent manner.Collectively, our findings suggest a role for BNST CRF signaling in the transduction and modulation of sex-specific behavioral responses to persistent pain. The protective role or CRF neuron population provides novel mechanisms that prevent addiction-induced negative effects in the context of pain, serving as potential therapeutic targets.

Disclosures: M. Xia: None. **K.M. Boyt:** None. **T.L. Kash:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.09/A47

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

Support: 1ZIANS009424
1ZIANS009422

Title: Neuregulin1 nuclear signaling suppresses specification of semilunar granule cells.

Authors: *P. RAJEBHOSALE[1], L. JIANG[2], K. R. JOHNSON[3], N. S. DESAI[4], L. W. ROLE[5], D. A. TALMAGE[6];
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Abstract: Dentate gyrus granule cells (GC) gate information coming into the hippocampus. Most GCs have a single primary dendrite extending into the molecular layer. A second population of GCs, known as semi-lunar granule cells (SGCs), have multiple splaying primary dendrites. SGCs are born embryonically, account for less than 10% of all GCs and 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 & ATACSeq (snRNASeq/ATACSeq) to define the molecular makeup of GC subtypes. Profiled cells with an SGC-like morphology were all located at the dorsal edge of the granule cell layer (GCL) consistent with their embryonic origin. SGCs differed in several electrical properties compared to typical GCs. snRNASeq also identified Penk expressing GCs, which showed SGC-like morphology. We also discovered several other marker genes for SGCs which we validated using multiplexed in situ hybridization with Penk. GCs with SGC-like morphologies have been found in overabundance in postmortem brains of people suffering from psychiatric disorders and in animal models thereof. A psychosis-associated missense mutation in NRG1 ($V_{321}L$) impairs cleavage of Nrg1 by gamma-secretase and membrane to nucleus signaling by the Nrg1 intracellular domain (ICD). Additionally, *Nrg1* was highly expressed in SGCs compared to other GCs. Thus, we next examined whether the $V_{321}L$ mutation altered GC properties and/or composition.We noted heterotopic SGCs located in the middle of the GCL in the V321L mice. Linear discriminant analysis revealed that morphologically, GCs and SGCs from mutant mice co-clustered with their respective WT counterparts. The electrical properties of SGCs matched those of WT SGCs, however, mutant GCs showed changes in their electrical properties matching those of SGCs. snRNASeq of V321L DG showed significantly higher numbers of SGCs defined by expression of Penk and other marker genes. Trajectory analyses revealed changes in the V321L DG that were in line with an

observation of altered terminal fate of GCs. Additionally, we found that even in WT mice, SGC marker gene start sites were accessible broadly to non-SGCs indicating the potential for expression and expansion of an SGC-like cell population. Thus, we conclude that Nrg1 nuclear signaling acts to actively suppress the SGC fate.

Disclosures: P. Rajebhosale: None. **L. Jiang:** None. **K.R. Johnson:** None. **N.S. Desai:** None. **L.W. Role:** None. **D.A. Talmage:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.10/A48

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

Support: NIH Intramural Research Program

Title: Neuregulin signaling in the physiology of fear learning related circuits

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Abstract: Type III Neuregulin 1 (Nrg1) is a bidirectional signaling molecule important for the establishment and maintenance of CNS and PNS synapses and is required for proper functioning of circuits engaged in sensory gating, short term memory and threat learning. Type III Nrg1 signaling engages both forward and multiple “back” signaling pathways and is required for cholinergic modulation of excitatory plasticity at cortical-BLA synapses. As cholinergic input from the nucleus basalis is also required for the establishment of a threat memory engram, we sought to determine which aspect(s) of Type III Nrg1’s bidirectional signaling motifs might be engaged in fear learning. Prior studies have shown that homozygous mutants of Type III Nrg1 die at birth, due to a lack of functional innervation of the diaphragm, presumably due to the requirement for forward and/or back signaling in normal cholinergic input- target interactions. Heterozygous mutants of Type III Nrg1 survive until adulthood but have diminished signaling in forward and in local and nuclear mediated back signaling processes, that underlie the observed sensory gating and threat learning deficits. We have now generated selective genetic mutations of Type III Nrg1 that mimic those found in human patients with psychosis and allow us to assess the relative contribution of γ-secretase-mediated nuclear back signaling vs local signaling pathways. Current studies focus on using patch clamp and optogenetic techniques to dissect the role of nuclear vs local back signaling by Type III Nrg1 during the maturation of specific synapses in hippocampus and amygdala, examining their plasticity and cholinergic modulation as it is involved in threat learning. In Type III Nrg1 heterozygous mice, we detect significant changes in spontaneous and evoked cortical- BLA glutamatergic transmission, in the cholinergic modulation of these synapses and in both NMDA and AMPA mediated synaptic currents, in comparison with WT littermates. In contrast, mice bearing a homozygous mutation of a critical

valine residue necessary for nuclear back signaling, revealed alterations in glutamatergic transmission that were more selective than those seen in the full heterozygous mutants. Continued study of the various selective mutations in Type III Nrg1 will help us to dissect which aspects of Type III Nrg1 signaling contribute to altered neuronal development, synaptic plasticity, and performance in assays of behavior.

Disclosures: L. Jiang: None. **L.W. Role:** None. **D.A. Talmage:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.11/A49

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

Support: T32-AG000114

Title: Impacts of social isolation on behavior and physiology in Drosophila melanogaster

Authors: *T. CHAKRABORTY[1], A. TORNES BLANCO[2], J. BAINS[3], R. RUCKER[1], Z. M. STRONG[1], Y. EMARA[4], D. PROMISLOW[5], S. D. PLETCHER[4];
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Abstract: Social isolation (SI) disrupts emotion, physiology, and behavior across species. *Drosophila melanogaster* recognizes and responds to SI variably along a spectrum of behavior ranging from aggression to sleep. Here we demonstrate that SI influences motivational drives and health metrics in *Drosophila*, and we outline new molecular mechanisms through which it does so. Behavioral measures at single-fly resolution revealed that SI isolation increased homeostatic, but not hedonic, feeding drive and promoted social interaction upon re-grouping. SI also compromised stress resistance and modulated aging. Temporal manipulations revealed that the effects of SI manifest after four days and are fully reversible upon changes in social environment. Dissection of the molecular mechanisms underlying the effects of SI revealed select neurons and neuropeptides that mediate their effects. We observed that inhibition of neurons that express the conserved neuropeptides NPF (the homology of mammalian NPY) or brain insulins, as well as loss of the neuropeptides themselves, protected flies from the effects of SI. SI increased the abundance of these molecules in the adult fly brain, and activation of NPF-or insulin-producing neurons was sufficient to mimic SI effects. Epistasis experiments suggest that NPF acts upstream of brain insulins in the same pathway, or overlapping pathways, to influence physiology and aging throughout the animal in response to social experience. Analysis of metabolomic responses in the brains of SI and group housed (GH) flies revealed NPF- and insulin-dependent changes. A notable decrease was observed in branched chain amino acids

(BCAAs), glucose, and trehalose metabolites during SI in wild type flies, but not in insulin and NPF mutants. This suggests that the brain orchestrates coordinated changes in BCAA biosynthesis, glycolysis, and gluconeogenesis in the brain and periphery in response to social perception. Overall, our findings provide a mechanistic link coordinating SI triggering upstream NPF signaling, resulting in downstream insulin regulation to alter metabolism, feeding, and health at a whole organismal level.

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Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.12/A50

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

Title: The impact of H_2S on the intrinsic excitability of CA1 hippocampal cells

Authors: *J. RAMIREZ C[1,2], A. PEREZ BARRAGAN[3], V. A. MARTINEZ-ROJAS[4], D. CENTURIÓN[5], E. J. GALVAN[6];
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Abstract: Hydrogen sulfide (H_2S), a poisonous gas known for its toxicity, is acknowledged as a beneficial molecule with positive effects on several physiological systems, including the central nervous system. In this sense, hippocampal neurons, involved in learning and memory, possess the molecular machinery to synthesize H_2S, which can act locally. However, its specific role in the intrinsic excitability of these cells has yet to be fully understood. To investigate the role of H_2S on the intrinsic excitability of CA1 pyramidal cells, the expression of the endogenous H_2S-generating enzymes were first demonstrated, and patch-clamp recordings on acute hippocampal slices was performed in the presence of exogenous H_2S. Perfusion of aminooxyacetic acid, a non-specific blocker of CBS, CSE, and CAT enzymes responsible for H_2S synthesis, elicited a divergent effect on neuronal excitability. In another series of experiments, the effect of increasing doses of H_2S revealed a concentration-dependent inhibitory modulation by exogenous H_2S, as sodium hydrosulfide perfusion, an H_2S donor, suppresses neuronal excitability by reducing neuronal firing frequency.In addition, the basal expression of the three enzymes responsible for H_2S synthesis (CBS, CSE, and 3-MST) were evaluated by the Western blot technique in resected sections of area CA1, that included strata oriens, pyramidale and radiatum. We found the presence of the three enzymes (CBS, CSE, and 3-MST), with the highest expression of CBS and 3-MST enzymes in area CA1.Collectively, these results indicate that a

local surge of H_2S modifies the active properties of CA1 pyramidal neurons. Exploring how H_2S specifically influences the intrinsic excitability of these neurons could uncover neuroprotective mechanisms beyond simple reactive oxygen species neutralization, delving into the regulation of ion channels and action potentials. This comprehensive approach offers crucial insights into understanding the role of H_2S in neuronal health and its potential therapeutic implications.

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Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.13/A51

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

Title: Manipulation of deoxyhypusine synthase acitivity affects neuronal homeostasis in rat primary cortical cultures

Authors: *P. CAVALLI[1], A. RAFFAUF[2], S. PASSARELLA[3], D. C. DIETERICH[4], P. LANDGRAF[5];
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Abstract: Deoxyhypusine synthase (DHPS) catalyzes the initial step of hypusine incorporation into the eukaryotic initiation factor 5A (eIF5A), leading to its activation. The activated eIF5A, in turn, plays a key role in regulating protein translation of selected mRNAs and therefore appears to be a suitable target for therapeutic intervention strategies. In the presented study, we analyzed the role of DHPS-mediated hypusination in regulating neuronal homeostasis using lentivirus-based gain and loss of function experiments in primary cortical cultures from rat. This model allows us to isolate and examine the impact of DHPS function on the composition of the synaptic compartment, which is associated with cognitive function and neurodevelopment. Our findings have revealed that shRNA-mediated DHPS knockdown diminishes eIF5A hypusination, resulting in notable alterations in neuronal dendritic architecture. Furthermore, in neurons the synaptic composition was altered and showed both reduced synaptophysin-1 (Syp-1) and homer-1 intensity, while DHPS overexpression construct slightly increases Syp-1 intensity. Additionally, DHPS knockdown cultures exhibited a pronounced reduction in other post-synaptic markers, such as Shank2 and PSD95. Therefore, we hypothesize that decreasing hypusination of eIF5A caused by reduced DHPS function may impair synaptic homeostasis due to a deficit in pre- and post-synaptic proteins. Finally, the decrease of eIF5A hypusination

correlates with changes of proteins involved in autophagy like ATG3, LC3, and TFEB, suggesting a direct link between DHPS mediated hypusination and dendritic growth, synaptic homeostasis, and autophagosome regulation.

Disclosures: P. Cavalli: None. **A. Raffauf:** None. **S. Passarella:** None. **D.C. Dieterich:** None. **P. Landgraf:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.14/A52

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

Support: NNSF 32130044
NNSF T2241002

Title: Extracellular K^+ accumulation leads to depolarization plateau and excessive neurotransmitter release during epilepsy

Authors: *W. DUAN[1], Q. HE[2], Y. SHU[3];
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Abstract: Epileptic seizure is a severe neurological disorder, also associates with mood disorders. Synaptic transmission supports the generation and propagation of epileptiform activity. Excessive excitatory neurotransmission causes rapid depolarization and then prolonged depolarization plateau (known as the depolarization block due to the inactivation of voltage-gated Na^+ channels) in individual neurons during epileptiform activity. However, it remains unclear how the depolarization plateau occurs and how abnormal synaptic transmission evolves. In this study, we employed electrophysiological and fiber photometry techniques to detect the generation of epileptiform activity and the release of neurotransmitters, including glutamate, GABA, and dopamine (DA) in neocortical slices (with Mg^{2+}-free bath solution) and a mouse model of epilepsy induced by kindling at the hippocampal CA3 region. During the spontaneous epileptiform activity in slices, we detected the occurrence of depolarization plateau in inhibitory interneurons as well as pyramidal cells (PCs). Interestingly, strong hyperpolarization induced by either negative current injections or optogenetic inhibition cannot abolish the depolarization plateau. In addition, intracellular application of an array of drugs cannot block the depolarization plateau. However, puffing high K^+ could induce similar depolarization plateau, suggesting an important role of high concentration of K^+ outside the cell. We then hypothesize that the high K^+ may lead to excessive release of neurotransmitters. Indeed, local puff of high K^+ to slices induces excessive release of both glutamate and GABA, as indicated by the barrages of excitatory and inhibitory postsynaptic currents. In mice with kindling, we observed abnormally strong release of DA in the neocortex during generalized seizures, as reflected by changes in the fluorescent DA

probe rDA3h. Together, these findings show that the abnormal accumulation of extracellular K^+ during epileptiform activity determines the occurrence of depolarization plateau in neocortical neurons and the uncontrollable synaptic release, suggesting that reducing extracellular K^+ concentration should be an effective but possibly the only way to prevent the generation of depolarization plateau, and thus to treat the epileptic seizures or the associated mood disorders.

Disclosures: W. Duan: None. **Q. He:** None. **Y. Shu:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.15/A53

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

Title: New modulators of Na,K-ATPase

Authors: *G. CHKADUA, E. NOZADZE, L. SHIOSHVILI, L. TSAKADZE, M. LELADZE, N. ARUTINOVA, S. DZNELADZE, N. METREVELI, M. JAVAKHISHVILI;
Iv. Beritashvili Ctr. of Exptl. biomedicine, Tbilisi, Georgia

Abstract: Abstract
Na, K-ATPase is an important enzyme that maintains Na^+, K^+ gradients across the cell membrane, which is required for many physiological functions in different organs and tissues. Because of its importance in cellular physiology, blocking the Na,K-ATPase can have serious physiological implications. This property makes it a target for different pharmacological uses, and medications that control the pump's function are utilized to treat a variety of medical ailments. Cytochrome c (Cytc) is a protein that has specific functions in the cell. It plays an important role in ATP synthesis and energy production in the mitochondria. However, in response to apoptotic stimuli, it is released into the cytosol and causes programmed cell death via the intrinsic apoptosis pathway. Aside from its role in conventional intrinsic apoptosis, Cytc performs various functions. Cytc participates in non-apoptotic actions that are less well understood than its role in apoptosis. In this in vitro investigation, we demonstrated for the first time the effect of Cytc on Na,K-ATPase. Cytc acts biphasically on Na,K-ATPase, activating at low concentrations (0.06ng/ml; 6ng/ml) and inhibiting at high concentrations (120ng/ml). The effect of Cytc on Na,K-ATPase is isoform/subunit specific. Cytc targets cysteine thiol groups in Na,K-ATPase.

Disclosures: G. Chkadua: None. **E. Nozadze:** None. **L. Shioshvili:** None. **L. Tsakadze:** None. **M. Leladze:** None. **N. Arutinova:** None. **S. Dzneladze:** None. **N. Metreveli:** None. **M. Javakhishvili:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.16/A54

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

Support: NIH P20 GM103475-19
American Diabetes Association 1- 19-IBS-300
NIH-NINDS R15-NS116478
NIH-NINDS SC2NS124907
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NIH-NINDS R01-NS-065201
NIH-NIMHD G12MD007583
National Science Foundation Grant 2122203

Title: Effects of hyperglycemia on Aquaporin-4 channels expression and the modulatory role of Metformin in rat astrocytes

Authors: *N. NIETO-TORRES[1], A. CAMACHO-BADILLO[1], A. DAVILA[1], D. E. RIVERA-APONTE[2], M. P. MÉNDEZ-GONZÁLEZ[3];
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Abstract: Diabetes is a chronic metabolic disorder characterized by high levels of glucose in the blood, affecting more than 450 million adults worldwide. Astrocytes are glial cells of the central nervous system, essential for maintaining ionic balance in the extracellular space. Their function is complemented by Aquaporin-4 (AQP4), a protein embedded in the astrocytes' endfeet, by regulating water permeability through the cell membrane. Previous studies have shown an increase in the expression of AQP4 in the retinas of diabetic rats, which contain large amounts of Müller cells, a type of glial cells in the eye, to foster glucose homeostasis. We hypothesize that astrocytes grown in hyperglycemic conditions will show reduced AQP4 mRNA and protein expression and that this phenomenon could be reversed with Metformin application, a drug known to reduce blood glucose levels. Therefore, the present study aims to determine the effect of hyperglycemic conditions on the expression of rat Aquaporin-4 channels and its response to Metformin treatment, a potential modulator. To test our hypothesis, RT-PCR and Western Blot will be performed using astrocytes cultured under normal (5mM) and hyperglycemic (25mM) conditions with and without Metformin application. AQP4 mRNA and protein expression increase is expected in cells grown under hyperglycemic conditions compared to those grown under normal conditions, and a downregulation in protein expression in cells grown under high glucose and administered with Metformin. These findings will be compelling for understanding the effects of high glucose on water absorption in astrocytes through AQP4 channels and potential strategies for clinical management.

Disclosures: N. Nieto-Torres: None. **A. Camacho-Badillo:** None. **A. Davila:** None. **D.E. Rivera-Aponte:** None. **M.P. Méndez-González:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.17/A55

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

Support: NIH / NIDA IRP: DA000069

Title: Discovery of the first chemical compounds targeting the human zinc transporter 3 (ZnT3, SLC30A3)

Authors: *Z. J. FRANGOS[1], O. SOLÍS CASTREJÓN[1], M. MICHAELIDES[2];
[1]Natl. Inst. on Drug Abuse, Baltimore, MD; [2]Biobehavioral Imaging & Mol. Neuropsychopharm., NIDA-NIH, Baltimore, MD

Abstract: Synaptic zinc is packaged into vesicles by zinc transporter 3 (ZnT3, SLC30A3), regulating its myriad of neuromodulatory effects via interaction with receptors and transporters in the central nervous system. Synaptic zinc and ZnT3 have been implicated in both neuropsychiatric and non-neuropsychiatric disorders. Despite this, there are currently no known ZnT3-targeting compounds which could unravel the role of synaptic zinc in disease states, or potentially be developed into therapeutics for brain diseases. Using a human ZnT3 Alphafold 2 model, we performed *in silico* screening and identified 50 putative ZnT3 ligands. We then developed a fluorescent assay to measure zinc accumulation in cells, allowing characterization of these ligands' activity *in vitro*. We created a stably expressed ZnT3 cell line by integrating the ZnT3 plasmid in human embryonic kidney (HEK)-293 cells by transposition. The assay consists of incubating the cells with putative ZnT3 ligands and Fluozin™-3, AM (a cell permeant selective zinc indicator), before being washed, then incubated, with ZnT3 ligands and $ZnSO_4$. Zinc uptake was determined by imaging and quantifying the level of fluorescence following incubation with $ZnSO_4$ and we identified compounds that could either increase, or decrease, uptake. Interestingly, some of the hits identified are existing treatments (e.g., antibiotics and diabetes medications) which suggests modulation of synaptic zinc may contribute to their mechanism of action. To characterize the *in vivo* activity of these hits, we plan to inject a genetically encoded fluorescent synaptic zinc sensor into the striatum and hippocampus of wild-type (WT) and ZnT3 knockout (KO) mice. We expect ZnT3 modulators to alter zinc dynamics in WT mice but not in KO mice, indicating their effects are specifically mediated by ZnT3 modulation. Through this study we plan to identify the first known modulators of human ZnT3 and demonstrate they have functional effects *in vivo*. These compounds may have the potential to be used in animal models to reveal how synaptic zinc contributes to the progression of specific neuropsychiatric and non-neuropsychiatric disease states and may form a new class of therapies to treat these conditions.

Disclosures: Z.J. Frangos: None. **O. Solís Castrejón:** None. **M. Michaelides:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.18/A56

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

Support: NIH award MH094527

Title: Capacity for protein kinase G dependent SERT phosphorylation supports sex dependent serotonin release in the nucleus accumbens following social interactions and MDMA administration

Authors: *C. MEINKE[1,2], A. STEWART[3,1], T. WELLS[1], S. WASILEWSKI[1], S. RAMAMOORTHY[4], R. D. BLAKELY[1,5];
[1]Dept. of Biomed. Science, Charles E. Schmidt Col. of Med., Florida Atlantic Univ., Jupiter, FL; [2]Intl. Max Planck Res. Sch. for Synapses and Circuits, Max Planck Florida Inst. for Neurosci., Jupiter, FL; [3]Neurosci. & Pharmacol., Univ. of Iowa, Iowa City, IA; [4]Pharmacol. and Toxicology, Virginia Commonwealth Univ., Richmond, VA; [5]Florida Atlantic Univ., Stiles-Nicholson Brain Inst., Jupiter, FL

Abstract: The serotonin (5-HT) transporter (SERT) mediates high affinity 5-HT clearance and dysregulation of SERT expression or function is linked to disorders like depression, anxiety, and autism spectrum disorder (ASD). SERT trafficking and function are regulated via multiple kinase-linked pathways, including PKC, p38α MAPK, and PKG. *In vitro* studies established that the conformationally dynamic residue Thr276 is the key site required for PKG-dependent SERT phosphorylation. To assess the *in vivo* impact of potential phosphorylation at SERT Thr276, we generated SERT Ala276 knock-in (KI) mice. Initial studies revealed sex-dependent changes in social behavior, where KI males exhibit reduced social dominance and KI females display decreased social preference. Here we evaluated social responses of adult WT and KI mice to a novel juvenile social stimulus while monitoring 5-HT dynamics in the nucleus accumbens (NAc) using the genetically encoded $GRAB_{5\text{-}HT}$ sensor. Our studies demonstrate decreased 5-HT release in female KI mice relative to WT during the first interaction, and a total loss of 5-HT release in the next interaction, whereas release persists in WT mice. The frequency of social interactions paralleled NAc 5-HT elevations, with female KI mice exhibiting fewer interaction bouts and an increased latency to interact with the juvenile conspecific relative to WT. In contrast, male WT and KI mice exhibited comparable social stimulus elicited 5-HT release during the first interaction, though release decreased compared to WT during the second interaction. Since 3,4-methylenedioxymethamphetamine (MDMA) is known to require SERT-mediated 5-HT release in the NAc for its prosocial effects, we further tested whether KI mice display changes in MDMA-induced 5-HT release. Interestingly, administration of 7.5mg/kg MDMA led to higher 5-HT release in both male and female SERT Ala276 KIs compared to their WT littermates. However, MDMA-induced 5-HT release resulted in increased locomotion and reduced anxiety in KI males, but not females, suggesting sex-differences in the neural substrates underlying MDMA

stimulated locomotion. Enhanced MDMA-driven 5-HT release in the NAc likely does not reflect a change in drug potency as the ability of MDMA to inhibit 5-HT uptake was genotype insensitive as revealed in *ex vivo* synaptosomal [^{3}H]5-HT uptake studies. Together, our studies demonstrate that a capacity to phosphorylate SERT Thr276 *in vivo* is important for the elaboration of social behavior as well as social and MDMA-induced 5-HT release in the NAc and supports a potential contribution of altered SERT phosphorylation to disorders linked to social behavior alterations such as ASD.

Disclosures: C. Meinke: None. **A. Stewart:** None. **T. Wells:** None. **S. Wasilewski:** None. **S. Ramamoorthy:** None. **R.D. Blakely:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.19/A57

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

Support: NIH Grant 5RO1NS111749

Title: Sex differences in metabolic regulation by $G_{i/o}$coupled receptor modulation of exocytosis

Authors: *M. YOUNG;
Pharmacol., Vanderbilt Univ., Nashville, TN

Abstract: <u>Sex Differences in metabolic regulation by Gi/o-coupled receptor modulation of exocytosis:</u>
Authors: Montana Young, Ryan P. Ceddia, David Reyes, Jackson B. Cassada, Analisa Thompson-Gray, Sheila Collins, Heidi E. Hamm
Presynaptic $G_{i/o}$ coupled GPCRs can act as negative feedback regulators of neurotransmitter release through two mechanisms via Gβγ effector modulation: decreased calcium influx and direct inhibition of membrane fusion by soluble N-ethylmaleimide- sensitive factor attachment protein (SNAP) receptor (SNARE). Previously, we discovered that truncation of the last three C-terminal amino acids of SNAP25 (SNAP25Δ3) prevents Gβγ-SNARE interaction effectively removing the braking mechanism on neurotransmitter release. We have demonstrated enhanced metabolic protection in male SNAP25Δ3 mice housed at room temperature (22°C) including increased adipose tissue beiging and glucose uptake and enhanced insulin sensitivity, rendering them resistant to diet-induced obesity (DIO). When male SNAP25Δ3 mice were housed at thermoneutrality (30°C) all metabolic protection was abolished, suggesting sympathetic tone is important for the phenotypes. Here, we find SNAP25Δ3 female mice have the same metabolic protection at RT (22°C), and also displayed enhanced metabolic protection from DIO compared to standard chow. However, female SNAP25Δ3 mice display persistent metabolic protection even when housed at thermoneutrality. In this study, we investigate the mechanisms behind this sex dependent phenotype persistence. Thermoneutral set point did not differ between sexes nor

genotype suggesting this effect is not due to a hypothalamic temperature regulation difference. Ovariectomizing the SNAP25Δ3 mice did cause weight gain, but these mice still displayed enhanced metabolic protection compared to the ovariectomized wild-type mice. Therefore, we hypothesize there is a sex hormone independent mechanism driving the persistent metabolic protection of female SNAP25Δ3 animals housed in thermoneutrality that is not present in the male SNAP25Δ3 mice.

Disclosures: M. Young: None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.20/A58

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

Support: R41 AG073144
NIH R21 AG073743
P20 GM103645

Title: Expression of the BMP co-receptor MuSK in neurogenic niches in the adult brain

Authors: C. XI[1], S. CHOI[1], L.-A. HASSELL[2], J. PAGE[1], S. OH[3], A. PAYNE[4], A. WEBB[5], *J. FALLON[1];
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Abstract: Adult neurogenesis, the generation of new neurons in adult brains, occurs in two neurogenic niches, the subventricular zone (SVZ) and the subgranular zone (SGZ). The SVZ is located in the wall of the lateral ventricle where neural stem cells (NSCs) give rise to new neurons that migrate to the olfactory bulb. NSCs in the SGZ of the dentate gyrus in the hippocampus generate granule cells that integrate into the local circuitry. Adult hippocampal neurogenesis (AHN) has critical functions including learning and memory, cognition, and emotional regulation. Bone morphogenetic proteins (BMPs) mediate negative signaling in the neurogenic niches that inhibit both the NSC activation and integration. Recently, we have shown that Muscle-associated tyrosine kinase (MuSK) binds BMPs via its Ig3 domain and shapes and augments BMP signaling. We term this mechanism the MuSK-BMP pathway. Knock-in mice constitutively expressing ΔIg3-MuSK have a normal lifespan and show increased AHN and enhanced hippocampal dependent cognition. MuSK is well established as a postsynaptic component of the neuromuscular junction. However, MuSK expression in the CNS has not been well characterized. Here we used immunocytochemistry with a panel of domain-specific monoclonal antibodies and show that MuSK is localized in both the SVZ and the SGZ. Co-immunostaining with SOX2 and GFAP showed that MuSK is expressed by NSCs in both niches.

MuSK is also present in a subpopulation of ependymal-like cells in the SVZ. Additionally, MuSK is expressed in cultured NSCs isolated from the adult hippocampus. These data provide critical support for further investigating the role of the MuSK-BMP pathway in regulating adult neurogenesis.

Disclosures: C. Xi: None. **S. Choi:** None. **L. Hassell:** None. **J. Page:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bolden Therapeutics. **S. Oh:** None. **A. Payne:** None. **A. Webb:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bolden Therapeutics. **J. Fallon:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Bolden Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bolden Therapeutics.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.21/A59

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

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

Title: Methamphetamine activates small GTPase Rac1 independently from RhoA and Trace Amine-Associated Receptor 1 (TAAR1)

Authors: *Z. E. KAEGI[1], S. M. UNDERHILL[2], S. G. AMARA[3];
[1]Lab. of Mol. and Cell. Neurobio., NIH, Washington, DC; [2]Lab. of Mol. and Cell. Neurobio., Natl. Inst. of Mental Hlth., Bethesda, MD; [3]Natl. Inst. of Mental Hlth., NIMH, Bethesda, MD

Abstract: Amphetamines (AMPHs) are a broad class of psychostimulants that include important treatments for attention deficit hyperactivity disorder. Despite their therapeutic qualities, however, AMPHs have strong abuse potential that can lead to detrimental outcomes. Our lab has previously established that AMPHs induce internalization of the dopamine transporter (DAT) in dopamine neurons by activating trace amine-associated receptor 1 (TAAR1) inside the cell. We demonstrated that AMPH and methamphetamine (METH) activate small guanosine triphosphatase (GTPase) RhoA in a TAAR1-dependent manner to initiate DAT internalization. Interestingly, we also observed that AMPHs activate another Rho-family GTPase, Rac1, but Rac1 is not required for DAT internalization. Here, we explore three potential pathways of Rac1 activation after METH exposure. We first inhibited three different Rac1 guanine nucleotide exchange factors (GEFs): Tiam1, TrioN, and P-Rex1 using Rac1 inhibitors NSC 23766 (blocks Tiam1 and TrioN) and 1A-116 (blocks P-Rex1). We also tested an alternative inhibitor, EHT 1864, which inhibits activity by binding directly to Rac1. Data from fluorescence resonance energy transfer (FRET) imaging show that EHT 1864 is the best inhibitor of METH induced

Rac1 activity, 1A-116 moderately inhibits Rac1, and NSC 23766 does not inhibit Rac1 activity. This indicates that P-Rex1 may be important for METH induced Rac1 activation, but TrioN and Tiam1 are not. Next, we tested whether RhoA activation is necessary for Rac1 activation by measuring Rac1 activity after pre-treatment with a RhoA inhibitor. Using Pak1-GST beads, we show that Rac1 activity was not affected by the RhoA inhibitor, suggesting that RhoA and Rac1 act independently after METH exposure. Finally, we analyzed whether TAAR1 is necessary for Rac1 activation by comparing METH induced Rac1 activity in TAAR1 knockout cells with wildtype cells. Pulldown assays with Pak1-GST beads and FRET imaging data show that Rac1 activity does not differ between TAAR1 knockout cells and wildtype cells after METH exposure, demonstrating that unlike RhoA, Rac1 activation is TAAR1 independent. Taken together, our data support the notion that AMPHs activate Rac1 via an alternative pathway independent of TAAR1 and RhoA. However, the regulators of Rac1 activation and its downstream effects remain unknown. Further exploration into the mechanisms of action behind AMPHs is therefore crucial to our understanding of these how these drugs contribute to beneficial and harmful outcomes.

Disclosures: Z.E. Kaegi: None. **S.M. Underhill:** None. **S.G. Amara:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.22/A60

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

Support: USDA/CRIS 51000-064-01S
American Heart Association Grant 23POST1030352
Texas Children's Research Scholar funds

Title: Lac-Phe reduces appetite through K_{ATP} channel-mediated inhibition of AgRP neurons

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[1]Pediatrics, USDA/ARS Children's Nutr. Res. Ctr., Baylor Col. of Med., Houston, TX;
[2]Pediatrics, Section of Neurol., Jan and Dan Duncan Neurolog. Res. Inst. at Texas Children's Hosp., Baylor Col. of Med., Houston, TX

Abstract: The lactate-derived N-lactoyl-phenylalanine (Lac-Phe) was recently identified as an exercise-induced circulating metabolite that reduces feeding and obesity, the mechanisms responsible for its appetite-suppressing effects remains unknown. In current study, we demonstrate that Lac-Phe directly inhibits Agouti-related peptide (AgRP)-expressing neurons in the arcuate nucleus of the hypothalamus (ARH), leading to an indirect activation of anorexigenic neurons in the paraventricular nucleus of the hypothalamus (PVH). Further, we show that Lac-Phe inhibits AgRP neurons via activating the ATP-sensitive potassium (K_{ATP}) channel, and inhibition of K_{ATP} channels in the ARH attenuates the hypophagic effect of Lac-Phe. Finally, we

identify the lactate dehydrogenase (LDH) as a molecular target of Lac-Phe, which functions as an inhibitor of the LDH enzymatic activity in AgRP neurons, resulting in feeding suppression. Remarkably, augmenting LDHB expression in AgRP neurons neutralizes the effects of Lac-Phe on both appetite suppression and exercise-induced prevention of weight gain in obese mice. Thus, our findings elucidate the complex neurobiological mechanisms by which Lac-Phe modulates appetite, underscoring its potential as an innovative anti-obesity treatment.

Disclosures: H. Liu: None. **H.K. Wong:** None. **Y. He:** None. **Y. Xu:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.23/A61

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

Support: Campus Celaya-Salvatierra, Universidad de Guanajuato

Title: Effect of Kefir on weight, glucose and brain-derived neutrophic factor levels in sucrose-fed mice

Authors: *C. SANDOVAL SALAZAR[1], A. MORALES-VARGAS[2], P. VILLALOBOS GUTIÉRREZ[3], O. GUTIEREZ-CORONADO[3], V. BELTRÁN CAMPOS[1], D. AYALA-CAMARENA[2];
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Abstract: The consumption of foods with a high sucrose content contributes to thedevelopment of obesity. It promotes fat accumulation, impairs glucose levels, impairscommunication between the gut-brain axis, and promotes an imbalance ofmolecules that modulate cell growth and development, such as Brain-DerivedNeurotrophic Factor (BDNF). Scientific research should include the development ofprevention strategies through the consumption of foods with prebiotic and probioticproperties, such as kefir, a fermented drink made from kefir grains, which has beenshown to lower blood sugar and have antioxidant effects. The aim was to investigatethe effect of Kérfir on weight, glucose levels and BDNF in C57BL/6 mice. Forty malemice of the C57BL/6 strain were used and divided into four groups (n=10). The firstgroup received a standard diet (Purina Rodent Chow: protein 23%, fat 4.5%,carbohydrates 72.5%) + water (SD); the second group received SD + kefir (SDK);the third group received SD + 20% sucrose solution (SS), the fourth group receivedSD + 20% sucrose solution + kefir (SSK). The kefir was administered in an amountof 4 ml/kg, which corresponds to approximately ¼ cup of kefir for a person with adiet of 2000 kcal/day. The treatment was carried out for 8 weeks. Weight and glucosewere recorded at the beginning and end of the experiment. BNDF levels weremeasured using the nanodot blot method with immuno-detection and Image Studiosoftware. One-way ANOVA and Tukey's post hoc test were used, considering a $p \leq 0.05$ for statistical significance.

The weight results showed no differences betweenthe groups. Significant differences were found in the glucose level at the end oftreatment between the SD (101±12.47) and SS (127.5±16.57) groups (p=0.001); theSD (101±12.47) and the SSK (119±5.82) group (p=0.006); the SS (127.5±16.57) andthe SSK (119±5.82) group (p=0.015); and the SDK (86±7.29) and the SSK(119±5.82) group (p=0.043). With regard to BDNF expression, there were statisticallysignificant differences between the group receiving SSK kefir (0.447±0.093)compared to the SD (0.302±0.024), SDK (0.310±0.021) and SS (0.247±0.055)groups p=0.0459. Conclusions: 1) The consumption of kefir has no effect on bodyweight. 2) Kefir consumption increases BDNF expression. 3) Further research isneeded on the use of kefir and the probiotic effect. 4) It is necessary to determinethe interaction between the gut microbiota and probiotics such as kefir to understandtheir specific mechanism on the gut-brain axis.

Disclosures: C. Sandoval Salazar: None. **A. Morales-Vargas:** None. **P. Villalobos Gutiérrez:** None. **O. Gutierez-Coronado:** None. **V. Beltrán Campos:** None. **D. Ayala-Camarena:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.24/A62

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

Support: Consejo Nacional de Humanidades Ciencias y Tecnologías CF-2023-I-905
Universidad de Sonora

Title: Clicking levodopa and protocatechuic acid into chitosan exerts biomedical functionalities in brain cells

Authors: *M. MONTIEL-HERRERA[1], O. ORTEGA-FIMBRES[2], F. PÉREZ DELGADO[4], D. MONGE-SANCHEZ[5], M. GARCÍA VILLA[3], D. FERNANDEZ QUIROZ[1], C. G. CASTILLO[6], J. DOMÍNGUEZ-AVILA[7], G. GONZALEZ AGUILAR[8];
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Abstract: This work presents a proof-of-concept that biomacromolecules such as N-(Levodopa) and N-(Protocatechuic acid) chitosan derivatives traverse brain cell membranes and induce biomedical functionalities in brain cells. These chitosan derivatives were synthesized by click chemistry and characterized by FT-IR, and 1H-NMR. Our results show that synthesized polymeric solutions and nanoparticles of N-(Levodopa) and N-(Protocatechuic acid) chitosan

were both internalized by rat brain cells modulated by extracellular Ca^{2+} and Na^{1+}. Moreover, Ca^{2+} imaging recordings performed in brain cells revealed the potential biomedical effect of these chitosan derivatives. The present study shows that N-(Levodopa) and N-(Protocatechuic acid) chitosan derivatives may serve as a potential molecular reservoirs of biomedical drugs to treat degenerative disorders of the nervous system.

Disclosures: M. Montiel-Herrera: None. **O. Ortega-Fimbres:** None. **F. Pérez Delgado:** None. **D. Monge-Sanchez:** None. **M. García Villa:** None. **D. Fernandez Quiroz:** None. **C.G. Castillo:** None. **J. Domínguez-Avila:** None. **G. Gonzalez Aguilar:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.25/A63

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

Support: ERC Grant Avian Mind

Title: Calcium-binding protein expression differentiates between primary input areas of the DVR and the visual Wulst in pigeons (Columba livia)

Authors: *K. B. HASELHUHN[1], M. ZIEGLER[2], O. GUNTURKUN[3], N. ROOK[4]; [1]Biopsychology, Ruhr Univ. Bochum, Bochum, Germany; [2]Ruhr-University Bochum, Bochum, Germany; [3]Fac. of Psychology, Dept. Biopsychology, Ruhr-Universität Bochum, Bochum, Germany; [4]Biopsychology, Ruhr-University Bochum, Bochum, Germany

Abstract: For well over a century, neuroscientists have been highly interested in studying the structure of the avian brain as avian species manifest cognitive capacities on par with mammals, although their forebrain architecture is markedly different. While mammalian cognitive functions predominantly arise from the structure and circuitry of the six-layered neocortex, avian forebrains are organized in a simpler nuclear configuration. The avian forebrain consists of the dorsal ventricular ridge (DVR) alongside the visual Wulst, a quasi-laminar structure. While the Wulst is widely recognized as sharing certain parallels with the neocortex, the DVR, with its multifaceted nuclei, lacks a direct homolog in mammals. However, recently, it was suggested that the neuronal circuitry of the avian Wulst and the sensory systems within the DVR show a laminar and columnar organization comparable to the organization of the mammalian cortex. Nevertheless, detailed descriptions of the neurochemical profiles of the avian DVR and Wulst with respect to potential cortical counterparts in mammals remain less explored. In this study we investigated the expression of the calcium-binding proteins calbindin, calretinin and parvalbumin in the different sensory systems of DVR and within Wulst and compared them to their expression patterns in the corresponding mammalian cortical layers. Our most striking finding was that similar to mammalian layer 4, calretinin was not expressed in the primary input layer of the visual Wulst. However, calretinin was heavily expressed in the entopallium, nucleus basalis

and field L2 that are the primary input areas of the DVR. Thus, our data support the idea that the visual Wulst might be truly homologous to the neocortex whereas the DVR shares certain similarities while only being a functional analogue.

Disclosures: K.B. Haselhuhn: None. **M. Ziegler:** None. **O. Gunturkun:** None. **N. Rook:** None.

Poster

PSTR148: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR148.26/A64

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

Support: NIH Grant 5R01HD100823

Title: Distinct molecular subtypes and anatomical projections of rhombomere 4-derived Pet1 neurons in the mouse brain

Authors: *P. DERR, Y. CHANG, G. MADDALONI, B. W. OKATY, N. STURROCK, S. M. DYMECKI;
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Abstract: *Pet1* (aka *Fev)* encodes an ETS-family transcription factor required, in most cases, for the differentiation of serotonin (5-Hydroxytryptamine, 5-HT)-producing neurons (Scott ***et al***., 2005). Here we report on *Pet1* neurons derived from the 4th embryonic rhombomere (Alonso ***et al.***, 2013); we refer to them as r4-*Pet1* neurons. We used dual-recombinase-dependent fluorescent reporters (Brust ***et al.***, 2014; Niederkofler ***et al.***, 2016) to capture and visualize *Pet1* neurons (Jensen ***et al.***, 2008) that have expressed *Hoxb1*, a patterning transcription factor for rhombomere 4 (O'Gorman, 2005). We performed single-cell RNA sequencing on manually sorted r4-*Pet1* neurons revealing 3 potential neuron subtypes comprising this population. While the expression level of many genes differs across these r4-*Pet1* neuron clusters, a distinguishing hallmark is seen in neurotransmitter expression profile. The largest subgroup has the molecular signature *Pet1*$^{+}$, *Tph2*^{low}, *Penk*$^{+}$, *Vglut3*$^{+}$, indicating a neurotransmitter phenotype involving enkephalin and glutamate. The next most abundant neuron subgroup has the molecular signature *Pet1*$^{+}$, *Tph2*^{low}, *Penk*$^{+}$, *Gad2*$^{+}$, suggesting GABAergic and enkephalinergic neurotransmission. A minority cluster expresses *Pet1* and *Tph2*^{high}, indicating a 5-HTergic phenotype. The anatomical correlates of these r4-*Pet1* neuron subgroups are being mapped in mouse and human tissue. We visualized r4-*Pet1* synaptic boutons *en masse* using a dual recombinase Synaptophysin-eGFP reporter. The descending projections target regions involved in cardiorespiratory control, including the Prebötzinger complex (**PBC**), the Nucleus of the Solitary Tract (**NTS**), the Hypoglossal Nucleus (**12N**), and the Parabrachial Nucleus (**PBN**). Ascending r4-*Pet1* fibers innervate the Extended Amygdala (**EAM**), the Ventral Tegmental Area (**VTA**), the Dorsal Raphe Nucleus (**DRN**), the Paraventricular Nucleus of the Thalamus (**PVT**), and the Substantia

Nigra (**SN**). We immunodetected Syp-GFP (r4-*Pet1* neuron boutons) along with 5-HT, PENK, VGLUT3, or VGAT in each target region. r4-*Pet1* Boutons within the PVT, NTS, and DRN predominantly colocalize with VGLUT3 and thus are likely innervated by the *Pet1*$^{+}$, *Tph2*^{low}, *Penk*$^{+}$, *Vglut3*$^{+}$ group. r4-*Pet1* Boutons in the 12N and PBC are predominantly comprised of 5-HT and are likely derived from the *Pet1*$^{+}$, *Tph2*$^{+}$ group. Other target regions have mixed neurochemical bouton phenotypes. Our data suggests three molecularly distinct subgroups of r4-*Pet1* neurons that maintain unique and partially overlapping innervation profiles. These results suggest distinct functional circuits and contribute to our understanding of *Pet1* neuron diversity in the mammalian brain.

Disclosures: P. Derr: None. **Y. Chang:** None. **G. Maddaloni:** None. **B.W. Okaty:** None. **N. Sturrock:** None. **S.M. Dymecki:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.01/A65

Topic: B.04. Synaptic Transmission

Title: Sv2a deletion in human ipsc-derived neurons leads to reduced synapse numbers and impaired synaptic transmission

Authors: *V. S. LOURENÇO[1,2], M. VERHAGE[3,4], R. F. TOONEN[1,2];
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Abstract: The vertebrate-specific synaptic vesicle protein 2 family of glycoproteins consists of three paralogs: SV2A, SV2B, and SV2C. SV2A is expressed in glutamatergic and GABAergic neurons and its absence results in recurring seizures in mice and early death. Mutations in the human SV2A gene also lead to epilepsy and SV2A is the molecular target of frequently prescribed anti-epileptic drugs (levetiracetam/Keppra). Previous work in rodents suggests that SV2A facilitates transmitter release, however the function of SV2A function in human neurons is unknown. Here, we CRISPR/Cas9 engineered null mutant SV2A (SV2A-/-) human induced pluripotent stem cell lines (BIONi010-C-13) and investigated single-cell and network cultures of iPSC-derived neurons with the (isogenic) CRISPR-treated line as a control. Glutamatergic neurons were generated by expression of NGN2 and dual SMAD/WNT inhibition and maintained in culture for 6 weeks. SV2A-/- neurons showed normal dendrite morphology and expression of the presynaptic marker Synaptophysin and dendrite marker MAP2, but synapse numbers were reduced by 25%. Whole-cell patch clamp recordings in single-cell cultures revealed a 40% reduction in EPSC amplitude of SV2A-/- neurons and no alterations in short-term plasticity or in the frequency and amplitude of spontaneous events. Estimations of the readily releasable pool of vesicles suggest a normal pool size. However, pool replenishment rates

were 2.5 times slower in SV2A-/- neurons. In mass cultures, SV2A-/- neurons showed reduced network frequency and calcium spike prominence during calcium imaging. Together, our study shows that SV2A modulates synapse formation/stabilization and controls release pool replenishment in human glutamatergic neurons resulting in lower network activity and evoked release amplitudes.

Disclosures: V.S. Lourenço: None. **M. Verhage:** None. **R.F. Toonen:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.02/A66

Topic: B.04. Synaptic Transmission

Support: Charles H. Revson Foundation Postdoctoral Fellowship
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Fresco and Burnett Family Foundations and the Vulnerable Brain Project
New York University Langone Medical Center Rodent Genetic Engineering Laboratory (P30CA016087)

Title: Functional specialization of somatostatin-expressing hippocampal interneurons

Authors: *G. GRANT[1], R. P. MACHOLD[2], E. R. NEBET[2], J. STICH[2], M. HANANI[2], K. KULLANDER[3], R. W. TSIEN[2], S. CHAMBERLAND[2,4];
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Abstract: Hippocampal somatostatin-expressing (Sst) GABAergic interneurons (INs) exhibit considerable anatomical and functional heterogeneity. Recent single-cell transcriptome analyses have provided a comprehensive Sst-IN subpopulations census, a plausible molecular ground truth of neuronal identity whose links to specific functionality remain incomplete. Here, we designed an approach to identify and access subpopulations of Sst-INs based on transcriptomic

features. Four mouse models based on single or combinatorial Cre- and Flp- expression differentiated functionally distinct subpopulations of CA1 hippocampal Sst-INs that largely tiled the morpho-functional parameter space of the Sst-IN superfamily. Notably, the Sst;;Tac1 intersection revealed a population of bistratified INs that preferentially synapsed onto fast-spiking interneurons (FS-INs) and were sufficient to interrupt their firing. In contrast, the Ndnf;;Nkx2-1 intersection identified a population of oriens lacunosum-moleculare (O-LM) INs that predominantly targeted CA1 pyramidal neurons, avoiding FS-INs. These neurons differed from previously studied Chrna2 cells, which share a similar O-LM anatomy but showed no targeting preference between CA1 pyramidal neurons and FS-INs. Anatomically, we found that Sst;;Nos1 cells sometimes project to the subiculum and Sst;;Tac1 cells sometimes project to CA2. Motivated by these morpho-functional distinctions, we investigated the microcircuit role of these interneurons. Interestingly, we found differences in the recruitment profiles of Sst-IN subtypes, with Sst;;Tac1 cells distinctly receiving excitatory inputs from both CA3 and CA1. Overall, our results provide a framework to translate neuronal transcriptomic identity into discrete functional subtypes that capture the diverse specializations of hippocampal Sst-INs.

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Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.03/A67

Topic: B.04. Synaptic Transmission

Title: Structure-function analysis of TRAPP complexes in the Drosophila nervous system

Authors: *J. LEE[1], L. LIANG[2], R. S. THAKUR[1], K. M. O'CONNOR-GILES[3];
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Abstract: Communication in the nervous system is critical for motor and cognitive functions such as movement, learning, and memory. Synapses are the primary site of communication between neurons and are composed of signal-sending (presynaptic) and signal-receiving (postsynaptic) cells. At the presynaptic membrane, synaptic vesicles (SVs) containing neurotransmitter cargo are docked and organized for release. However, little is known about how SVs are recruited and positioned. Ultrastructural analysis of synapses reveals protein tethers linking SVs to presynaptic membranes, but the composition of these vesicle tethers remains unknown. Transport Protein Particle (TRAPP) is a multisubunit protein complex that, in other cellular contexts, tethers endoplasmic reticulum-derived vesicles to vesicular tubular clusters for protein cargo processing. Disruptive variants in TRAPP subunits have been implicated in motor dysfunction, intellectual disability, and developmental delay. However, it remains unknown why symptoms predominantly affect the nervous system given the universal role of the complex. We

hypothesize that TRAPP complexes play an important role in SV recruitment and positioning at presynaptic terminals.
We investigated the in vivo nervous system role of TRAPP complex through studies at the Drosophila glutamatergic larval neuromuscular junction. We find that core TRAPP subunits are expressed at synapses of the larval ventral nerve cord and at the neuromuscular junction. TRAPP subunits strongly co-localize with SVs and loss of TRAPP subunits leads to decreased levels of SV proteins at presynaptic terminals. Notably, synaptic vesicle proteins are observed in neuronal cell bodies, suggesting that loss of TRAPP function causes a transport deficit which leads to cargo accumulation. Overall, our findings suggest a role for TRAPP complexes in the transport of SVs and possible maintenance of SVs at presynaptic terminals. Ultimately, an increased understanding of the TRAPP will provide insight into unknown disease mechanisms while answering long-standing questions about synaptic vesicle tethers.

Disclosures: J. Lee: None. **L. Liang:** None. **R.S. Thakur:** None. **K.M. O'Connor-Giles:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.04/A68

Topic: B.04. Synaptic Transmission

Support: NIH Grant 1RF1MH130468-01A1

Title: Synapse-seq: Simultaneous acquisition of single-neuron transcriptome and neuroanatomy

Authors: *S. M. GARCIA[1], M. KIM[2], M.-J. DOLAN[2], J. LUU[3], A. URKE[4], B. E. DEVERMAN[5], B. A. STEVENS[6], E. MACOSKO[7];
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Abstract: Synapse-seq: Simultaneous acquisition of single-neuron transcriptome and neuroanatomy.
Synapses are the points of communication between neurons, controlling the flow of information across neural circuits. The number of synapses (synapse quantity) between cells is tightly regulated by processes that are both productive and eliminative. Today, state of the art techniques for examining synapses include IHC and EM. Results from the application of these techniques have shown dysregulation of synapse quantity has been implicated in a wide range of neurodevelopmental and neurodegenerative diseases such as schizophrenia, autism, Alzheimer's disease and frontotemporal dementia (Salter and Stevens 2017). However, there is no tool available to link the data with single-cell RNAseq to synaptic density. Despite a clear role in disease, the mechanisms leading to aberrant synapse gain or loss are not well understood.

Our goal is to develop a new tool to link single-cell RNA seq with synaptic information. In this Poster, I present Synapse-Seq, a novel technology that links single-cell RNA sequencing with synaptic information by trafficking barcodes to the synapses. The technology is based on an RNA-protein complex that is made up of two parts - a protein component and an RNA component. The protein component contains a targeting that allows trafficking to the presynapse. The RNA component contains an mRNA barcode that uniquely labels each cell. Critically, the protein and RNA component each also has a domain that allows them to specifically bind to each other, thereby allowing the labeling of presynaptic termini with RNA barcode.
In the second part of my poster presentation, I will describe how we read out our barcodes by using spatial transcriptomics and bulk RNA sequencing. Specifically, I will describe the findings from our robust bulk RNA sequencing protocol. The regions of interest were those of sparse projections that could not be captured by Slide-seq effectively. The bulk RNA sequencing protocol consists of nested PCRs with gene-specific primers and purification of the final product through DNA fragment cleanup from tissue sections of 100 um dissections.
Using Synapse-seq, we can connect synapse loss to gene expression and reveal which neural circuits develop synaptic abnormalities. This could lead to a new understanding of, and potentially treatments for, neurodegenerative disease.

Disclosures: S.M. Garcia: None. **M. Kim:** None. **M. Dolan:** None. **J. Luu:** None. **A. Urke:** None. **B.E. Deverman:** None. **B.A. Stevens:** None. **E. Macosko:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.05/A69

Topic: B.04. Synaptic Transmission

Support: NIH Grant MH118258

Title: Activation of GHSRs Induces A Chemical LTP at the Perforant Path-Granule Cell Synapses and Potentiates Spatial Learning and Memory

Authors: *S. LEI[1], C. ORAEGBUNA[2];
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Abstract: Ghrelin is a gut hormone that affects growth hormone release, energy homeostasis and acts as a neuromodulator by activating the growth hormone secretagogue receptors (GHSRs). GHSRs are expressed in different parts of the brain including the dentate gyrus (DG) of the hippocampus. The granule cells (GCs) in the DG receive glutamatergic innervation from the perforant-path (PP) pathway originating from the entorhinal cortex. We studied the effects of GHSR activation on glutamatergic transmission at the PP-GC synapses. Our results indicate that activation of GHSRs induced a robust chemical LTP at the PP-GC synapses. Activation of

GHSRs decreased both the coefficient of variation and paired-pulse ratio of AMPA EPSCs, suggesting that activation of GHSRs increases presynaptic glutamate release. We further used the Y maze spontaneous alternation test to assess the effects of GHSRs on working memory. Our results indicate that microinjection of ghrelin into the DG significantly increased the alternating sequences compared to the saline control group, suggesting that activation of GHSRs augments spatial memory. Ghrelin-mediated memory enhancement was blocked by adenylate cyclase and Epac2 inhibitors, suggesting that cAMP and Epac2 are involved in GHSR-mediated augmentation of spatial memory. Our findings provide a cellular and molecular model to explain the roles of GHSRs in cognitive functions.

Disclosures: S. Lei: None. **C. Oraegbuna:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

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Program #/Poster #: PSTR149.06/A70

Topic: B.04. Synaptic Transmission

Support: KAKENHI 24K18217
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KAKENHI 22H02721

Title: Contrasting reduction of presynaptic RRP and Ca^{2+} influx by distinct cannabinoid receptors highlighted by direct recordings from axon terminal of cerebellar Purkinje cell

Authors: *T. INOSHITA, S.-Y. KAWAGUCHI;
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Abstract: Neurotransmitter release at chemical synapses is fundamental to neuronal signaling. It is known that transmitter release is negatively regulated by cannabinoids at various synapses in the CNS. Two types of cannabinoid receptors, CB1 and/or CB2 have been shown by axonal Ca^{2+} imaging experiments to reduce Ca^{2+} influx into a presynaptic bouton, resulting in reduction of presynaptic exocytosis of synaptic vesicles. On the other hand, G protein-coupled receptor 55 (GPR55) was recently identified as a new type of cannabinoid receptors, and suggested to affect synaptic transmission at both the presynaptic and postsynaptic sides. However, the mechanism how GPR55 controls synaptic function remains elusive. To study this issue in detail, we used cerebellar Purkinje cells (PCs), which allowed us to perform direct patch-clamp recordings from axon terminals presumably expressing GPR55. Surprisingly, activation of GPR55 suppressed transmitter release in PC boutons not by affecting presynaptic Ca^{2+} influx, but by reducing the size of readily releasable pool (RRP) of vesicles. This result was supported by fluorescence imaging experiments using VAMP-pHluorin, showing that GPR55 reduces the total size of recycling pool of synaptic vesicles without affecting the total number of vesicles present at the PC boutons. Thus, the GPR55-mediated reduction of presynaptic transmitter release in PC

boutons contrasts to the well-known CB1/CB2-mediated one at other synapses. Notably, in spite of the potential expression of CB2 in PCs, previous slice patch clamp studies demonstrated the lack of CB1/CB2-mediated synaptic modulation. When exogenously expressed using AAV, CB2 reduced the size of synaptic outputs in cultured PCs through decreasing presynaptic Ca^{2+} current without changing the RRP size at terminals. Taken all these results together, the present study identified subtype-specific two negative controls of presynaptic transmitter release by cannabinoid receptors: decreasing the RRP size by GPR55 and suppressing the presynaptic Ca^{2+} influx by CB2.

Disclosures: T. Inoshita: None. **S. Kawaguchi:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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Topic: B.04. Synaptic Transmission

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Title: The Ca^{2+}sensors of synchronous and asynchronous release at hippocampal mossy fiber synapses

Authors: *S. JAMRICHOVA, P. JONAS;
Inst. of Sci. and Technol. Austria, Klosterneuburg, Austria

Abstract: Synaptic transmission is a complex series of events, which requires activation of presynaptic Ca^{2+} channels, Ca^{2+} influx and diffusion, and Ca^{2+} binding to release sensors. Synaptotagmin 1 (Syt-1) has been previously identified as the primary Ca^{2+} sensor for fast synchronous transmitter release, while synaptotagmin 7 (Syt-7) was suggested to mediate slower, asynchronous release. However, recent reports have come to conflicting conclusions on the mechanisms of vesicle release modes, confusing our understanding of Ca^{2+} sensing in transmission. To understand the action of synaptotagmins at central synapses, we performed subcellular paired patch-clamp recordings between hippocampal mossy fiber boutons and CA3 pyramidal neurons in acute slices from conditional Syt-1 (Syt-$1^{fl/fl\ x\ Prox1\text{-}Cre}$) and constitutive Syt-7 knockout mice (Syt-$7^{-/-}$). To analyze the biophysical mechanisms of synaptic transmission, presynaptic terminals were stimulated non-invasively in the cell-attached configuration and excitatory postsynaptic currents (EPSCs) were recorded from postsynaptic cells under voltage-clamp conditions (Vandael et al., 2021, Nature Protocols 6, 2947). We found that the deletion of Syt-1 from dentate gyrus granule cells abolished stimulus-evoked synchronous EPSCs (207.5 ± 66.7 pA, control (7 pairs); 12.5 ± 2.4 pA, Syt-$1^{fl/fl\ x\ Prox1\text{-}Cre}$ (16 pairs), $p < 0.0001$). In parallel, the

asynchronous release component was enhanced, consistent with a clamping function of Syt-1 at hippocampal mossy fiber synapses. In contrast, ablation of Syt-7 had only minimal effects on synaptic transmission; both vesicle pool dynamics and synaptic facilitation remained largely unaffected. To determine the effects of Syt-1 deletion on mossy fiber synaptic transmission under physiological conditions, we examined the properties of unitary excitatory postsynaptic potentials (EPSPs) in wild-type and Syt-$1^{fl/fl\ x\ Prox1\text{-}Cre}$ synapses in the current-clamp configuration. In Syt-1 conditional knockout, 50-Hz trains of 3 stimuli triggered spikes in postsynaptic CA3 pyramidal cells (4 out of 6 paired recordings), although to a smaller degree than in wild-type synapses. Whereas the detonator properties of the synapse were maintained in Syt-1 conditional knockout synapses, the precision of timing of postsynaptic spikes was reduced (mean latency to AP peak: 5.7 ± 0.46 ms, control (4 pairs); 39.6 ± 24.9 ms, Syt-$1^{fl/fl\ x\ Prox1\text{-}Cre}$ (3 pairs), $p < 0.002$). Our results identify Syt-1 as the main Ca^{2+} sensor of synchronous release at the hippocampal mossy fiber synapse, and demonstrate that Syt-1 plays a key role in regulating the timing of signaling in the hippocampal trisynaptic circuit.

Disclosures: S. Jamrichova: None. **P. Jonas:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.08/A72

Topic: B.04. Synaptic Transmission

Support: The Swedish Research Council
Hjärnfonden
Research project 95444211 from Saint-Petersburg University

Title: The role of membrane invaginations in synaptic vesicle recycling during high-frequency activity

Authors: *O. SHUPLIAKOV[1,2], N. V. NIFANTOVA[2], A. SHISHKOV[2], L. BRODIN[1]; [1]Neurosci., Karolinska Institutet, Stockholm, Sweden; [2]Saint-Petersburg State Univ., Saint-Petersburg, Russian Federation

Abstract: Large plasma membrane invaginations formed in chromaffin cells following high-frequency stimulation are the primary site for clathrin-coated pit generation during secretory vesicle recycling (Arpino et al., 2022, iScience 25(2):103809). Large membrane invaginations also form at the periactive zone in central synapses during high-rate activity. The goal of our study was to investigate the role of these membrane invaginations in vesicle recycling in central synapses. The giant reticulospinal synapse in lamprey was used as an experimental model. Clathrin-mediated endocytosis was inhibited by compounds that perturb clathrin-cage assembly, Pitstop 2, and dynamin-dependent vesicle fission, GTPγS. Syndapin function was perturbed by microinjection of Fab-fragments of antibodies against its SH3-domain. Presynaptic membrane

invaginations around active zones in synapses were induced by stimulation at 5 Hz for 20 min and studied using electron microscopy. Pre-embedding immunogold technique was utilized to detect syndapin and dynamin. Sections were analyzed in a Tecnai 12 electron microscope (FEI). 3D reconstructions were generated using Amira software. Our experiments with Pitstop 2 and GTPγS showed that the membrane invaginations were the sites for clathrin-coated pit generation. Localization of syndapin and dynamin in uninjected synapses demonstrated that syndapin accumulated at membrane invaginations. Dynamin labeling occurred on both membrane invaginations and clathrin-coated pits. Analysis of 3D reconstructions of synapses revealed that perturbation of the syndapin SH3-domain interaction resulted in a statistically significant increase in the number of free endosomes at endocytic zones as compared to stimulated control synapses. Thus, syndapin appears to regulate budding of the invaginations at the periacitve zone. Our study uncovers a mechanism preventing the budding of presynaptic membrane invaginations, that are the site for clathrin-coated pit generation, during high-rate activity. We suggest that this mechanism promotes the reformation of uniformly sized vesicles.

Disclosures: O. Shupliakov: None. **N.V. Nifantova:** None. **A. Shishkov:** None. **L. Brodin:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

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Program #/Poster #: PSTR149.09/A73

Topic: B.04. Synaptic Transmission

Support: NIH Grant NS094184
NIH Grant NS113922

Title: Morphological analysis of driver inputs from layer 5 cortical axons in subcortical regions and thalamic axons in cortex

Authors: *V. SAMPATHKUMAR[1,2], B. J. CARROLL[1], K. P. KOSTER[1], A. J. MILLER-HANSEN[3], S. SHERMAN[1], N. B. KASTHURI[1,2];
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Abstract: Glutamatergic afferents in thalamus and cortex can be divided into drivers and modulators. Drivers are thought to be the main vehicle for transferring information. One difference between the two types is that presynaptic terminals of modulators are, on average, smaller than those of drivers. However, whereas modulator pathways have uniformly small terminals, those of drivers are much more variable, including both small and large terminals. This raises the question as to whether driver pathways include different axon classes, with some having only small or large terminals or whether individual axons have the range of variable terminal sizes seen in the population. To address this, we used large volume serial electron

microscopy of labeled layer 5 (L5) terminals in thalamus and caudoputamen (CP) in mice, inputs known to be drivers, and analyzed the terminal size distribution of individual axons. More specifically, we used Rbp4-cre transgenic mice having cre in L5 cells, and labeled these with cre-dependent APX into somatosensory (S1), motor (M1), and visual (V1) cortices to reconstruct their synaptic terminals in the posterior medial nucleus (POm, from S1 and M1), pulvinar (Pul, from V1), and CP (from S1 and M1). We reconstructed 150 L5 corticofugal axons in POm, CP, and Pul across 5 mice. Our main finding is that terminal sizes among individual axons vary greatly in size, effectively representing the variability seen in the entire pathway of driver axons. We thus found no evidence of distinct categories of driver axons. Expanding upon these findings, we are currently investigating thalamocortical driver axons from POm to S2 and pulvinar to V2 to extend these observations to thalamocortical driver inputs. Preliminary results indicate a similar pattern of driver axons with both large and small terminals. In summary, these findings suggest that the variability in the morphology of driver inputs is not due to a subset of afferents with varying morphology, but rather reflects the intrinsic properties of individual axons.

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Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.10/A74

Topic: B.04. Synaptic Transmission

Title: Inhibition of presynaptic calcium channels in cortical inputs to the nucleus accumbens shell by metabotropic CB2 receptors and induced binge-eating behavior in rats

Authors: *I. CONDE ROJAS[1], J. O. SUAREZ-ORTIZ[2], F. CORTÉS SALAZAR[2], G. B. FLORAN[3], V. LÓPEZ-ALONSO[4], J. MANCILLA-DIAZ[4], R. ESCARTIN-PEREZ[4];
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Abstract: Neurophysiological and neurochemical factors contribute to the expression of eating disorder symptoms, with the endocannabinoid system playing a central role. The present report aimed to investigate the role of CB2 cannabinoid receptors (CB2r) in modulating neurotransmitter release in cortico-accumbens pathway projections from the anterior cingulate cortex (ACC) neurons to medium spiny neurons (MSNs) in the nucleus accumbens shell (NAc-Sh). Current evidence supports the role of this circuit in the coordination of decision-making processes regarding the positive and rewarding consequences of stimuli. Despite the above, the regulatory mechanism by which rCB2 may presynaptically regulate the activity of neurons in the

cortico-accumbens pathway has not been described. Here, we used electrophysiological techniques in the NAc-Sh slice preparation (male Wistar rats) to provide evidence that activation of rCB2 (GW 405833, 1μM) decreased glutamate release and that rCB2 antagonism (AM630, 100 nM), but not CB1 cannabinoid receptor antagonism (AM281, 100 nM), prevented this effect. We also observed that selective inhibition of P/Q-type Ca2+ channels with ω-agatoxin-TK (200 nM) blocked the inhibitory effect of rCB2 and, conversely, that the inhibitory effects of rCB2 did not require functional L- and N-type Ca2+ channels (1μM). Additionally, we examined whether laboratory rodents exhibit altered reward circuit activity due to binge-like behavior induced by the 10% sucrose intermittent access protocol and whether these changes involved rCB2. Our findings suggest a cannabinoid component capable of modifying neuronal activity within the reward circuit, supported by the observation that rCB2 regulates glutamate release in MSNs, particularly those innervated by the ACC, suggesting a possible mechanism by which rCB2 contributes to the processing of information necessary for decision making.

Disclosures: I. Conde Rojas: None. **J.O. Suarez-Ortiz:** None. **F. Cortés Salazar:** None. **G.B. Floran:** None. **V. López-Alonso:** None. **J. Mancilla-Diaz:** None. **R. Escartin-Perez:** None.

Poster

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Topic: B.04. Synaptic Transmission

Support: R01MH113349
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R25GM109436

Title: Mechanisms of RIM-BP liquid-liquid phase separation with RIM

Authors: *A. M. RIOS, J. W. ANDERSEN, P. S. KAESER;
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Abstract: Synaptic transmission is initiated when calcium entry through voltage-gated calcium channels drives secretion of neurotransmitters via fusion of synaptic vesicles with the plasma membrane. The transmitters are then sensed by select postsynaptic cells. Neurotransmitter release is regulated by active zone proteins, including Rab3-interacting molecules (RIM) and RIM-binding protein (RIM-BP). RIM-BP and RIM form self-assembled condensates via liquid-liquid phase separation capable of clustering presynaptic calcium channels *in vitro*. Here we determine the protein sequences and domains needed for liquid-liquid phase separation of RIM-BP with RIM. To investigate this, we generated a series of mutant versions of RIM-BPs. We assessed their ability to form condensates and the liquid properties of these condensates in transfected cells. We also tested whether these RIM-BP mutants co-condense with RIM. Ultimately, these studies reveal the mechanisms of RIM-BP phase separation and its dependence

on RIM. This work will allow for generating new models on the assembly of protein complexes at the presynaptic active zone that mediate the calcium-triggering of neurotransmitter release.

Disclosures: A.M. Rios: None. **J.W. Andersen:** None. **P.S. Kaeser:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.12/A76

Topic: B.04. Synaptic Transmission

Title: Molecular mechanisms of synaptic release machinery at sensory neurons

Authors: *L. ROJAS[1], C. LEE[2], J. RHEE[3];
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Abstract: The importance of DRG neurons in the perception, transmission and regulation of sensory signals has been widely recognized from a physiological perspective. However, our understanding the machinery for releasing synaptic vesicles (SVs) at the synapse where signal transmission occurs remains incomplete. To specifically investigate the molecular mechanisms of SV priming and fusion, we used a technique in which genetically modified mouse neurons are cultured on astrocytes islands of approximately 200 microns in diameter. Glutamatergic DRG neurons were cultured alone or co-cultured with glycinergic or GABAergic spinal cord neurons to mimic a simplified but physiologically relevant network. Interestingly, functional and biochemical experiments revealed the absence of glutamate receptors in cultured mouse DRG neurons. Therefore, we recorded synaptic release properties using a dual patch technique on co-cultured DRG and spinal cord neurons. The importance of Munc13s in regulating SV priming has been highlighted by previous research showing complete silencing of synaptic activity in Munc13-1/2-deficient CNS neurons (Munc13 DKO). However, in this experiment, while Munc13 DKO DRG neurons had severely impaired SV priming, excitatory postsynaptic currents (EPSCs) could still be recorded. Both EPSC amplitude and the number of primed vesicles were significantly reduced compared to controls, indicating a severe impairment in the priming step, although some priming processes still occurred. In addition, EPSCs from Munc13 DKO neurons showed significantly reduced release rate and delayed release kinetics, measured from their post synaptic partner cell. It is worth noting that the synaptic puncta density assessed with confocal microscopy, was similar between control and DKO neurons, so an impairment in synapse development could not underlie the results. We found that unlike CNS neurons, DRG neurons possess other types of priming factors besides Munc13-1 and Munc13-2 for SV priming. In addition to controlling all-or-none priming activity, Munc13s' function also regulates the state of primed SVs, thereby influencing the timing and rate of SV fusion.

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Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.13/A77

Topic: B.04. Synaptic Transmission

Title: Molecular basis for Gβγ-SNARE mediated inhibition of neurotransmission

Authors: *A. R. EITEL[1], H. E. HAMM[2];
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Abstract: G-protein βγ heterodimers (Gβγ) liberated upon activation of presynaptic inhibitory G-protein Coupled Receptors (Gi/o GPCRs) prevent neurotransmission downstream of Ca2+ influx through direct interactions with the ternary N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex. The ternary SNARE complex is composed of synaptosomal-associated protein, 25kDa (SNAP-25), syntaxin-1A, and synaptobrevin-2. Previous work from the Hamm lab identified regions of both Gβγ and t-SNARE that are critical for the interaction, but a sufficient understanding of the molecular mechanism remains elusive due to lack of structural data for the complex. To address this, we have expressed and purified a pre-fusion ternary SNARE mimetic containing a C-terminal truncation of synaptobrevin-2 which prevents full zippering of the SNARE complex. This partially zippered SNARE construct has a higher affinity for Gβ1γ2 than the fully zippered version as determined by microscale thermophoresis (MST). We then structurally characterized the complex using crosslinking mass spectrometry, size-exclusion chromatography, and electron microscopy. Our preliminary results revealed two separate binding sites on Gβγ for pre-fusion SNARE—one comprising the Ga-Gβγ interface, and one involving the N-termini. Interestingly, we also found that both the amino and carboxy termini of ternary SNARE interact with Gβγ. These results are consistent with previous biochemical data and suggest a higher degree of stoichiometry between Gβγ and ternary SNARE. To investigate further, we are in the process of determining the high-resolution structure using cryo-EM. Future studies will focus on the functional stoichiometry of the interaction as well as effects of Gβγ on other SNARE-effector interactions.

Disclosures: A.R. Eitel: None. **H.E. Hamm:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

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Topic: B.04. Synaptic Transmission

Support: NIH grant R01EY031411
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Title: A circuit-specific role of ribbons in mediating synaptic depression in the retina

Authors: *A. SCHULTZ[1,2], M. HOON[3,4,5], R. SINHA[1,6,5];
[1]Dept. of Neurosci., Univ. of Wisconsin, Madison, Madison, WI; [2]Molecular & Cellular Pharmacology Training Program, University of Wisconsin, Madison, Madison, WI; [3]Ophthalmology and Visual Sci., Univ. of Wisconsin, Madison, Madison, WI; [4]Department of Neuroscience, University of Wisconsin, Madison, Madison, WI; [5]McPherson Eye Research Institute, University of Wisconsin, Madison, Madison, WI; [6]Ophthalmology and Visual Sciences, University of Wisconsin, Madison, Madison, WI

Abstract: A common mechanism by which sensory circuits adapt is by modulating the gain of synaptic transmission. The visual system has evolved several mechanisms of synaptic gain control starting in the retina. Retinal tissue is comprised of a primary excitatory circuit: photoreceptors to bipolar cells (BC) to ganglion cells (GC). Glutamate release from photoreceptors and BCs is mediated by a ribbon complex in the axon terminal that replenishes vesicles at sites of exocytosis. While it has been demonstrated that facilitation and depression—two conserved modes of presynaptic gain control—of glutamate release are essential to global retinal function, how different retinal circuits utilize these mechanisms for visual adaptation and the underlying role of ribbons have not been extensively studied. Using patch clamp electrophysiology in ex vivo mouse retinal preparations, we recorded excitatory inputs in response to contrast-varying visual stimuli from three GC subtypes in the alpha family: ON-sustained, OFF-sustained, and OFF-transient. Synaptic depression, due to depletion of the readily releasable pool (RRP) of synaptic vesicles, can be caused by very weak stimuli at inner retinal ribbon synapses that operate at high gain. Thus, we used a paired-flash stimulus paradigm to elicit synaptic depression and measured the time course of synaptic recovery. To test the role of ribbons, we also performed these experiments in mice lacking the ribbon structural protein RIBEYE, which in photoreceptors reduces RRP size and may disrupt vesicle-active zone coupling. Because GC types receive inputs from distinct BCs that differ in ribbon abundance, we believed the kinetics of synaptic depression and the associated effects of RIBEYE-KO would differ between GC types. We found that, while excitatory inputs to ON-sustained and OFF-transient GCs require several hundred milliseconds to recover from flash-evoked depression, OFF-sustained GC inputs depress minimally, suggesting these circuits may be governed by different synaptic machinery. In RIBEYE-KO retina, excitatory inputs to ON- and OFF-sustained GCs recovered more slowly from synaptic depression than their wild-type counterparts and were unchanged in OFF-transient GCs. Together, our results reveal circuit-level heterogeneities in glutamate release probability and kinetics of visual adaptation, and a circuit-specific role for ribbons in setting synaptic gain.

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Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

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Topic: B.04. Synaptic Transmission

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Postdoctoral fellowship from DGAPA, UNAM

Title: Fusion proteins involved in somatic exocytosis from dense-core vesicles

Authors: *J. LAGUNA MACÍAS[1], N. SAROJ[2], C. WINCHELL[3], D. A. WEISBLAT[4], F. F. DE-MIGUEL[5];
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Abstract: Our study aimed to identify candidate proteins of the fusion complex for extrasynaptic dense core vesicles. Extrasynaptic exocytosis of transmitters and peptides releases vast amounts of signaling molecules upon streams of action potentials or long depolarizations, following a mechanism significantly different from that for synaptic release. One major difference is that extrasynaptic release occurs from dense core vesicles that must be transported to the plasma membrane for fusion. Another difference is that the calcium that triggers extrasynaptic exocytosis is released from the endoplasmic reticulum. Therefore, high-affinity calcium sensors are expected to replace the low-affinity calcium sensors at synapses. The large soma of serotonergic Retzius neurons from the leech Hirudo verbana was employed to identify candidate proteins forming the fusion complex for somatic exocytosis from dense-core vesicles. The transcriptome of isolated Retzius neurons contained mRNA sequences for four fusion proteins. Two key proteins were the high-affinity calcium sensors CAPS-1 (calcium-dependent activator protein for secretion 1) and SYT7 (synaptotagmin 7). Another protein was the SNARE protein STX12 (syntaxin 12), and the fourth protein was the exocyst complex protein EXOC4. Our blast analysis indicates that these four proteins are conserved from E. coli to the human brain, suggesting their consistent involvement in non-synaptic exocytotic events. The expression of each protein was confirmed in Retzius neurons through in situ hybridization, immunofluorescence, and Western blot techniques. Furthermore, the RNA expression was selectively disrupted by intracellular microinjection of RNA interference sequences into the neuronal soma in ganglia that were isolated and cultured. Our data suggests that the fusion complex responsible for extrasynaptic exocytosis of serotonin from dense-core vesicles involves an assembly of fusion proteins that are unconventional compared to synchronous synaptic release. This research has been funded by a Human Frontier Science Program Organization

(HFSP) grant to DAW and FFDM (RPG0060/2019), and by grants from the Dirección General de Asuntos del Personal Académico (DGAPA) (AG200823) of the Universidad Nacional Autónoma de México (UNAM), and the National Council of Humanities, Sciences, and Technology (CONAHCYT), to FFDM (CY319711) . AL receives a doctorate fellowship from CONAHCYT (CVU666673), and NS receives a postdoctoral fellowship from DGAPA, UNAM.

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Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.16/B1

Topic: B.04. Synaptic Transmission

Support: DAAD
TUBITAK

Title: Impact of Diet on Synapse Morphology, Synaptic Protein Intensity, and Locomotor Behavior in Drosophila

Authors: *R. KARATEPE;
Univ. of Hlth. Sci., Istanbul, Turkey

Abstract: A well-balanced diet is important for neuronal health and has big impacts on behavior, as some nutrients can exacerbate neurological diseases and dietary therapies are well-known for treatment of neurological disease.
In this study, our aim was to investigate how dietary intake affects synapse morphology, synaptic protein levels, and locomotor behavior at type 1b neuromuscular junctions. To achieve this, we used Drosophila melanogaster as our experimental model. We exposed these flies to two distinct diets and then used immunohistochemistry to examine changes in synapse structure.
Our findings revealed noteworthy alterations in both presynaptic and postsynaptic components. Larvae fed with the first diet displayed smaller and rounder active zones at neuromuscular junctions. Additionally, these larvae showed increased levels of both presynaptic protein bruchpilot (brp) and postsynaptic glutamate receptors. Moreover, these larvae displayed a preference for bending of their upper abdominal segments instead of forward movement compared to larvae fed with the second diet.
These findings underscore the pivotal role of diet in influencing neuronal function and locomotor behavior, establishing a direct connection between dietary intake, synaptic activity, and locomotor outcomes. These observed changes provide valuable insights into how dietary factors might impact synapse morphology, synaptic protein abundance, and ultimately, locomotion. Understanding the effects of diet on synaptic properties is crucial for unraveling the mechanisms governing brain health and locomotor behavior. Further investigations in this domain hold the

potential to contribute to the development of dietary interventions, particularly in the context of neurological diseases.

Disclosures: R. Karatepe: None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

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Title: Presynaptic structural and functional plasticity are coupled by convergent Rap1 signaling

Authors: *S. LEE;
Sch. of Dent. Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Dynamic presynaptic actin remodeling drives structural and functional plasticity at synapses, but the underlying mechanisms remain largely unknown. Previous work has shown that actin regulation via Rac1 guanine exchange factor (GEF) Vav signaling restrains synaptic growth via bone morphogenetic protein (BMP)-induced receptor macropinocytosis, and mediates synaptic potentiation via mobilization of reserve pool vesicles in presynaptic boutons. Here, we find that Gef26/PDZ-GEF and small GTPase Rap1 signaling couples the BMP-induced activation of Abelson kinase to this Vav-mediated macropinocytosis. Moreover, we find that adenylate cyclase Rutabaga (Rut) signaling via exchange protein activated by cAMP (Epac) drives the mobilization of reserve pool vesicles during post-tetanic potentiation (PTP). We discover that Rap1 couples activation of Rut-cAMP-Epac signaling to Vav-mediated synaptic potentiation. These findings indicate Rap1 acts as an essential, convergent node for Abelson kinase and cAMP signaling to mediate BMP-induced structural plasticity and activity-induced functional plasticity via Vav-dependent regulation of the presynaptic actin cytoskeleton.

Disclosures: S. Lee: None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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Topic: B.05. Synaptic Plasticity

Support: NIH Grant AA029811

Title: A novel form of presynaptic homeostatic plasticity: self-tuning of presynaptic calcium channel levels in response to neurotransmitter release

Authors: A. XIONG[1], ***H. KIM**[2];
[1]Cell Biol. & Anat., Chicago Med. Sch., Rosalind Franklin Univ., North Chicago, IL; [2]Cell Biol. & Anat., Chicago Med. School/Rosalind Franklin Univ., North Chicago, IL

Abstract: The abundance of CaV2 voltage-gated calcium channels is linked to presynaptic homeostatic plasticity (PHP), a process that recalibrates synaptic strength to maintain the stability of neural circuits. While PHP is widely known to be caused by a deficit in postsynaptic receptors (transsynaptic homeostatic plasticity), it is not clear whether other types of PHP are also present. Here, we uncover a novel form of PHP in *Caenorhabditis elegans*, in which PHP is not elicited by a postsynaptic deficit but rather induced by an inverse regulatory mechanism present between the efficiency of neurotransmitter release and the abundance of UNC-2/CaV2 channels. Gain-of-function *unc-2SL(S240L)* mutants, which carry a mutation analogous to the one causing familial hemiplegic migraine type 1 in humans, showed markedly reduced channel abundance despite increased channel functionality. However, perturbations in the efficiency of synaptic vesicle (SV) exocytosis reduced UNC-2SL channel levels. Conversely, loss-of-function *unc-2DA(D726A)* mutants, which harbor the D726A mutation in the channel pore, exhibited a marked increase in channel abundance, which is reduced when the efficiency of SV exocytosis was enhanced. Together, these findings demonstrate that the efficiency of neurotransmitter release inversely impacts the abundance of presynaptic CaV2 channels, which in turn indirectly influences neurotransmitter release efficiency. Importantly, this homeostatic regulation of UNC-2 channel levels is accompanied by the structural remodeling of the active zone. This remodeling, marked by parallel changes in select core active zone protein levels, is required for the effective homeostatic regulation of UNC-2. These findings highlight a self-tuning PHP regulating UNC-2/CaV2 channel abundance along with active zone reorganization, ensuring synaptic strength and stability.

Disclosures: A. Xiong: None. **H. Kim:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

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Title: Investigation of activity-dependent glucose metabolism in cortical excitatory neurons

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Abstract: The human brain, which constitutes only 2% of the total body weight, accounts for approximately 20% of the total body's glucose consumption. Most of this glucose is classically thought to function as an energy source for ATP synthesis via oxidative phosphorylation (OXPHOS) in the mitochondria, which supports repetitive neuronal activities at synaptic boutons. However, recent studies have shown that blocking OXPHOS in mammalian neuronal cultures has limited effects on presynaptic ATP levels, even under extreme, non-physiological levels of action potential-triggered stimulation. Therefore, the question remains as to how critical axonal mitochondria are involved in ATP generation in mammalian neurons in the central nervous system. In this study, we demonstrated that the majority (~80%) of axonal mitochondria in cortical pyramidal neurons (CPNs) both *in vitro* and *in vivo*, lack mitochondrial DNA (mtDNA). Since mtDNA encodes parts of the essential respiratory chain complex proteins, our results suggest that the majority of axonal mitochondria do not play a major role in the ATP supply (https://doi.org/10.1101/2024.02.12.579972). Our findings are consistent with observations in mammalian brains indicating that physiological neural activity increases the uptake of glucose but not as much of oxygen, suggesting that most activity-dependent glucose uptake is metabolized in a non-oxidative manner. Then, how do neuronal mitochondria metabolize glucose? To explore this, we conducted a ^{13}C isotope-based metabolic flux analysis using [U-$^{13}C_6$]glucose in primary cultured mouse cortical neurons. Interestingly, we found that a significant portion of the carbon from [U-$^{13}C_6$]glucose contributed to the *de novo* synthesis of glutamate. The percentage of [U-^{13}C]-labeled glutamate increased markedly with picrotoxin treatment, indicating that glucose is converted to glutamate in an activity-dependent manner. Thus, we hypothesize that during neuronal activity, glucose primarily contributes to glutamate synthesis rather than oxidative ATP synthesis. In this conference, I would like to discuss this hypothesis in more detail.

Disclosures: Y. Du: None. **T. Ishiyama:** None. **Y. Sugiura:** None. **T. Lewis:** None. **D. Virga:** None. **I. Testa:** None. **F. Polleux:** None. **Y. Hirabayashi:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.20/B5

Topic: B.04. Synaptic Transmission

Support: NSERC
CIHR
CRC

Title: Molecular and ultrastructural bases of distinct release modalities at a central synapse

Authors: *R. CHAN[1,2], M. GURMA[1,2], A. DARBANDI[3], R. L. SCHALEK[5], J. W. LICHTMAN[5], W. S. TRIMBLE[4], M. ZHEN[1,3,6], L.-Y. WANG[1,2];
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Abstract: Sensory experience and cognitive ability are made possible by the diverse strength and plasticity of synapses in the brain. Yet, we lack a fundamental understanding of the mechanisms underlying functional synaptic heterogeneity. We address this at the mature glutamatergic calyx of Held synapse in the mouse brainstem (P16-19, either sex), where morphologically diverse nerve terminals contain 3-4 main stalks, but variable number of bouton-like swellings. Our previous work suggests that the filamentous protein Septin 5 (Sept5) differentiates spatial coupling of calcium influx to neurotransmitter release at active zones (AZs) in stalks and swellings, diversifying whole-terminal release probability (Pr). Sept5 is known to bind the SNARE protein syntaxin-1 to inhibit synaptic vesicle (SV) exocytosis. However, how Sept5 functions at the subsynaptic level to support heterogeneous Pr remains enigmatic. We hypothesize that Sept5 acts as a wedge that prevents the full zippering of the SNARE complex, rendering lower Pr at swellings compared to stalks. In structural approaches, we employed automated tape-collecting ultramicrotome scanning electron microscopy (ATUM-SEM), reconstructing calyx terminals in 3D to compare presynaptic AZ ultrastructure, and super-resolution expansion microscopy (ExM) to compare postsynaptic AMPA receptor topography at stalks and swellings. In functional approaches, we performed quantal analysis of miniature excitatory postsynaptic currents (mEPSCs) from patch clamp recordings at morphologically diverse WT and Sept5 KO synapses. Additionally, we designed two membrane-permeable TAT-peptides conjugated with putative binding motifs from Sept5 to disrupt its interaction with SNAREs. Preliminary EM analysis showed fewer docked SVs, smaller docked SV size, and less compound SVs at swelling AZs. ExM showed potentially smaller AMPA receptor cluster size at swellings. mEPSC analysis revealed that quantal size was inversely correlated with the number of swellings, and this correlation was eliminated at Sept5 KO synapses. One of two peptides mirroring Sept5 interface motifs altered the quantal size distribution in WT but not Sept5 KO synapses. Our findings implicate Sept5 as a key molecular substrate that enables bimodal release modalities within the same synapse. Varying the proportion of these modalities diversifies both evoked and spontaneous forms of neurotransmission, expanding coding capacity within a single synapse population. Ongoing nanoscopic analyses and molecular tests will elucidate the precise mechanisms of Sept5 in pivoting synaptic heterogeneity.

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Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

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Topic: B.04. Synaptic Transmission

Support: NIH K01 Grant to HL
NSF/IOS Grant to HL

Title: Differential overexpression of the vesicular acetylcholine transporter and its effects on organismal survival and locomotion during the lifespan in Drosophila

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Abstract: Impairment in cholinergic neurotransmission is associated with normal and pathological aging, making cholinergic release a subject of sustained interest. The vesicular acetylcholine transporter (VAChT) is present in many species and is responsible for the packaging of ACh for exocytotic release. Although there is a plethora of knowledge about the molecular machinery that regulates ACh, the exact manner in which VAChT, an essential component of ACh regulation, alters ACh-linked neuronal function is not fully understood. Here, we use the overexpression of VAChT as a tool to increase the amount of ACh released into the synaptic cleft. And we are measuring the effect of that altered state on synaptic activity using two key behavioral circuits, locomotion and cognition. Previously, we showed that vast increases in VAChT expression cause severe behavioral deficits, including a sharp decline in lifespan. Our current study is focused on testing the hypothesis that more moderate increases in VAChT expression will not only lead to less severe effects but also beneficial ones. To test this idea, we screened eight VAChT overexpressing lines with varying levels of increased expression. We report the intriguing results that while strong increases in VAChT produced a corresponding decrease in lifespan, a less drastic overexpression of the protein led to a less steep decline in lifespan. Moreover, we show that in agreement with our previous published findings, our preliminary data indicate that VAChT overexpression caused an age-dependent decrease in locomotion ability in all lines tested. Further, immunohistochemical analysis showed that at least one VAChT overexpressor showed a strong increase in localization of the protein to punctae in cholinergic neurons of the optic lobe, indicative of increased presence in synaptic vesicles. We report findings from a super resolution microscopic analysis of these stained specimens to measure effects of differential VAChT overexpression on synapse structure. We also present measure of acetylcholine level in flies that differentially overexpress VAChT compared to controls. Taken together, these data indicate that morphological and behavioral effects of

VAChT overexpression are driven by the levels of the protein's expression and inform further studies aimed at identifying precisely which dial in VAChT expression could lead to a beneficial effect on synaptic neurotransmission.

Disclosures: K. Rosikon: None. **A. Atheby:** None. **H.O. Lawal:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

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Topic: B.04. Synaptic Transmission

Support: R01NS099457
P30DA056410
NS131728
RRF-2.3.1-21-2022-00011

Title: Nanoscale molecular basis of the synaptic cannabinoid tone

Authors: *B. BARTI[1], B. DUDOK[2], K. KENESEI[3], M. ZÖLDI[4], V. MICZÁN[5], G. Y. BALLA[6], D. ZALA[7], C. SAGHEDDU[8], M. KISFALI[9], B. TOTH[10], M. LEDRI[11], E. VIZI[12], M. MELIS[13], L. BARNA[9], Z. LENKEI[14], I. SOLTESZ[15], I. KATONA[16];
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Abstract: Different temporal scales define various forms of CB_1 cannabinoid receptor (CB_1R)-mediated synaptic plasticity. While the retrograde signaling pathway underlying the phasic forms of endocannabinoid-mediated plasticity is well-described, the molecular mechanism of tonic cannabinoid signaling remains highly debated. Here, we show that tonic CB_1R activity calibrates release probability in a target cell-dependent manner at interneuron-pyramidal cell connections in the mouse hippocampus. Unexpectedly, the synaptic cannabinoid tone remained intact in the absence of endocannabinoid-producing enzymes. Instead, by developing a workflow for physiological, anatomical, and molecular measurements at the same unitary synapse, we

demonstrate that the nanoscale stoichiometric ratio of CB_1Rs to the release machinery is sufficient to predict synapse-specific release probability. Accordingly, selective decrease of extrasynaptic CB_1Rs does not affect synaptic transmission, whereas in vivo exposure to the phytocannabinoid Δ^9-tetrahydrocannabinol disrupts the intrasynaptic nanoscale stoichiometry and reduces synaptic variability. This finding implies that individual synapses leverage the nanoscale stoichiometry of constitutively active presynaptic receptor coupling to the release machinery to optimize their synaptic weights.

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Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.23/Web Only

Topic: B.04. Synaptic Transmission

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Title: Mimicking the pathological hypomethylation of RNA binding protein FUS induces synaptic dysfunction in hippocampus CA1 neuron.

Authors: S. KIM, ***F. KONG**, M. TONEVA, J. ULE, S. J. MITCHELL, M.-D. RUEPP, K. K. CHO;
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Abstract: Fused in sarcoma (FUS) is an RNA-binding protein, which under physiological conditions mainly located in the nucleus, from where it shuttles to the cytoplasm to play key roles in RNA translation, transport, and transcription. Mutations and posttranslational modification of FUS give rise to cytoplasmic pathophysiological condensates associated with neurodegeneration (amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Specifically, in FTLD the pathological condensation of FUS is driven by the hypomethylation of arginine residues in FUS. These hypomethylated FUS condensates have been observed in multiple brain regions, including the hippocampal pyramidal layer. Collectively

indicated a role for hypomethylated FUS in inducing cognitive impairments associated with FTLD.
However, if, and how, hypomethylated FUS condensates can induce synapse weakening remains unknown. In this study, FUS constructs were engineered to mimic hypomethylation by increasing 16 arginine residues in the low complexity domain of FUS (FUS_{16R}). We then capitalise on this tool to reveal how hypomethylated FUS condensates dysregulate synaptic function in hippocampus CA1 neuron.
In organotypic hippocampus slice culture, expression of FUS_{16R} induced widespread dendritic condensates in CA1 neurons, conversely wild-type FUS (FUS_{WT}) only produced nuclear condensates. Surprisingly, the FUS_{16R} condensates exhibited spontaneous movement in the dendrites, which was enhanced upon neuronal depolarisation. Furthermore, stimulation of single dendritic spines resulted in a recruitment of FUS_{16R} condensates to the spine. It was of interest, therefore, whether the movement and synapse localisation of FUS_{16R} condensates can affect synaptic function in CA1 neuron. We observed that dendritic FUS_{16R} condensates produced a robust synapse weakening phenotype; resulting in the loss of synapses, reduced synaptic function, and deficits in local network connectivity. Furthermore, FUS_{16R} condensates impaired multiphoton glutamate uncaging induced single spine structural plasticity. Whereas overexpression of FUS_{WT} did not cause any synaptic deficits(doi: 10.1186/s40478-023-01703-w).Collectively, our study illustrates that mimicking hypomethylation of FUS resulted in the formation of dendritic condensates which were dynamic and recruited to active spines and were able to induce significant synapse dysfunction. Thereby, potentially revealing the pathophysiology associated with FUS induced neurodegenerative disease.

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Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.24/B8

Topic: B.04. Synaptic Transmission

Support: 1R35GM149211

Title: Novel role for OSBP regulating synaptic transmission in neurons

Authors: *M. CASAS PRAT, Z. KOVACS, R. E. DIXON, E. J. DICKSON;
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Abstract: The intricate functionality of the mammalian brain relies on precisely coordinated neuronal connectivity, occurring at synapses within nanometer-scale junctions. Synaptic activity heavily depends on the regulated release of neurotransmitters from presynaptic neurons to the plasma membrane of postsynaptic cells. Cholesterol has been widely reported as an essential

component in the molecular and structural organization of lipid rafts, ion channels and exocytic proteins, facilitating neurotransmitter release at these synaptic clefts. However, the precise mechanisms by which neurons modulate cholesterol levels at presynaptic nanodomains in response to external stimuli remain unclear. The Oxysterol Binding Protein (OSBP) is a key regulator of cholesterol at Endoplasmic Reticulum (ER) - Trans Golgi Network (TGN) contact sites, facilitating cholesterol transfer from the ER to the TGN in exchange for PI(4)P via interactions with ER-resident VAP proteins. Using spatial proteomic analyses, we unbiasedly determined the local interactome of OSBP in the murine brain and reported for the first time that OSBP is found in close proximity to various synaptic proteins, including Bassoon and Piccolo. Super resolution imaging of cortical neurons not only further confirmed such OSBP close localization to these presynaptic proteins, but also showed OSBP proximity to Synaptophysin, a presynaptic vesicle protein; indicating that OSBP could be a cholesterol modulating protein at presynaptic domains. Analysis of OSBP's distribution revealed it dynamically relocates to synaptic vesicles upon membrane depolarization. Notably, inhibiting OSBP or disrupting its interaction with ER-resident VAP proteins impairs neurotransmitter release and synaptic vesicle fusion, as demonstrated by transfecting neurons with a GFP-tagged synaptic vesicle release indicator, Synapto-pHluorin. These findings underscore the critical role of OSBP in neurotransmitter release and neuronal excitability control, offering insights into potential therapeutic strategies targeting OSBP to address disorders of dysfunctional neuronal excitability.

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Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

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Topic: B.04. Synaptic Transmission

Support: National Natural Science Foundation of China (Key Project), Grants: 32130044

Title: Functional autapses selectively develop in pyramidal neurons of the hippocampal subiculum

Authors: *X. PAN[1,2], W. KE[3,4], Y. SHU[3,4];
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Abstract: Autapses are synaptic contacts formed by a single neuron between the axon and its own soma or dendrites. In the neocortex, autapses selectively form in certain types of GABAergic inhibitory interneurons and glutamatergic pyramidal cells (PCs). They provide self-inhibition in inhibitory interneurons and self-excitation in PCs, regulating the spike timing in interneurons and firing pattern (such as burst firing) in PCs. Similar morphological and electrophysiological features are observed in hippocampal PCs, it remains unclear whether they form functional autapses. Here we performed whole-cell recordings from both hippocampal and neocortical PCs in C57 mouse (aged P5-70) brain slices perfused with artificial cerebrospinal fluid containing 8 mM $SrCl_2$, which allows the detection of autaptic responses by postponing the release of neurotransmitter glutamate. To our surprise, autaptic responses are only observed in a subpopulation of subiculum PCs (30-60%), but not in CA1, CA2, and CA3 PCs. Consistently, analysis of the axon and dendrite morphology reveals that, as compared to PCs in the other hippocampal regions, PCs in the subiculum exhibit greater morphological complexity, with extensive overlap between the axonal and dendritic fields. Additionally, we explore the emergence of subiculum autapses in PCs during development, and find that they develop earlier compared to those in the medial prefrontal cortex (mPFC). Hippocampal subiculum autaptic responses can be detected in P5, the youngest mice in our experiments, whereas those in the mPFC are not typically recorded until P10. Moreover, similar to those in the mPFC, autapses in subiculum PCs also exhibit projection specificity, with nucleus accumbens (NAc)-projecting PCs (~45.7%) have a higher probability of forming autapses compared to the amygdala-projecting PCs (~6.7%). Together, our results indicate that glutamatergic autapses selectively form in a subgroup of subiculum PCs from early developmental stages to adult, suggesting that they could provide selfexcitation in these PC populations and participate in hippocampal information processing.

Disclosures: X. Pan: None. **W. Ke:** None. **Y. Shu:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.26/B10

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

Support: UNAM-PAPIIT-IG200121
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UNAM-PASPA-BECA SABATICA
CONAHCYT-BECA SABATICA
FULBRIGHT-GARCIA ROBLES FELLOWSHIP
NIMH-IRP NIH MH002386

Title: A Calyx-like synapse in the central amygdala with unique neurochemical features

Authors: *L. ZHANG[1], V. S. HERNANDEZ[1], D. GIRALDO-GOMEZ[2], S. Z. JIANG[3], J. LEON CONTRERAS[4], R. HERNANDEZ PANDO[5], F. FERRAGUTI[6], L. E. EIDEN[3]; [1]Physiology, Sch. of Med., Natl. Autonomous Univ. of Mexico, Mexico City, Mexico; [2]Unit of Microscopy, Natl. Autonomous Univ. of Mexico, Mexico City, Mexico; [3]Section on Mol. Neurosci., Natl. Inst. of Mental Hlth., IRP, NIH, Bethesda, MD; [4]Exptl. Pathology, Natl. Inst. of Med. Sci. and Nutr. "Dr. Salvador Zubiran", Ciudad de México, Mexico; [5]Exptl. Pathology, Natl. Inst. of Med. Sci. and Nutr. "Dr. Salvador Zubiran", Mexico, Mexico; [6]Pharmacol., Innsbruck Med. Univ., Innsbruck, Austria

Abstract: Using immuno-electron microscopic methods (FIBSEM-TEM) we have previously reported PACAPergic calyceal axon terminals in the ventro-lateral capsular subdivision of the central amygdala (Zhang et al, SfN 2021) in rat. To further investigate the origin and molecular constituents of these peculiar calyceal terminals, we used fluorogold retrograde and AAV5-EF1a-DIO-hChR2(H134R)-eYFP anterograde tracing in Adcyap1-2A-cre mice and freeze-fracture replica immunolabeling (FRIL) to investigate its molecular signature for both presynaptic and postsynaptic components. We report here that this Calyx of Held-like giant synapse terminal has mixed glutamatergic and cholinergic synaptic specifications (Gray type I and II), seen for the first time, at the ultrastructural level, in a telencephalic structure. CeC PKCd neurons, co-expressing VGAT, PAC1, and VPAC2, have been identified as the main post-synaptic target cells. To identify the neuronal population serving as the presynaptic source of this synapse, we studied first the molecular features of the axon terminal, which is characterized by the co-expression of multiple neurotransmitter vesicular transporters, including VGLUT1, VGLUT2 and VAChT, as well as the neuropeptides CRGP and NTs, and calretinin. Fluorogold (FG) retrograde tracing from CEA labeled a cellular subpopulation in the brainstem parabrachial/Kölliker-Fuse (KF) complex expressing the mRNAs Slc17a7, Slc17a6, Chat, Adcyap1, Calb2, Calca and Nts. Highly restricted Cre-dependent eYFP anterograde viral vector labeling within this region of the parabrachial complex, in PACAP-cre mice, revealed Calyx-like ring patterns in CeC. FRIL employed in tissue specimens from these mice localized glutamatergic AMPA receptor in apposition to GFP in amygdalar synaptic structures. AMPA receptor immunoreactivity was localized post-synaptically in relationship to YFP immunoreactivity suggesting a glutamatergic axosomatic relationship between these PACAPergic projection neurons and their targets in central amygdala. Our observations suggest that signaling from brainstem PB/KF complex to amygdala occurs via a unique synaptic connection characterized by high-fidelity transmission, yet diverse regulation, potentially contributing to general behavioral adaptation to aversive stimuli.

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Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.27/B11

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

Support: NSF Grant 2131750
NIH Grant # P20 GM103446

Title: Neuronal Vesicular Acetylcholine Transporter Expression Alters Acetylcholine Dynamics in Drosophila

Authors: *R. NEMAT[1], A. ATHEBY[2], K. ROSIKON[2], H. O. LAWAL[3];
[1]Delaware State Univ., Dover, DE; [2]Biol. Sci., Delaware State Univ., Dover, DE; [3]Biol., Delaware State Univ., Dover, DE

Abstract: Acetylcholine (ACh) neurotransmission is necessary for the regulation of essential life functions such as locomotion and cognition. As a result, increases or decreases in neuronal cholinergic signaling lead to an impairment in learning and memory, and normal locomotive functions. Although much about how acetylcholine is regulated is known, the mechanism through which changes in cholinergic signaling effects changes in ACh-linked behavior is not fully understood. Here, we are interested in using the vesicular acetylcholine transporter (VAChT), which mediates the packaging and transport of acetylcholine (ACh) for exocytotic release, as a tool to understanding the mechanism through which acetylcholine release is regulated. We use both an overexpression of VAChT (using a construct that we have reported on previously) and mutants in *Vacht* that cause varying decreases in the gene's expression, to increase or decrease (respectively) the amount of ACh released into the synaptic cleft. And we are measuring the effect of that altered state on synaptic activity using a biochemical approach. We have optimized an assay that allows us to reliably measure cholinergic pathway components ACh and choline from as little as five *Drosophila* heads. Using this assay, we report the finding that consistent with its role in mediating ACh release, VAChT overexpression lines have an elevated total head ACh levels. Moreover, we report a significant increase in choline, the byproduct of the extracellular breakdown of ACh. In VAChT overexpressing flies, to determine the effect of altered VAChT expression of ACh level in vivo, we conducted an immunohistochemistry assay to determine the spatial localization of ACh and Vacht in both Vacht mutants and overexpressed specimens, using a spinning disk confocal microscopy and we found significantly elevated ACh staining at or near the plasma membrane of cholinergic neurons, as well as strong co-localization with Bruchpilot, an active zone marker. Our data aligns with the results of our neurochemistry assay, indicating an elevated level of Ach in the overexpressed line and reduced level of Ach in mutant line. Collectively, these findings offer significant insights into the outcomes of VAChT expression disruptions and enhance our understanding of how the vesicular acetylcholine transporter facilitates the exocytotic release of ACh in Drosophila.

Disclosures: R. Nemat: None. **A. Atheby:** None. **K. Rosikon:** None. **H.O. Lawal:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR149.28/B12

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

Support: Palm Health-Sponsored Program in Computational Brain Science and Health

Title: Detection of biphasic synaptic currents from single vesicle release using machine learning

Authors: *C. CEBALLOS[1], R. D. PENA[1,2];
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Abstract: The traditional view of synaptic physiology established that most neurons release only one neurotransmitter or neuromodulator. With the advance of experimental techniques, it has recently been identified that a small percentage of neurons can release more than two neurotransmitters, a phenomenon known as co-transmission. Among them, an interesting case is where glutamate and GABA are co-transmitted. A question that remains largely unexplored is the existence of co-release, i.e., the release of both neurotransmitters packaged in the same synaptic vesicle. A particular technique used for these purposes is electrophysiological recordings of synaptic currents. Two methods have been proposed: inducing single vesicle release using optogenetic stimulation of single synaptic terminals or recordings of spontaneous release from single vesicles (i.e., miniature events or minis). The analyses and identification of these minis are difficult because amplitudes are small, sometimes close to the recording noise (~5 pA). To identify the spontaneous release of neurotransmitters from single presynaptic vesicles, recordings are performed holding the neuron voltage as far as possible of the reversal potential of the receptor, which increases the driving force and the amplitude of the event. However, this is not possible for the cases of biphasic minis derived from GABA/glutamate co-release. The rationale is that the recording must be at voltages between the reversal potential of AMPA and $GABA_A$ receptors (i.e., approximately -30 mV). This results in events with smaller amplitude than those from conventional recordings, impairing detection. We overcome this limitation by training an artificial neural network that identify biphasic minis embedded in electrical noise. The models are trained and validated using a standard 80-20 split of the samples from actual patch-clamp recordings. The samples were increased in number with data augmentation techniques. We fine-tuned the models' hyperparameters, including the number of layers and neurons, kernel size, learning rate, and choice of optimizers. Techniques such as dropout, penalties, and early stopping were employed to mitigate overfitting. The performance detection by this new method was similar to that obtained by a human observer but with the significant advantage of taking just a few seconds compared to almost 20 min by the human observer.

Disclosures: C. Ceballos: None. **R.D. Pena:** None.

Poster

PSTR149: Presynaptic Mechanisms, Organization, and Structure

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Topic: B.04. Synaptic Transmission

Support: NTU (113L893104)
NSTC (NSTC 112-2311-B-002-007-MY3)

Title: The roles of PKA-mediated SNAP-25 phosphorylation in regulating calcium-dependent exocytosis

Authors: *Y.-L. SU, C.-T. WANG;
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Abstract: Calcium-dependent exocytosis involves a series of steps, such as docking, priming, calcium entry, and fusion, involving the assembly and disassembly of fusion machinery, i.e., the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complex, including Synaptobrevin (mainly Syb2), Syntaxin (mainly Stx1), and SNAP-25 (synaptosome-associated protein of size 25 kDa; mainly SN25b). Upon calcium entry, the calcium sensor protein, Synaptotagmin (mainly Syt1), binds to multiple calcium ions and SNARE complex to trigger membrane fusion. Moreover, during transient oscillations of cell signaling, SNARE proteins may undergo phosphorylation via oscillations of kinase activity, such as protein kinase A (PKA). Only SN25 undergoes PKA-mediated phosphorylation among SNARE proteins at threonine 138 (T138). Our previous studies suggest that PKA-mediated SN25b phosphodeficiency destabilizes exocytotic fusion pores. However, it remains unclear how PKA-mediated SNAP-25 phosphorylation alters SNARE formation. In this study, we examine how PKA-mediated SN25 phosphorylation regulates SNARE interaction. We overexpressed the control vector (Ctrl), wild-type SN25b (SN25b), or its PKA-phosphodeficient mutant (SN25b-T138A) in secretory cells, PC12 cells. Proximity-ligation assays (PLA) were used to determine the interaction of SNAREs after 1-min treatment of KCl in the presence or absence of forskolin (FSK), an adenylate cyclase activator. As a result, additional FSK increased the SN25-Stx1 interaction in control cells compared to applying KCl alone. Moreover, additional FSK increased the SN25-Stx1 interaction in SN25b-overexpressing cells compared to KCl alone. By contrast, cells overexpressing SN25b-T138A did not show an increase in the SN25-Stx1 or SN25-Syb2 interaction upon additional FSK application compared to KCl alone. In addition, the levels of SN25-Syt1 interaction remained similar among all groups. These results suggest that PKA-mediated SNAP-25b phosphodeficiency may reduce its interaction with the other SNAREs, Stx1 and Syb2. Thus, PKA-mediated phosphorylation of the release machinery is sufficient to rapidly regulate the capability of SNARE assembly.

Disclosures: Y. Su: None. **C. Wang:** None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR150.01/B14

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Title: Generating in vitro genetic epilepsy models for functional phenotypic discovery

Authors: *J. ROBERTS, A. LIU, B. FAIRES, H. NAMI, S. SEE, J. SERRATS, A. KAYKAS, R. BAROR, A. OLIVEIRA PISCO, C. O'HARA;
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Abstract: We used an RNP based engineering system on iPSC-derived cortical neurons to generate pooled screening compatible genetic epilepsy disease models (such as KCNQ2 encephalopathy) for MEA phenotyping and functional validation. Our results using the commercially available Neural Metrics software showed that KCNQ2-KO neurons exhibited increased mean firing rate and number of bursts compared to WT neurons. In addition, we used linear modeling to reveal KCNQ2-KO specific phenotypes. These methods showed clear separation between KCNQ2-KO and WT with top features being burst and network-burst level features on high dimensional readouts.To further validate our model, we explored causal disease biology of KCNQ2 encephalopathy and tested multiple compounds from the literature in our KCNQ2-KO disease model. For example, functional enhancement of small conductance(SK) and big conductance(BK) Ca-mediated K channels have been shown to contribute to the in vitro KCNQ2 encephalopathy bursting phenotype. Using an automated, high-throughput, and low volume dosing technique, we tested the effects of Apamin (an SK channel antagonist) and Paxilline (a BK channel antagonist) on KCNQ2-KO and WT neurons. Our results indicate that Paxilline addition leads to a significant decrease in numerous neural metrics in a dose-dependent manner, achieving both functional validation and phenotypic reversion of our KCNQ2-KO disease model. In conclusion, we generated a cellular disease model for KCNQ2 encephalopathy in MEA showing phenotypic relevance and susceptibility to known reversion compounds. In the future, we intend to use these methods to identify and assess the efficacy of novel therapeutic targets that will be discovered via the insitro target discovery platform.

Disclosures: J. Roberts: A. Employment/Salary (full or part-time):; insitro. **A. Liu:** A. Employment/Salary (full or part-time):; insitro. **B. Faires:** A. Employment/Salary (full or part-time):; insitro. **H. Nami:** A. Employment/Salary (full or part-time):; insitro. **S. See:** A. Employment/Salary (full or part-time):; insitro. **J. Serrats:** A. Employment/Salary (full or part-time):; insitro. **A. Kaykas:** A. Employment/Salary (full or part-time):; insitro. **R. Baror:** A. Employment/Salary (full or part-time):; insitro. **A. Oliveira Pisco:** A. Employment/Salary (full or part-time):; insitro. **C. O'Hara:** A. Employment/Salary (full or part-time):; insitro.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR150.02/B15

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: RO1 DC018797

Title: Sound-derived plasticity and heterogeneity of MNTB principal neurons in the mouse auditory brainstem

Authors: *H.-G. BAE, J. KIM;
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Abstract: The medial nucleus of the trapezoid body (MNTB) is a key component of the superior olive complex (SOC) in the mouse auditory brainstem, critical for sound localization. MNTB principal neurons have traditionally been characterized by their single/phasic spiking behavior in response to depolarizing current injections, which allows for high-frequency action potential generation with temporal fidelity. Interestingly, our recent findings reveal notable heterogeneity in these spiking patterns. Approximately 10% of post-weaning (P20) MNTB neurons exhibit a tonic/bursting spiking pattern at a frequency of ~120 Hz when subjected to 300 pA depolarizing current injection, independent of their position along the tonotopic axis. Utilizing the Patch-seq technique, which combines electrophysiological recordings with single-cell sequencing, we discovered that MNTB neurons with different spiking behaviors (single/phasic vs. tonic/bursting) do not show strong genetic differentiation, indicating they belong to the same genetic cell type. Interestingly, Patch-seq data analysis showed a reduction of *Kccn2*, encoding SK channel, in tonic/bursting neurons compared with single/phasic firing neurons, suggesting that SK channel plays a role in determining firing patterns in MNTB neurons. Furthermore, we studied whether this heterogeneity is attributable to neuronal plasticity rather than a heterogeneous cell population. Notably, sound experience during the critical period (P13-P19) modifies the heterogeneity of physiological properties, resulting in increased MNTB neurons displaying tonic/bursting spiking (10% to 40 increased). This suggests that the spiking pattern heterogeneity in MNTB neurons is associated with sound-evoked neural activity in the auditory brainstem. Our results challenge the traditional view of MNTB neurons as physiologically static, proposing instead that they are dynamically plastic in response to sound stimulation and play a role in processing auditory information.

Disclosures: H. bae: None. **J. Kim:** None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR150.03/B16

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: Illinois State University Firebird and Birdfeeder grants

Title: Inter-individual differences and adaptive resilience against spreading depolarization

Authors: *E. NELSON[1], G. CROWE[1], W. STEIN[2], A. HARRIS[3];
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Abstract: Spreading depolarization (SD) is a slowly propagating wave of neuronal hyperexcitability that travels across large areas of the cortex in disorders such as ischemia, stroke, and traumatic brain injury. Accompanied by swelling of neurons, large changes in ion homeostasis, and neuronal inactivity (spreading depression), SD can have debilitating consequences even in otherwise healthy brains. Its effects are probably best known for causing visual auras that precede the headache pain in about 30% of all migraineurs. While SD propagation has been studied for decades, key questions about its initiation remain, including whether there are similarities or differences in how SD is elicited between individuals, whether repeated SD events facilitate or diminish the likelihood of subsequent SD events, and whether SD originates from a singular location or multiple sites. To address these questions, we have developed a high-throughput assay using fluorescent calcium imaging of SD in adult and larval fruit flies (*Drosophila melanogaster*) with pan-neuronal GCaMP expression. Rapid cooling from room temperature to 0^{o}C reliably elicited SD in adult brains and larval nervous systems. On average, SD occurred at 4.6±2.9°C (N=8) in adults and at 3.2±2.0°C (N=30) in larvae. However, there was substantial inter-animal variability in the temperature at which SD was elicited (range: 1.7 - 9.7°C in adults, 0.2 - 9.3°C in larvae). Inter-individual differences were directly tested in a paired approach that measured the temperature of SD occurrence in several (3 - 6) L2 larvae under identical conditions. We found a significant interaction between SD temperature and animal identity ($P<0.001$, One Way RM Anova), suggesting that inter-individual differences in resilience against SD exist. Through repeated coolings that elicited SD, we found that increasingly colder temperatures were required to elicit subsequent SD events ($P<0.001$, RM Anova on Ranks, $\chi^2 = 31.22$). For example, the first SD event occurred on average at 3.2±2.0°C (N=30), while the fifth repetition required cooling to 0.4±1.0°C (N=10). The need for a significantly stronger temperature perturbation suggests that repeated SD events may make the nervous system more resilient against future SD events. Finally, by tracking SD spread in adult brains, we found that SD was on average elicited at 3.5 distinct initiation sites per animal (N=6). The location of the leading initiation point varied across animals, suggesting that SD initiation is individualized. Overall, our results indicate that SD provides adaptive resilience against future SD events and originates in multiple brain areas that vary between animals.

Disclosures: E. Nelson: None. **G. Crowe:** None. **W. Stein:** None. **A. Harris:** None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR150.04/B17

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: KAKENHI 21K06434, 24H02336

Title: Simulation test for depolarization-assisted unblock of spike propagation along hippocampal mossy fiber

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Abstract: Reliable propagation of action potentials along the axons is supported by dense expression of voltage-dependent Na^+ channels on the axonal membrane. Hippocampal mossy fibers consist of *en passant* axons with multiple large boutons, and therefore the conduction might be potentially blocked at the large and less excitable boutons where the safety factor for spike propagation is presumed to be low. Although the experimental study with direct electrophysiological recordings revealed the presence of high-level Na^+ channels in a majority of boutons, it is also demonstrated that some boutons express fewer Na^+ channels and therefore are the possible sites for the conduction block. In this study, the possibility of conduction block at large and less excitable boutons was tested with computer simulation using the model of hippocampal mossy fiber. Assuming the equal size of boutons throughout the mossy fiber axon, action potential triggered by stimulation at the soma reliably propagated orthodontically to the distal end of the axon even though the Na^+ conductance was removed from one bouton. If the size was increased to larger than twice in one bouton, conduction was blocked by the removal of Na^+ conductance from the bouton. It should be noted that slight depolarization at the bouton restores conduction over the site of the block. These findings suggest that the variety of Na^+ conductance levels among boutons may potentially cause the conduction block at the large and less excitable bouton, and unblock by local small depolarization due to changes in the microenvironment surrounding the axon. This form of depolarization-assisted unblock at the large less excitable bouton may serve as a gatekeeper of information flow through the neuronal network.

Disclosures: H. Kamiya: None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

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Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIH Grant R01-NS126043
NIH Grant R01-MH117042
HBI Postdoc Pioneers Grant

Title: Optical electrophysiology reveals the role of cAMP on dendritic computations and associative plasticity in the hippocampus

Authors: *P. PARK[1], B. LEE[2], X. WU[2], D. WONG-CAMPOS[2], A. E. COHEN[2,3];
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Abstract: Neuromodulatory systems affect the formation and stability of memory. cAMP, a key molecular mediator of neuromodulation, plays a critical role in learning and memory.[1] The precise mechanisms underlying these effects remain unclear. Here we studied the role of cAMP on single-neuron computations in hippocampal brain slices using optogenetic modulation of cAMP and dendritic voltage imaging. We co-expressed Voltron2, a chemigenetic voltage indicator,[2] and bPAC, a light-sensitive cAMP generator,[3] in a sparse subset of mouse hippocampal CA1 neurons.[4] We developed a new instrument that combines micromirror-patterned blue (488 nm) and orange (594 nm) illumination for stimulation and readout of membrane potentials, and two-photon (2P) structural imaging. We observed a slow increase in subthreshold membrane depolarization (< 10 mV in 30 sec) triggered by bPAC stimulation, and an associated increased probability of plateau potentials in dendrites and complex spikes at the soma. We are now measuring the effect of cAMP on dendritic excitability in live animals using microprism-based imaging and a virtual reality system to understand the role of cAMP in dendritic function, associative plasticity, and place cell formation *in vivo*.
1. Kandel, E. R. The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. *Mol Brain* **5**, 14 (2012); 2. Abdelfattah, A. S. *et al.* Sensitivity optimization of a rhodopsin-based fluorescent voltage indicator. *Neuron* **111**, 1547-1563 (2023); 3. Stierl, M. *et al.* Light modulation of cellular cAMP by a small bacterial photoactivated adenylyl cyclase, bPAC, of the soil bacterium Beggiatoa. *J.Biol.Chem.* **286**, 1181-1188 (2011); 4. Park, P. *et al.* Dendritic voltage imaging reveals biophysical basis of associative plasticity rules. BioRxiv (2023).

Disclosures: P. Park: None. **B. Lee:** None. **X. Wu:** None. **D. Wong-Campos:** None. **A.E. Cohen:** None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

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Program #/Poster #: PSTR150.06/B19

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: Ministerio de Ciencia e Innovacion, Spain Project PID2021-123994NB-C21
AGAUR 2021 SGR 00606
ICREA ACADEMIA

Title: Neural coding of weak periodic inputs into symbolic temporal spike patterns

Authors: M. MASOLIVER[1], J. TIANA ALSINA[2], ***C. MASOLLER**[1];
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Abstract: Neuromorphic photonics is an ultrafast and energy-efficient optical computing paradigm that can revolutionize information processing and artificial intelligence systems.
To develop photonic neural networks, we need to identify laser systems that emit optical pulses that precisely mimic neuronal activity, and implement in these lasers the coding mechanisms used by neuronal populations to process information, in particular, to process weak inputs in noisy environments.
We have shown that in stochastic single-neuron models (FitzHugh Nagumo and Morris Lecar) the relative timing of spikes can encode information from a weak, subthreshold sinusoidal input. Using a symbolic method for analyzing spike sequences, known as ordinal analysis, we have found more expressed and less expressed spike patterns, whose frequency of occurrence in the spike sequence varies with both the amplitude and frequency of the input.
Thus, these findings suggest that the frequencies of the different spike patterns in the spike sequence fired by a neuron in response to a weak sinusoidal input, may encode information of the input.
In this study, we analyze if neuronal populations are also capable of using this encoding mechanism and whether it can be implemented in a diode laser that emits optical pulses whose statistical properties are equivalent to neuronal spikes.
We find that neuronal populations are able to encode weak sinusoidal inputs into symbolic spike patterns and, furthermore, can encode weak inputs that cannot be encoded by one or a few neurons. Importantly, we found that a few random connections facilitate the encoding of weak inputs.
Taken together, our findings suggest that encoding weak input information into the frequencies of occurrence of spike patterns is a coding mechanism that can be used by neuronal populations, exploiting the presence of noise.
We also present experiments performed with a diode laser and characterize the statistical properties of the optical spike patterns and the spike rate, when sinusoidal signals of different frequencies are added to the laser current. Our analysis reveals similarities, but also differences, with spike sequences generated by the stochastic FitzHugh Nagumo model. Using ordinal analysis and machine learning, we found that sequences of optical spikes emitted in response to low- or high-frequency inputs tend to be located in different regions of a three-dimensional feature space, suggesting that some information about the signal that was applied to the laser current can be recovered from the analysis of the emitted spike sequence. This finding can lead to a new neuro-inspired way of information coding using sequences of optical spikes.

Disclosures: M. Masoliver: None. **J. Tiana Alsina:** None. **C. Masoller:** None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

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Program #/Poster #: PSTR150.07/B20

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIH-NINDS Grant NS062771

Title: A lower induction threshold for intrinsic than for synaptic plasticity in L2/3 pyramidal neurons of the mouse primary somatosensory cortex

Authors: *D. HUANG, C. HANSEL;
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Abstract: On the single-cell level, synaptic and nonsynaptic adaptions are the principal mechanisms of neuroplasticity. Although synaptic mechanisms that mediate synaptic strength (SS) and nonsynaptic properties that define intrinsic excitability (IE), i.e., membrane excitability directly shape functional output, whether SS and IE are equally susceptible to experience-induced modulation remain poorly understood. The current study uses whole-cell patch clamp recordings to investigate the induction threshold for long-term synaptic vs. intrinsic plasticity in somatosensory cortex (S1) pyramidal neurons in layers 2/3. The induction threshold is identified as the minimum duration of synaptic or somatic tetanization at 50 Hz to incite long-term changes in SS or IE, respectively. As a benchmark for neuronal plasticity, we applied synaptic tetanization to identify that 1-second tetanus was sufficient to produce long-term IE potentiation. Somatic tetanization required 7 seconds to incite a persistent potentiation in IE. Synaptic plasticity had the highest induction threshold, and sustained SS potentiation was not observed until after 10 seconds of synaptic tetanization. Furthermore, muscarinic acetylcholine receptor activation has been shown to contribute to cholinergic-mediated modulation of neuroplasticity through potentiating N-methyl-D-aspartate receptor activity or inhibiting activation of calcium-activity small conductance potassium channels, respectively; therefore, we investigated whether it may also play a role in regulating the induction threshold of synaptic and intrinsic plasticity. Not surprisingly, pharmacological activation of muscarinic receptors by bath application of Oxotremorine M (7 μM) during tetanization decreased the induction threshold for synaptic and intrinsic plasticity. Our results provide evidence showing an asymmetry in the induction threshold that disproportionately skewed lower towards intrinsic plasticity compared to synaptic plasticity. In addition, muscarinic acetylcholine receptor-mediated M-type potassium current may play an essential role in modulating molecular mechanisms that regulate the induction threshold in neuroplasticity. Collectively, these results provide new insight suggesting a discrepancy in synaptic and intrinsic plasticity thresholds that crucially govern mechanisms in learning and memory.

Disclosures: D. Huang: None. **C. Hansel:** None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

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Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIH NINDS Grant R35 NS097185
NIH NINDS Grant F31 NS127499
NIH Grant T34GM145507

Title: Slow spike-frequency adaptation in mouse external globus pallidus neurons

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Abstract: External globus pallidus (GPe) neurons fire continuously and inhibit each other with a continuous barrage of synaptic inputs. This inhibition de-regularizes GPe firing but has a smaller than expected effect on firing rate. Likewise, GPe neurons signal transient changes in their inputs with transient changes in spike timing but have stable average rates over a range of input conditions. We injected GPe neurons, in mouse brain slices, with 20 second depolarizing or hyperpolarizing currents to compare their transient firing rate change (at the onset and offset of currents) to the steady state response to the same input. GPe neurons exhibited four types of slow spike-frequency adaptation in response to current injection. Firing rate increased and then slowly decayed in response to a depolarizing current - classical spike-frequency adaptation. At the offset of a depolarizing current, firing rate decreased below baseline and slowly recovered. During a hyperpolarizing current, rate initially decreased and then partly recovered while the current remained. At the offset of a hyperpolarizing current, rate rebounded above baseline and slowly decayed back. Through all this, GPe neurons remained oscillators whose responses were governed by their phase resetting curves (PRC). Currents responsible for spike frequency adaption may alter the underlying oscillation of a neuron and change its phase-dependent sensitivity to inputs. We measured PRCs of individual GPe neurons at different rates during constant current injection. Changes in rate moderately changed PRC shape, but the average PRC was unchanged, accounting for the linear instantaneous frequency-intensity curve and its constant slope at all firing rates and levels of adaptation. The steady state frequency-intensity curve governing the response to a prolonged constant input was also linear and had a much smaller slope, indicative of the adaptation process and a reduced sensitivity of the neuron to sustained input. The adaptation process had a time constant of approximately 5 seconds, making the neurons relatively insensitive to inputs averaged over several seconds. With hyperpolarizing current $\leq$ -100 pA it was possible to silence GPe neurons for the full 20 seconds. This made it possible to test if adaptation was spike-dependent or was enhanced by hyperpolarization during periods of silence. We injected GPe neurons with long hyperpolarizing currents over varying amplitudes that halted firing and recorded the rebound response following each current. The change in the rebound firing across the range of silencing currents was small, suggesting adaptation arises primarily from spike-triggered currents.

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Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

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Program #/Poster #: PSTR150.09/B22

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: R01DA041705

Title: Nonlinear dynamic mechanisms for subpopulation specific differences in synaptic integration by midbrain dopamine neurons

Authors: *C. J. KNOWLTON[1], J. ROEPER[2], C. C. CANAVIER[3];
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Abstract: The in vivo firing patterns in midbrain dopamine (DA) neurons consists of bursts, pauses, and pacemaker-like spiking which convey reward-related and/or movement-related signals. We consider three broad categories of DA neurons: DLS-projecting, DMS-projecting, and medial shell/core projecting VTA DA neurons. We apply mathematical methods based on separation of time scales to gain insights into neural dynamics of pacing, bursting, and responses to inhibition of three different subpopulations. Two types of bursts have been observed in DA neurons in patch clamp recordings in anesthetized mice: rebound bursts that follow a large hyperpolarization and plateau bursts (Otomo et al., 2020). Two types of responses to prolonged hyperpolarization have also been observed: immediate rebound bursts and ramp responses. Burst oscillations can be understood in terms of slow variables that control transitions between an up state and a down state. These states require a regenerative current that is on in the up state and off in the down state and creates an unstable branch in the voltage nullcline. For the DA neuron models, phase plane analyses reveal that the nullcline for the slow K+ conductances (eg. SK and Kv4) together with the unstable middle branch of the voltage nullcline separate the dynamics between fast spiking and slow ramp-like responses. For the regularly pacing subpopulations, the ramp like portion of the ISI is controlled by Kv4 through the close spacing of voltage and Kv4 inactivation nullclines - creating a strongly restorative region. While the deep SK-driven AHPs of this population strongly recruits these Kv4 channels, the shallower AHPs of the less regular populations largely remain away from this restorative region, rendering those populations more susceptible to noise and specifically susceptible to plateau bursts if the AHP is reduced. Transient currents such as Ca,T along with synaptic currents such as NMDA can extend and shift the unstable middle branch, allowing for transient periods of burst firing. Simulated DLS-projecting neurons selectively exhibit rebound bursts in vitro immediately upon release from hyperpolarization, whereas the DMS and medial shell/core projecting populations respond with ramps of varying duration (longest in the VTA) preceding resumption of firing.

Disclosures: C.J. Knowlton: None. **J. Roeper:** None. **C.C. Canavier:** None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

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Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: VA Merit Award I01 BX005396
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Title: Impact of unitary synaptic inhibition on spike timing in ventral tegmental area dopamine neurons

Authors: *M. H. HIGGS, M. J. BECKSTEAD;
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Abstract: Midbrain dopamine neurons receive convergent synaptic input from multiple brain areas, which perturbs rhythmic pacemaking to produce the complex firing patterns observed *in vivo*. This study investigated the impact of single and multiple inhibitory inputs on ventral tegmental area (VTA) dopamine neuron firing in mice of both sexes using novel experimental measurements and modeling. We first measured unitary inhibitory postsynaptic currents (uIPSCs) produced by single axons using both minimal electrical stimulation and minimal optical stimulation of rostromedial tegmental nucleus (RMTg) and ventral pallidum (VP) afferents. We next used perforated-patch recordings to determine the phase resetting curve (PRC), the reversal potential for $GABA_A$ receptor-mediated IPSCs, and the average inter-spike membrane potential trajectory during pacemaking. We then combined these data to develop a phase oscillator model of a VTA dopamine neuron, simulating the effects of unitary inhibitory postsynaptic conductances (uIPSGs) on spike timing and rate. The effect of a uIPSG on spike timing was predicted to vary according to the neuron's position within the inter-spike interval (ISI), or phase. Synchronous compound IPSGs were predicted to pause dopamine neuron firing for a duration greatly exceeding the IPSG time course, but limited by sublinear summation. In contrast, more physiological asynchronous uIPSGs summate supralinearly by stalling the neuron in the depolarized portion of the firing cycle, where inhibition is most effective. Our results indicate that small fluctuations in the inhibitory synaptic input arriving from the many afferents to each dopamine neuron are sufficient to produce highly variable firing patterns like those observed *in vivo*.

Disclosures: M.H. Higgs: None. **M.J. Beckstead:** None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

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Program #/Poster #: PSTR150.11/B24

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Title: Intrinsic rebound burst excitability is unique among dopamine midbrain neurons for those projecting to the dorso-lateral striatum

Authors: *S. STOJANOVIC[1], C. J. KNOWLTON[2], R. EGGER[1], C. C. CANAVIER[2], J. ROEPER[1];
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Abstract: Projection-defined subpopulations of midbrain dopamine (DA) neurons exhibit significant variability in their rebound properties. *In vitro* patch-clamp experiments revealed that only dorsolateral striatum (DLS)-projecting DA SN neurons displayed intrinsic rebound bursting (> 10 Hz, 2-3 APs, mean rebound delay 86ms, 3-fold frequency gain compared to background rate, n = 20, N = 3). In contrast, DA neurons projecting to the dorsomedial striatum (DMS) and lateral shell of the nucleus accumbens (lNAc) returned to their pacemaker rate (frequency gain < 2) after variable delays. We investigated the biophysical mechanisms underlying rebound bursting in DLS-DA SN neurons. We found that bath application of a selective T-type Ca^{2+} channel inhibitor (70 µM, NNC 55-0396) eliminated rebound bursting by significantly reducing rebound gain (3-fold) and prolonged rebound timing (mean rebound frequency [RF]: 4.11Hz, mean rebound delay [RD]: 192ms, n = 13, N = 4). In contrast, the T-type Ca^{2+} channel inhibitor had less effects on rebound gain and delay in DMS- and lNAc-projecting DA SN neurons (DMS: RF: 2.51Hz, RD: 461ms, n = 10, N = 3; lNAc: RF: 1.81Hz, RD: 653ms, n = 15, N = 3). Moreover, inhibition of SK3 channels (300nM, apamin) amplified the differences in rebound gain between DLS-DA and the other two projections, while exhibiting lower effect on rebound timing (DLS: RF: 26.43Hz, RD: 151ms, n= 19, N= 3; DMS: RF: 5.91Hz, RD: 443ms, n= 23, N= 6; lNAc: RF: 3.68Hz, RD: 685ms, n= 11, N= 3). In contrast, K_v4 channel inhibition (1µM AmmTX3) removed projection-specific differences in rebound gain and timing (DLS: RF: 4.02Hz, RD: 34ms, n= 15, N= 3; DMS: RF: 6.05Hz, RD: 48ms, n= 10, N= 3; lNAc: RF: 5.19Hz, RD: 25ms, n= 10, N= 3). Furthermore, our investigation revealed a strong influence of tonic D_2- and $GABA_B$-mediated signaling (G_i-coupled GPCRs) in preventing rebound bursting. These effects were mediated via open GIRK-channels in DLS-projecting DA SN neurons. In addition, we performed computational modeling using realistic morphologies of SN DA neurons combined with our *in vitro* findings. After confirming intrinsic differences linked to specific projection sites, we tested how these neurons might integrate synaptic signals in a simulated, balanced in vivo state. Transient synaptic $GABA_B$-sIPSC induced similar degrees of transient membrane hyperpolarization in all three models of projection-specific SN DA neurons. However, a substantial increase in rebound firing following the termination of synaptic $GABA_B$ inhibition was only observed in DLS-DA neurons. To test these model predictions in the intact brain, we are currently carrying out *in vivo* patch-clamp recordings in defined DA SN neurons.

Disclosures: S. Stojanovic: None. **C.J. Knowlton:** None. **R. Egger:** None. **C.C. Canavier:** None. **J. Roeper:** None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR150.12/B25

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Title: Nmdar activation driven plateau potentials in dendrites differ among d1r and d2r spiny projection neurons

Authors: *S. MITRA[1], W. W. LYTTON[2], S. D. ANTIC[3], M. MIGLIORE[4];
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Abstract: We performed computer simulations of striatal spiny neurons (SPNs) using physiologically plausible multi-compartment cell models to explore the generation of plateaus in SPN dendrites using NEURON simulation software. D1R and D2R SPN's used the same channel distributions though they had different morphologies, with D1R cells having greater arborization of the dendritic tree. Our simulations showed that NMDA-generated dendritic plateau potentials could occur in dendrites with spread to the soma. Plateaus occurred in both D1R and D2R SPN's. Fast sodium and potassium channels with small time constants were reciprocally distributed along dendrites. High speed sodium channels were preferentially concentrated near the soma and were distributed with decreasing density going from proximal to distal along dendrites, with the opposite for potassium channels. Low threshold calcium channels were distributed preferentially in distal dendrites. Glutamatergic stimulation was provided in a section of a dendrite with NMDA and AMPA receptors present in synaptic locations, with NMDA receptors distributed in extrasynaptic sites, to model for glutamate spillovers. Glutamatergic pulses were provided with gradually increasing intensity; with higher intensity stimulation leading to dendritic plateaus with spread to other dendrites and soma. The D1R SPN had wider plateaus compared to D2R SPN plateaus. In D1R SPN, high intensity stimulation produced sustained plateaus that did not terminate abruptly and instead gradually terminated over seconds. In D2R plateaus terminated for all intensities. Plateaus in D1R SPN's had a maximum amplitude of -20 mV at the stimulation site with an average width of 300 ms with recording performed at the site of stimulation, while D2R SPN had a maximum amplitude of -13 mV and an average width of 300ms at the same stimulation intensity. The plateaus showed an initial sodium spike, followed by a brief rise and decay. The decay from the peak was faster in D2R cells compared to D1R cells. The plateaus were dependent on the distribution of sodium, calcium and potassium channels. Identification of the mechanisms and roles of plateaus in SPNs may assist in development of new therapeutic approaches to Parkinson's disease.

Disclosures: S. Mitra: None. **W.W. Lytton:** None. **S.D. Antic:** None. **M. Migliore:** None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR150.13/B26

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: JST, CREST Grant Number JPMJCR21P4, Japan
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EU Horizon Europe Programme under the Specific Grant Agreement No. 101147319 (EBRAINS 2.0 Project)

Title: Simulation based inference of striatal network model parameters from calcium imaging slice data reveals origin of pathological cell assembly dynamics in Parkinsonian and dyskinetic conditions

Authors: A. CORREA LUCES[1], A. P. PONZI[3], V. CALDERON[4], ***R. MIGLIORE**[2];
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Abstract: Under normal conditions the principal cells of the striatum, medium spiny neurons (MSNs), show structured cell assembly activity patterns which alternate sequentially over exceedingly long timescales of many minutes. It is important to understand this activity since it is characteristically disrupted in multiple pathologies, such as Parkinson's disease and dyskinesia, and thought to be caused by alterations in the MSN to MSN lateral inhibitory connections and in the strength and distribution of cortical excitation to MSNs. To understand how these long timescales arise we extended a previous network model of MSN cells to include synapses with short-term plasticity, with parameters taken from a recent detailed striatal connectome study. We first confirmed the presence of sequentially switching cell clusters using the non-linear dimensionality reduction technique, Uniform Manifold Approximation and Projection (UMAP). We found that the network could generate non-stationary activity patterns varying extremely slowly on the order of minutes under biologically realistic conditions. Next, we used Simulation Based Inference (SBI) to train a deep net to map features of the MSN network generated cell assembly activity to MSN network parameters. We used the trained SBI model to estimate MSN network parameters from ex-vivo brain slice calcium imaging data. We found that best fit network parameters were very close to their physiologically observed values. On the other hand, network parameters estimated from Parkinsonian, decorticated and dyskinetic ex-vivo slice preparations were different. We found the MSN-MSN collateral inhibition was reduced in Parkinsonian preparations. This moved the network state towards the border with a highly pathological winners-take-all (WTA) network dynamical regime. Proximity to this state induced metastable switching with extended dwell times in the vicinity of WTA state. These findings are in good agreement with empirical studies, (e.g., Jáidar et al. Journal of Neuroscience 30.34 (2010): 11326-11336) showing that the dynamics of Parkinsonian striatal microcircuit become entrained into a dominant network state, reminiscent of Parkinson's Disease symptoms. We also found that MSN cells from dyskinetic preparations after extended L-Dopa treatment (LID) had higher excitability. This partially restored the network dynamics away from the WTA regime, but as a side-effect also created more rapid irregular switching of cell assemblies, reminiscent of

dyskinesia symptoms. Our work may provide a pipeline for diagnosis of basal ganglia pathology from spiking data as well as for the design pharmacological treatments.

Disclosures: A. Correa Luces: None. **A.P. Ponzi:** None. **V. Calderon:** None. **R. Migliore:** None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR150.14/B27

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Title: Subthreshold membrane potential oscillations in the mouse, non-human primate, and human across brain regions

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Abstract: We examined the intrinsic subthreshold membrane potential oscillations across a comprehensive set of transcriptomically defined excitatory and inhibitory cell types in acute brain slices from mouse, non-human primate (NHP), and human in multiple brain regions. Subthreshold oscillation power was efficiently captured between the resting membrane potential and spiking using a ramp stimulus acquired during Patch-seq experiments. Time-frequency analysis methods, including continuous wavelet transform, were employed to extract frequency content. In mouse, distinct frequency-power profiles were observed among transcriptomically identified inhibitory cell subclasses (Somatostatin, VIP, and Parvalbumin), correlating with potassium current density and gene expression. Interestingly, the frequency profiles of the inhibitory cell types also correlate with their previously described contribution to network oscillations. We compare these observations in mouse to those in the NHP and human.

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Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR150.15/B28

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NICHD Z01-HD001205-27 Intramural Research Award

Title: Cross-species electrophysiological comparison of fast-spiking inhibitory neurons across multiple brain regions

Authors: *L. HEWITT[1], R. CHITTAJALLU[2], A. CACCAVANO[3], G. A. VARGISH[4], N. MCLEAN[5], X. YUAN[6], S. HUNT[7], M. A. ELDRIDGE[8], B. B. AVERBECK[9], K. A. ZAGHLOUL[10], K. A. PELKEY[11], C. J. MCBAIN[12];
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Abstract: Mouse models have been historically used in neuroscience research to determine potential therapeutic targets for human neurophysiological disease. However, there is a surprising lack of literature describing the overlap in the underlying function of mouse neurons compared to other species, including non-human primates and humans. It has become increasingly valuable to characterize similarities and innovations in non-human primate and human brain tissue in comparison to the rodent to determine the translational relevance of mouse physiology. Inhibitory neurons are vastly diverse and exhibit numerous sub-types which gate network activity in a spatial-temporal manner. The sparse representation of GABAergic neuron subtypes has made them inherently difficult to study without targeted labeling of the genetic and molecular identification of these cells. Baseline electrophysiological parameters of GABAergic neurons are critical to proper brain function yet remain to be examined between species. Additionally, immense amounts of research indicate these inhibitory neurons are altered in disease states in the mouse brain, however; whether these neurons exhibit similar aberrant properties in humans and non-human primates is poorly understood. We investigated these properties using a viral targeting technique, regional specific injections of the virus S5E2, which selectively labels fast-spiking (FS) parvalbumin neurons in tandem with whole-cell patch clamp electrophysiology recordings. We recorded FS neurons in hippocampus and cortex across three species: mouse, macaque, and humans, in addition to recordings of striatal FS neurons in the mouse and macaque. Our data indicate S5E2 neurons express the hyperpolarized activated cyclic nucleotide gated channel (HCN) in human and macaque hippocampus and cortex. In contrast, the typical sag and rebound response to hyperpolarizing current steps is absent in mouse FS neurons. HCN channels are essential for shaping the subthreshold integrative properties, influence the resting membrane potential, intrinsic excitability of neurons, and have been well studied in mice across several brain areas and specific cell types. Interestingly, analysis from FS neurons in the striatum show a lack of HCN in macaque, similar to data collected in the mouse. Our data indicate the convergence and divergence of physiological properties exist in a region and species dependent manner. Ultimately, the data from this study will expand our understanding of how divergent FS neuron physiological parameters are between species, and across brain regions.

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Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR150.16/B29

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NICHD Z01-HD001205-27 Intramural Research Award
NINDS Center on Compulsive Behaviors Graduate Student Fellowship

Title: Identification and interrogation of novel long-range projecting hippocampal somatostatin interneurons across species

Authors: *A. VLACHOS[1], N. MCLEAN[1], A. CACCAVANO[1], G. A. VARGISH[1], L. T. HEWITT[1], X. YUAN[1], S. HUNT[1], E. FURLANIS[3], Y. WANG[3], M. DAI[3], J. WU[3], S. ANTONUCCI[2], H. SILBERBERG[2], C. E. LE PICHON[2], M. A. ELDRIDGE[4], B. B. AVERBECK[5], G. J. FISHELL[6], K. A. PELKEY[1], C. J. MCBAIN[1];
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Abstract: Though numerically sparse, a remarkably heterogeneous population of GABAergic inhibitory interneurons critically regulates information flow and processing amongst projection neurons of forebrain circuits. Uncovering interneuron subtype-specific characteristics is imperative in understanding cellular components of cognitive processes underlying behavior. Furthermore, while interneuron diversity is best characterized in common experimental model organisms, such as rodents, the degree of conservation/divergence of discrete interneuron subpopulations in higher species is understudied but required to understand human brain function in health and disease. Subpopulations of somatostatin-expressing interneurons (SST-INs) continue to be uncovered, comprising distinct molecular profiles, electrophysiological properties, morphologies, and functions, including orchestration of network rhythms amongst principal cells. Using a novel cell-type specific labelling adeno-associated virus (*Hpse* cis-acting regulatory enhancer, S9E10) injected into the hippocampus of mice and macaques, we discovered a subpopulation of previously undescribed hippocampal SST-INs with unique molecular, anatomical, and electrical properties. S9E10 labelled SST-INs co-stain for SST but not parvalbumin and show a bimodal distribution of maximum firing frequencies that span from regular-spiking (<100 Hz) to fast-spiking firing patterns (>200 Hz), while maintaining a notable I_h current in most cells recorded in the stratum oriens of the hippocampus, suggesting two subpopulations of SST-INs within the virally labelled cohort in both mouse and macaque. Tissue clearing in mice reveals labelled SST-INs also form extrahippocampal projections to the medial septum and ventral diagonal band (MSvDB) which demonstrate GABAergic output to MSvDB cells, indicating a role for long-range signaling across the septo-hippocampal pathway. Through

transcriptomic experiments, cell reconstructions, and electrophysiological assays our study aims to characterize the molecular, morphological, and physiological properties of these novel SST-INs, investigate their functional role in local and long-range circuits, and their conservation across rodents and non-human primates. Identification of this unique cell type sets the foundation to probe its role in network rhythms, such as theta oscillations and sharp-wave ripples, which are prominent across the septo-hippocampal axis and implicated in crucial processes including learning and memory consolidation.

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Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR150.17/B30

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: R01 NS10533
F32 FNS119282A

Title: Molecular and biophysical mechanisms of plasticity of intrinsic excitability in L2/3 somatostatin interneurons

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Abstract: Sensory experience drives robust plasticity in sensory cortex, including in inhibitory circuits. Mechanisms of inhibitory circuit plasticity are poorly understood and are likely specific to interneuron type. Recent work in somatosensory cortex (S1) showed rapid weakening of L2/3 parvalbumin (PV) interneuron intrinsic excitability (IE) following 1 day of whisker deprivation, that is mediated by changes in $K_V1.1$ expression and depolarization of spike threshold (Gainey et al., 2018). Here we focus on somatostatin (SST) interneurons, which provide dendritic inhibition to pyramidal cells and may gate their plasticity. We asked whether L2/3 SST cells in S1 exhibit similar plasticity of intrinsic excitability response to whisker deprivation, and if this involves the same cellular mechanisms as in PV cells. We patched L2/3 SST cells in S1 slices and used whole-cell current-clamp recordings to measure IE. 3 days of D-row deprivation decreased SST IE, depressing F-I curves by ~40% by reducing input resistance and elevating rheobase. Unlike in PV cells, 1-day deprivation was not sufficient to drive F-I curve depression. Next, we asked if deprivation reduced SST IE by the same molecular mechanisms as known for PV cells.

Quantitative immunofluorescence showed no change in $K_V1.1$ protein expression in SST cells (including specifically in Martinotti- or non-Martinotti cells) following 3 days of whisker deprivation, compared to sham-deprived mice. This suggests distinct biophysical mechanisms for plasticity in SST cells versus PV cells. Labeling of the axon initial segment (AIS) with AnkyrinG, showed, paradoxically, that 3 day deprivation caused a significant increase in AIS length. In pyramidal cells, increased AIS length correlates with increased cell excitability and is presumed to involve Na_V channel insertion (Jamaan et al., 2021). We hypothesize that in SST cells, AIS lengthening reflects a predominant increase in non-K_V1 K_V channels in the AIS, which would reduce excitability. We are currently investigating whether $K_V7.2/7.3$ channels are upregulated, which mediate the mAChR-sensitive K current (M-current) in SST interneurons.Overall, this work shows that whisker deprivation reduces SST IE in S1 cortex through cell-type specific molecular mechanisms relative to PV cells.

Disclosures: B.M. Bohannon: None. **D. Feldman:** None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR150.18/B31

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Title: Restoring neuronal network balance via chemogenetic inhibition of hippocampal somatostatin interneurons in a mouse model of Alzheimer's disease

Authors: *M. ABDOLLAHI NEJAT, A. B. SMIT, R. E. VAN KESTEREN;
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Abstract: Imbalances in neuronal networks resulting from alterations in both excitatory and inhibitory signaling pathways are thought to play a role in the pathogenesis of Alzheimer’s disease (AD). Here, we contribute to the growing evidence of neuronal network dysfunction as an early symptom in AD by highlighting alterations in the excitability of hippocampal CA1 somatostatin (SST) interneurons in the APP/PS1 mouse model for amyloidosis. Specifically, we show that SST interneurons are hyperexcitable at ~16 weeks of age, coinciding with the previously reported hyperexcitability of PV interneurons and impairments in spatial learning and memory. Interestingly, while hippocampal PV interneurons exhibit a biphasic response and become hypoexcitable at ~24 weeks of age, SST interneurons remain hyperexcitable. We further show that SST neuron hyperexcitability, similar to PV neurons, depends on soluble amyloid-beta and that early intervention targeted at restoring SST interneuron activity results in long-lasting restoration of SST neuron excitability. Interestingly, targeting SST interneurons also rescued PV interneuron function on the long term. In addition, we show that another subclass of presumably VIP-positive hippocampal interneurons is unaffected in this model. Taken together, these findings suggest that the imbalances in hippocampal neuronal networks observed in AD may involve the dysfunction of both SST and PV interneurons, and that early intervention targeted at

restoring SST interneuron activity rescues overall network function and could thus have clinical implications in terms of preventing network and memory decline in AD.

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Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

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Program #/Poster #: PSTR150.19/B32

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NSF Grant Neuronex 2015276

Title: Parvalbumin interneuron diversity in mouse visual and prefrontal cortices

Authors: *T. MIYAMAE[1], Y. NISHIHATA[2], O. L. KRIMER[1], D. HOWARD[3], N. XU[4], S. TRIPATHY[5], G. GONZALEZ-BURGOS[1];
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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, the fraction of all GABA neurons that PV cells represent differs among sensory and association areas: PV cells are >40% of all GABA neurons in visual cortex (VC), but <20% in prefrontal cortex (PFC). Surprisingly, other than their different proportions, little is known about VC-PFC regional differences in PV cell properties. Here we compare the properties of mouse VC and PFC PV cells, using patch clamp electrophysiology and patch-seq in acute slices. All PV cells currently analyzed (VC n=57; PFC n=53), exhibited Fast Spiking (FS) electrophysiological properties. Moreover, PV cells in VC slices showed two FS phenotypes distinguished by their long (>70 ms) or short (<70 ms) delay to fire the first spike at rheobase (dFS and cFS, respectively), as in somatosensory cortex (Neuron 58:387,2008; Science 349:1216, 2015). In VC slices most PV cells were dFS (dFS: 43/57, 75.5%; cFS: 14/57, 24.5%) and analysis of 16 membrane properties showed that cells classified as dFS or cFS by the delay at rheobase differed in 5 other membrane properties, cFS being more excitable than dFS cells. In PFC slices PV cells could also be divided into cFS and dFS groups via the first spike delay at rheobase. However, contrasting with VC, PFC slices contained nearly equal proportions of dFS and cFS neurons (dFS: 28/53, 52.8%; cFS: 25/53, 47.2%). Interestingly, in PFC PV cells with long or short first spike delay did not differ in most of their membrane properties suggesting the grouping of PV cells in PFC is more complex than 2 groups simply defined by the first spike delay. To further assess the electrophysiological diversity of PV neurons, we began applying dimension reduction using Uniform Manifold Approximation and Projection (UMAP).

Preliminary analysis showed that cFS and dFS cells from VC slices were spatially segregated in the UMAP space forming 2 groups that differed in various properties. Interestingly, the PFC UMAP space contained at least 2 dFS groups and one cFS group. Preliminary studies showed the feasibility of generating high-quality patch-seq samples suitable for bioinformatic analysis, which identified excitatory and inhibitory neurons with 100% accuracy, and correctly predicted the identity of 95% of the PV neurons. Ongoing analysis of the patch-seq data is comparing the expression of marker genes (e.g. Pvalb), and transcription factors that may contribute to the electrophysiological diversity of PV neurons in VC and PFC (e.g. Etv1, encoding Er81).

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Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR150.20/B33

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIH R01NS105333
NIH R01NS134639
NSF Graduate Research Fellowship Program

Title: Rapid Bidirectional Plasticity of PV Interneuron Intrinsic Excitability by Sensory Experience

Authors: *A. M. NIETO[1,2], D. E. FELDMAN[3];
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Abstract: Parvalbumin (PV) interneurons are rapidly plastic in response to sensory experience, including by regulation of PV intrinsic excitability (PV_{IE}), which is often a more rapid form of plasticity than synaptic changes. In whisker somatosensory cortex (S1), 1 day of whisker deprivation weakens PV_{IE} in layer 2/3 of deprived columns, leading to disinhibition of pyramidal cells that homeostatically restores pyramidal cell mean firing rate. However, whether this mechanism is bidirectional (that is, whether PV_{IE} increases in response to elevated sensory use) is unknown. Using whole cell current clamp electrophysiology in acute slices from PV-Cre;TdTomato mice (P18-23), we measured intrinsic excitability of layer 2/3 PV neurons in the D column of S1 following 1-3 days of elevated use of the single row of D whiskers, achieved by trimming non-D whiskers (D-Sparing), combined with environmental enrichment (EE). We observed a significant upward shift in PV neuron F-I curves in D-Spared/EE mice compared to age-matched control mice with all whiskers intact (full whisker experience; FWE) in standard housing (SH) conditions. This effect appeared to emerge as early as 24-hours after D-Sparing/EE onset. This increased spiking was accompanied by a hyperpolarization of spike threshold,

complementary to what was observed previously following whisker deprivation. This implies that up- and down-regulation of PV_{IE} is caused by a bidirectional regulation of spike threshold. Neither D-Sparing alone (under SH conditions), nor EE alone (in animals with FWE) was sufficient to induce changes in PV_{IE}, suggesting PV_{IE} up-regulation requires both competition between whiskers and environmental enrichment. No differences in passive membrane properties were found between any groups. Overall, these findings are consistent with a bidirectional homeostatic role of PV neurons in maintaining mean cortical firing rates amidst fluctuating sensory input. Furthermore, we hypothesize that this bidirectional modulation of PV_{IE} may be impaired in autism spectrum disorders (ASDs), as PV hypofunction and impairments in excitatory/inhibitory balance are common features in genetic models of ASDs. We are currently investigating whether bidirectional PV_{IE} plasticity is absent in the *Tsc2+/-* ASD model.

Disclosures: A.M. Nieto: None. **D.E. Feldman:** None.

Poster

PSTR150: Intrinsic Properties and Modulation of Neuronal Firing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR150.21/B34

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Italian National Recovery and Resilience Plan (NRRP), M4C2 (project IR0000011, CUP B51E22000150006, EBRAINS-Italy)
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EU Horizon Europe Program under the specific Grant Agreement 101147319, EBRAINS 2.0 project

Title: Computational modeling of T-type Ca^{2+} and persistent Na^{+} currents in modulating excitability of ventral medial entorhinal cortical stellate neurons

Authors: *E. GIACALONE[1,2], A. P. TOPCZEWSKA[3], W. PRATT[4], A. C. DOLPHIN[4], M. M. SHAH[5], M. MIGLIORE[1];
[1]Natl. Res. Council, CNR, Palermo, Italy; [2]Dept. of Mathematics and Computat. Sci., Univ. of Palermo, Palermo, Italy; [3]Sch. of Pharm., [4]UCL, London, United Kingdom; [5]Univ. Col. London, London, United Kingdom

Abstract: The medial entorhinal cortex (mEC) is a significant component of the hippocampal formation, impacting episodic memory, spatial memory, and spatial navigation. Membrane properties and firing frequency of mEC stellate layer II neurons vary along the dorsal-ventral axis, with dorsal neurons being less excitable than ventral neurons. This difference is partially due to higher inhibitory conductance densities in dorsal neurons. Experimental findings reveal an increase in T-type Ca^{2+} currents along the dorsal-ventral axis in mEC layer II stellate neurons. Furthermore, long depolarizing stimuli trigger T-type Ca^{2+} currents, which interact with

persistent Na^+ currents, leading to membrane voltage elevation and spike firing specifically in ventral neurons. To explore the effects of T-type Ca^{2+} currents further, a computational model of a ventral stellate neuron was developed. The model incorporated known ion channel conductances and experimental measurements of T-type Ca^{2+} current kinetics and density obtained from the soma. The computational modelling suggests that T-type Ca^{2+} currents work in conjunction with subthreshold Na^+ currents, enhancing the membrane resistance (R_N) of ventral wild-type stellate neurons and increasing their action potential firing rates. This modulation of ventral neuron excitability likely plays a role in mEC circuit activity and functions.

Disclosures: E. Giacalone: None. **A.P. Topczewska:** None. **W. Pratt:** None. **A.C. Dolphin:** None. **M.M. Shah:** None. **M. Migliore:** None.

Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.01/B35

Topic: B.07. Network Interactions

Support: NIH DIRP ZIAMH002797
BRAIN initiative U19 NS107464-01

Title: Random forests and branching trees: machine learning approaches for optogenetic stimulation recordings

Authors: *S. PAJEVIC, T. L. RIBEIRO, D. PLENZ;
Section on Critical Brain Dynamics, Natl. Inst. of Mental Hlth. (NIMH), NIH, Bethesda, MD

Abstract: In the observed formation of scale-invariant parabolic avalanches (Ribeiro, Capek et al., 2023), it is important to understand the contribution and interactions of local groups of neurons. To address this we conducted optogenetic stimulation experiments, using a low-repetition, high-power laser (Carbide/Orpheus; LightConversion) and a high-resolution spatial light modulator (MeadowLark) to achieve successful 2-photon (2P) excitation of individual neurons expressing the opsin ChrimsonR in the layer II/III pyramidal neurons of widefield-identified primary visual cortex (V1) of awake mice. During and after holographic stimulation (100 ms; <10 mW per target) of each of the 32 different pyramidal neurons co-expressing the opsin (~100 trials per group), the activity of ~150-300 neurons was recorded using 2P imaging in a ~450 μm x 450 μm area (100 - 200 μm depth) at ~45 Hz framerate. Acquired images were denoised and deconvolved yielding time-series of spike activations for each of the imaged neurons. A subset of non-stimulated cells responded significantly to the perturbation (> 92% baseline spike count), which we attribute to indirect, cascading propagation of activity in the network in the form of branching trees. Here, we use machine-learning strategies to analyze these stimulation recordings. In the first stage, we use classification algorithms (e.g., random forest, deep neural networks) in which we aim to predict which neurons were stimulated based

on the response of other neurons, that are potential descendants in the activation cascade. In most recordings, the predictability was much larger than the chance level (3%), and in some recordings, the prediction accuracy was greater than 65%. We discuss reasons some recordings had lower predictability and address this via randomized sampling of the target neurons to identify the reliable cells, allowing us to boost the accuracy significantly. We showed how this predictability changes as a function of time and distance from the stimulation site, yielding the highest predictability 50-60 ms after the onset of stimulation. All these findings allowed us to study the branching tree map of the cascading neural activity propagating in the network.

Disclosures: S. Pajevic: None. **T.L. Ribeiro:** None. **D. Plenz:** None.

Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.02/B36

Topic: B.07. Network Interactions

Support: NIH Grant R15NS116742
NSF Grant 1912352

Title: Cortex deviates from criticality during action and deep sleep: a temporal renormalization group approach

Authors: *S. SOOTER[1], A. FONTENELE[2], A. K. BARREIRO[3], C. LY[4], W. L. SHEW[5]; [1]Univ. of Arkansas Fayetteville, Fayetteville, AR; [2]Physics Dept., Univ. of Arkansas Fayetteville, Fayetteville, AR; [3]Mathematics, Southern Methodist Univ., Dallas, TX; [4]Statistical Sci. & Operations Res., Virginia Commonwealth Univ., Richmond, VA; [5]Physics, Univ. of Arkansas, Fayetteville, AR

Abstract: The hypothesis that the brain operates near criticality explains observations of complex, often scale-invariant, neural activity. However, the brain is not static, its dynamical state varies depending on what an organism is doing. Neurons often become more synchronized (ordered) during unconsciousness and more desynchronized (disordered) in highly active awake conditions. Are all these states equidistant from criticality; which is closest? The fundamental physics of how systems behave near criticality came from renormalization group (RG) theory, but RG for neural systems remains largely undeveloped. Here we developed a temporal RG (tRG) theory for analysis of typical neuroscience data and mathematically identified multiple types of criticality (tRG fixed points). We demonstrate tRG-driven data analysis methods to assess proximity to each fixed point based on relatively short time series. Unlike traditional methods for studying criticality in neural systems, our tRG approach allows time-resolved measurements of distance from criticality in experiments at behaviorally relevant timescales. We apply our approach to recordings of spike activity in mouse visual cortex, to show that the relaxed, awake state is closest to criticality. When arousal shifts away from this state - either

increasing in more active awake states or decreasing in deep sleep - cortical dynamics deviate from criticality.

Disclosures: S. Sooter: None. **A. Fontenele:** None. **A.K. Barreiro:** None. **C. Ly:** None. **W.L. Shew:** None.

Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.03/B37

Topic: B.07. Network Interactions

Support: NIH DIRP ZIAMH002797

Title: Improving critical exponent estimates of neuronal avalanches via multi-plane two-photon imaging in awake mice

Authors: *E. HUO, S. PAJEVIC, T. L. RIBEIRO, D. PLENZ;
Sect. Crit. Brain Dynamics, Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: Neuronal avalanches are scale-invariant cascades of propagated neuronal synchronization characterized by power laws in their size and duration. These spatiotemporal cascades evolve preferentially in superficial layers of the mammalian cortex and can be tracked using advanced technologies such as microelectrode arrays or 2-photon imaging (2PI). Recently, the scaling relationship between the mean size and duration of parabolic avalanches has been found to exhibit the critical exponent of 2 (Miller et al., 2019, nonhuman primate; Capek, Ribeiro et al., 2023, transgenic mouse). This exponent can be revealed in vivo when spatial subsampling, finite-size effects and temporal coarse-graining in the observation of avalanches are properly taken into account. However, of particular concern is the inability to infer the influence of neuronal activity above and below single-plane imaging and the bias it introduces in reconstructing critical exponents of avalanche dynamics. Here, we study the impact of incomplete spatial sampling of neuronal avalanches using conventional 2PI with a single optical plane compared to volumetric sampling with multiple planes. We utilized 2PI coupled to a piezoelectric actuator (P-725.xCDE2; Physik Instrumente) to capture neuronal activity of the primary visual cortex (layer 2/3) in awake mice expressing the genetically encoded calcium indicator GCaMP8s. Avalanches were reconstructed across volumes ranging from 50 to 200 µm, spanning 3 to 7 planes. We find that reconstructing avalanches using multiple imaging planes increased the critical scaling exponent towards its theoretical value of 2 (critical branching process), when compared to single-plane recordings. When using simultaneous dual-plane mesoscope imaging (Thorlabs), it enhanced temporal resolution of multiplane imaging over piezoelectric actuation (45.5 Hz/2 planes). Data obtained from these recordings showed similar and more robust improvements in estimating the scaling exponent. We also conducted simulations of cascading avalanche dynamics in a network of neurons embedded in a 3D lattice

network utilizing different network topologies. We studied the subsampling effects of planar imaging and compared them with our experimental findings. Our results showed that reconstructing avalanches from multiple planes significantly increases our ability to extract the correct critical exponents. In summary, the improved recovery of the critical scaling exponent in multiplane imaging experiments demonstrates that volumetric imaging can improve the observation of critical brain dynamics in the form of neuronal avalanches.

Disclosures: E. Huo: None. **S. Pajevic:** None. **T.L. Ribeiro:** None. **D. Plenz:** None.

Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.04/B38

Topic: B.07. Network Interactions

Support: HFSP-RGP0004/2019

Title: Nanoscale single-cell interfaces allow optical activation of spinal neurons and sensory-motor modulation in organotypic slices

Authors: *M. FONTANINI[1], B. TIAN[2], L. FRUK[3], L. BALLERINI[1];
[1]Neurobio., SISSA, Trieste, Italy; [2]Univ. of Chicago, Chicago, IL; [3]Chem. Engin. and Biotech., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: We exploited nanoscale photodiodes (nPDs), based on functionalised nanowires, to achieve minimally invasive light excitation of identified spinal neurons and to selectively manipulate ventral or dorsal activity in mouse organotypic slice cultures. This model of segmental microcircuit mantains the spinal cord dorso-ventral organisation and sensory-motor cytoarchitecture, providing easy access to molecular, pharmacological and optical manipulations. By NIR-light stimulation, Capacitative and Faradic currents are generated at the nPD-plasmalemma interface, exciting the contacting neuron. We potentiated the interface stimulating efficacy decreasing the distance between nPD and the cell surface, by chemically functionalising the nPD external shell with the transactivator of transcription (TAT) peptide. We explored the network effect(s) of single cell light-activation in respect to its ventral or dorsal localization, by simultaneous live imaging of either neuronal calcium signaling or glial calcium dynamics, using genetically encoded Ca^{2+} indicators, respectively GCaMP7f and GCaMP6f. Directing the NIR-stimulation (100 stimuli, 25 Hz) to the dorsal horn (DH), over caspaicin-sensitive neurons positive for the transient receptor potential cation channel subfamily V member 1 (TRPV1), we obtained a long-lasting (>20min) potentiation (wind-up) in the frequency of DH synaptic activity. When directing the stimulation to ventral horn (VH) glutamatergic interneuron, we obtained an increase in the synchronisation of the VH premotor network. We further explored differences in local (ventral or dorsal) astrocytes' responses to activation states when evoked in sensory or premotor circuits. In the VH the increased synchronization, due to single neuron

stimulation, was accompanied by an increase in GFAP+ cells Ca^{2+}-fluctuations frequency, while DH TRPV1-dependent wind-up did not affect the GFAP+ cell calcium dynamics. In both glial networks gap-junction mediated inter-astrocytic communication was crucial to regulate basal and reactive calcium signaling, with VH astrocytes becoming unresponsive to neuronal synchronization and DH ones becoming tunable after wind-up upon application of connexin-43 hemichannel inhibitor (GAP27). In summary, nPDs allow for non genetic wireless stimulation of single neurons with diverse impacts on neuronal and astrocytic networks basing on the identity and dorso-ventral localization of the stimulated cell.

Disclosures: M. fontanini: None. **B. Tian:** None. **L. Fruk:** None. **L. Ballerini:** None.

Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.05/B39

Topic: B.07. Network Interactions

Support: All work was directly funded by PTEN Research, a UK registered charity supporting research into PHTS.

Title: Microelectrode array (MEA) for assessing neuronal network restoration in PTEN knockdown primary hippocampal neurons.

Authors: C. PAPADOPOULOS[1], P. FRIESS[1], A. CARBONE[1], S. MODOLO[2], G. CALUSI[2], D. FEDERICO[2], ***P. ELVIN**[3];
[1]Evotec SE, Hamburg, Germany; [2]Pharmacometrics, Aptuit (Verona) Srl, an Evotec Co., Verona, Italy; [3]PTEN Res., Cheltenham, United Kingdom

Abstract: PTEN hamartoma tumour syndrome (PHTS) is a rare disease (incidence 1:200,000) arising from germline mutations in PTEN. Approximately 25% patients with PHTS may develop symptoms of autism spectrum disorder (ASD) and ~2% patients with idiopathic ASD have been found to harbour PTEN mutations. PTEN mutations lead to an upregulation of PI3K-Akt-mTOR signaling that has been associated with macrocephaly and structural and functional changes in hippocampal and cortical neurons in both human and murine CNS. Animal models with PTEN loss show neuronal hyperexcitability and seizure development. Our PTEN knockdown hippocampal neuron cell model showed upregulation of PI3K-Akt-mTOR signaling. Microelectrode array (MEA) *in vitro* assays offer valuable insights into cellular behavior, particularly in neurons, by measuring electrical activity noninvasively across a cell population over time using electrodes. We showed that AAV shRNA mediated PTEN knockdown neurons exhibit a consistent increase (e.g. burst duration and network burst duration) and decrease (network burst frequency) over time for a subset of neuronal network parameters linked to neuronal excitability. Rapamycin, an inhibitor of mTOR restored these functional parameters in PTEN knockdown cells to the level of non-target shRNA control cells. A structured statistical

framework was applied to evaluate the activity of rapamycin, accounting for experimental variability and potential neurotocixity over time. Applying the MEA assay to complement other measures of PTEN loss in neurons provides data that may further discriminate between inhibitors of PI3K signaling as drug repurposing candidates.

Disclosures: C. Papadopoulos: A. Employment/Salary (full or part-time):; Evotec SE. **P. Friess:** A. Employment/Salary (full or part-time):; Evotec SE. **A. Carbone:** A. Employment/Salary (full or part-time):; Evotec SE. **S. Modolo:** A. Employment/Salary (full or part-time):; Aptuit (Verona) Sri, an Evotec Co. **G. Calusi:** A. Employment/Salary (full or part-time):; Aptuit (Verona) Sri, an Evotec Co. **D. federico:** A. Employment/Salary (full or part-time):; Aptuit (Verona) Sri, an Evotec Co. **P. Elvin:** None.

Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.06/B40

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Measuring Excitatory and Inhibitory Neuronal Activities Using a Multi-channel, Spectrally Resolved Fiber Photometry Platform

Authors: *A. VARGHESE[1], T.-H. CHAO[2], R. J. NONNEMAN[3], Y.-Y. I. SHIH[4]; [1]Neurol., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Univ. of North Carolina Chapel Hill, Chapel Hill, NC; [3]Anat. and Neurobio., St. Louis Univ., UNC Chapel Hill, NC; [4]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Introduction:Understanding the balance between excitatory and inhibitory (E/I) neuronal activities is paramount for unraveling the complex mechanisms underpinning brain function. Spectral fiber photometry has emerged as a pivotal technique for assessing this balance by targeting specific cell types using optical probes. Our study introduces a multi-channel, spectrally resolved fiber photometry platform which can simultaneously measure dynamic neuronal activities in multi-brain regions. We elucidate the intricate patterns of neuronal response to oddball stimuli, specifically within the retrosplenial cortex (RSC), cingulate cortex (Cg), prelimbic cortex (PrL), and anterior insular (AI). By employing jRGECO indicators for excitatory neurons and GCaMP8m for inhibitory interneurons, the study provides a comprehensive peri-event time-frequency analysis, highlighting differential regional excitatory/inhibitory neuronal activations and deactivations. Methods: The rodent used for the control and oddball experiments consisted of Male Long Evans rats (n=7), recorded at 8-10 weeks. The rats were injected with AAV9-mDlx-jGCaMP8m-WPRE, AAV1.Syn.Flex.NES-jRGECO1a.WPRE.SV40, and AAV5.CamKII 0.4.Cre.SV40 at the S1 coordinate region, and were incubated for 2 weeks prior to fiber photometry recordings. The brain regions recorded included the retrosplenial cortex (RSC), cingulate cortex (Cg), prelimbic cortex (PrL) of the DMN and the anterior insula (AI) for the SN. Control or Oddball tones were pseudo-randomly

given with an interstimulus interval of 2 s.. Each session consisted of 580 controls and 20 oddballs, and a minimum initial sequence of 10 controls were given before pseudo-random presentation of oddballs, with a minimum of 10 control stimuli between any two oddballs.Results:Peri-event time-frequency analysis of excitatory-neuronal signal, utilizing jRGECO indicators, unveiled substantial deactivation within the RSC, Cg, and PrL, alongside activation in the AI following oddball stimuli. Conversely, examination of inhibitory-neuronal GCaMP8m signals demonstrated pronounced activation of inhibitory interneurons in both the RSC and AI in response to oddball stimuli.Conclusion: This study introduces a platform that facilitates the simultaneous examination of both excitatory and inhibitory neural activities in response to oddball stimuli across localized brain regions. The tool supports analysis into the dynamics of E/I balance and its circuit mechanisms, which provides insights to key areas of cognitive networks across large-scale brain networks.

Disclosures: A. Varghese: None. **T. Chao:** None. **R.J. Nonneman:** None. **Y.I. Shih:** None.

Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.07/B41

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

Support: NINDS Grant U19NS123719

Title: Visualizing Astrocytic Modulation of Neurotransmitter Signaling Using Synaptic Glutamate Indicator

Authors: *Y. JIN[1], N. ELAZAR[2], B. LYU[3], K.-A. CITRIN[4], J. SAVAGE[5], D. LUO[4,6], J. CAO[4], Y. WANG[4], G. YU[7], C. EROGLU[8], L. TIAN[4];
[1]Univ. of California, Davis/ Max Planck Florida Inst., Jupiter, FL; [2]Duke Univ., Durhan, NC; [3]Virginia Polytechnic Inst. and State Univ., Blacksburg, VA; [4]Max Planck Florida Inst. for Neurosci., Jupiter, FL; [5]Duke Univ. Neurobio. Grad. Program, Durham, NC, ; [6]Max Planck Florida Inst., Jupiter, FL; [7]Tsinghua Univ., Beijing, China; [8]Cell Biol Dept, Duke Univ, DUMC, Durham, NC

Abstract: Astrocytes, essential for the development and maturation of synaptic structure and function, have garnered increasing attention for their role in synaptic plasticity and cognition. Astrocytes possess elaborate processes that envelop or have close contact with pre- or post-synaptic partners, or both. Each astrocyte can interact with a diverse array of input neurons, spanning different genetic identities, brain regions and neurotransmitter properties, positioning them as potential integrators. Molecular and proteomic study have identified key regulators of astrocyte development and function, include cell adhesion molecules crucial for astrocyte morphology and synaptic plasticity. Glutamate serves as the predominant neurotransmitter to mediating astrocyte-neuron interaction, facilitating synaptic transmission and regulating

plasticity and behavior. To further elucidate neurotransmitter signaling through astrocytic modulation, we have developed molecular probe to monitor the structure and functional interaction of astrocyte-neurons. We engineered tissue specific scaffold to target a synaptic glutamate sensor, syniGluSnFR, to perisynaptic astrocytic processes (PAPs). Derived from the iGluSnFR series, syniGluSnFR allows for the visualization and detection of glutamate release specifically within synaptic clefts in defined circuits. By displaying extracellularly nonfluorescent halves of the sensor to presynaptic neurons and their associated astrocytes, we can visualize reconstituted sensor expression at neuron-astrocyte contacts using confocal microscopy, Additionally, we can record and analyze synaptic glutamate transient evoked by field stimulation in acute brain slices using two-photon microscopy, enabling examination of the heterogeneity across circuits and even within individual astrocyte. We have further applied the sensor to detect circuit-level glutamate changes resulting from astrocytic modulation of cell adhesion molecules, demonstrating its sensitivity and ability to capture structural alterations. In awake animals performing a reward memory task, we utilize two-photon microscopy to record glutamate transient in the hippocampus CA1 region, receiving input from contralateral CA3 pyramidal neurons. The development and application of this sensor allows us to visualize and record glutamate activity alongside two-photon microscopy, providing insight into the effects of protein compositions, environmental conditions, and neurological disorders on neurotransmitter and neuromodulator dynamic in astrocytes.

Disclosures: Y. Jin: None. **G. Yu:** None. **C. Eroglu:** None. **L. Tian:** None.

Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.08/B42

Topic: D.05. Auditory and Vestibular Systems

Title: High-efficiency single pulse two-photon stimulation for in vivo all-optical interrogation of neuronal circuits with acousto-optic deflectors

Authors: *Y. GOULAM HOUSSEN[1], M. PISONI[2], S. DIEUDONNE[3], B. BATHELLIER[2]; [1]Inst. de l'Audition, Paris, France; [2]Inst. Pasteur, Paris, ; [3]Ecole Normale Supérieure, Paris.

Abstract: Combining two-photon (2P) calcium imaging and digital holography is now the state-of-the-art approach to simultaneously record and perturb neuronal circuits in vivo. Typically, in this all-optical approach, neuronal manipulation is performed by employing digital holography to generate a “patterned’” illumination of the target neurons. Nonetheless, this technique is limited in terms of speed and throughput. An alternative strategy is offered by acousto-optic deflectors (AOD)-based 2P approaches (“ULoVE”, Villette et al. 2019; “3D-CASH” Akemann et al. 2022). Both ULoVE and 3D-CASH have been developed as ultrafast optical recording techniques, leveraging on the properties of AODs. However, we tested their efficiency in photostimulation. We could overcome digital holography limitations and combined simultaneous recording and

perturbation of neuronal circuits in awake head-fixed mice across single planes and three-dimensional volumes. The advantage of using these techniques lies in the enhancement of indicator excitation and opsin activation efficiency by sequential sampling the target neurons with extended volumes over a minimal dwell time. They grant single-cell resolution, with a temporal resolution of tens of microseconds, both for imaging and perturbation. When compared with digital holography, this results in the possibility to manipulate with exquisite precision the activity of a large population of neurons by performing random access ultrafast local scanning, without compromising the photon budget. First, using ULoVE, we validated stimulation of ChRmine-expressing neurons, which yielded robust responses to as brief as 70 µs illumination periods. After his encouraging result, we tested the stimulation efficiency using 3D-CASH, which allowed us to design the minimal photostimulation possible. By phase locking AODs modulation to the laser repetition rate (40 kHz), we could deliver high-energy single femtosecond laser pulses to different target locations, every 25 µs. We obtained responses with few pulses and even with a single-pulse stimulation, allowing for example the co-activation of 50 neurons in just 1.25 ms. Notably, such a precise and efficient perturbative approach is compatible with in vivo 2P voltage imaging, opening up the opportunity to unprecedented all-optical electrophysiological dissection of neuronal circuits in the intact brain.

Disclosures: Y. Goulam houssen: None. **M. Pisoni:** None. **S. Dieudonne:** None. **B. Bathellier:** None.

Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.09/B43

Topic: B.07. Network Interactions

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

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

Abstract: *In vitro* neuronal models are valuable tools for studying neural diseases and neurotoxicology. With the use of microelectrode arrays, the Maestro Pro can measure the electrical activity of neural cultures in a high throughput, label-free manner. Here, several assay parameters concerning the *in vitro* cell model and measurement approach were optimized using the Maestro Pro. The optimized assay was then used to characterize the response of neural cultures to neuroactive compounds. Three different cell model parameters were tested to determine the culture conditions best suited to detect neuroactive compound effects: cell density, time in culture at which to dose, and media type. Metrics representative of neural network activity, synchrony, and oscillation of rat cortical cultures in three different media types were assessed using the Maestro Pro over 21 days with cell densities ranging from 5,000 to 80,000

cells per well. Results showed that cultures consisting of 40,000 cells at Day 14 cultured in BrainPhys + SM1 were the optimal cell model for this application, because this condition induced a moderate level of baseline neural activity, allowing for the detection of increases or decreases in all three measures following compound dosing. After optimizing neural culture conditions for dosing, assay parameters including drug incubation time and recording time were interrogated. A drug incubation time of 1 hour and a recording length of 5 minutes were determined to be optimal after recording the response of our cell model to an example compound (4-aminopyrirdine). Finally, the optimized cell model and measurement protocol were used to assess the response of two excitatory compounds (bicuculine and SNC80), two inhibitory compounds (mefloquine and amitriptyline), and two anti-psychotic compounds (haloperidol and aripiprazole). The excitatory compounds increased activity and synchrony in the neural cultures, while decreasing oscillations. In contrast, dosing with the inhibitory or anti-psychotic compounds led to decreases in all three neural network characteristics. In total, this study shows that the Maestro Pro is a valuable tool for optimizing *in vitro* neural models and detecting electrophysiological changes from neuroactive compounds.

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

Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.10/B44

Topic: B.07. Network Interactions

Support: NIH 1RF1NS133755-01

Title: Wide-area all-optical electrophysiology probes functional connectivity across the cortex

Authors: *Y.-C. HUANG, A. E. COHEN;
Harvard Univ., Cambridge, MA

Abstract: To facilitate sensory processing, cognition, and behavior, the brain must coordinate activity among widely distributed regions. Thus, there is great interest to simultaneously observe high-speed electrical activity in genetically defined neuronal populations across many brain regions in behaving animals. Correlated dynamics do not, on their own, reveal causal influences, due to common inputs and recurrent feedbacks. Thus, one would also like to deliver targeted perturbations (activation or silencing) and map how these influences propagate through the brain. We employed all-optical electrophysiology (Optopatch) to monitor spontaneous and

optogenetically evoked membrane voltage dynamics in Layer 2/3 pyramidal cell populations across multiple brain regions in awake, head-fixed mice. A voltage indicator 'Voltron2' and channelrhodopsin actuator 'CheRiff' were co-expressed in Layer 2/3 pyramidal cells across the entire mouse cortex. In a first set of experiments, voltage-dependent fluorescence signals were imaged through a wide-field window using a high-speed camera, while locomotion and facial motion were monitored using a video camera. Brain-wide correlations in regional activity were analyzed. In a second set of experiments, targeted optogenetic stimuli were delivered via a digital micromirror device (DMD). We mapped the spatial and temporal dynamics of responses to focal stimuli targeted sequentially in a grid across the cortex. Together, these experiments provide high-resolution cortex-wide functional correlation and functional connectivity maps.

Disclosures: Y. Huang: None. **A.E. Cohen:** None.

Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.11/B45

Topic: B.07. Network Interactions

Support: Abe Bresver Chair in Functional Neurosurgery at the Hospital for Sick Children

Title: Connectivity of the centromedian nucleus of the thalamus: Insights from brain stimulation and intracranial electroencephalography

Authors: *K. MITHANI[1], S. M. WONG[2], G. IBRAHIM[3];
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Abstract: The centromedian nucleus of the thalamus (CM) is an increasingly common target for deep brain stimulation (DBS) in the treatment of drug-resistant epilepsy. CM-DBS is thought to exert its anti-seizure effects by modulating large-scale thalamocortical networks, but its connectivity in humans remains elusive. We acquired rare intracranial electroencephalographic recordings from four children undergoing stereoelectroencephalography, including electrodes implanted into the CM, to map its connectivity using *in vivo* brain stimulation. Single-pulse electrical stimulation was delivered to the CM at 1 Hz (pulse-width 100 μs, 20-pulse trains) in escalating 1 mA intervals up to a maximum of 6 mA or until the occurrence of after-discharges. Intracranial electroencephalographic data was recorded at a sampling rate of 2048 Hz and preprocessed using a fourth-order zero-phase bandpass Butterworth filter (0.5-250 Hz) and a notch filter (60 Hz & harmonics) to remove powerline artifacts. Single-trial voltage responses were cross-correlated by computing semi-normalized dot products to identify the canonical response at each channel. Each channel's projection weights were then tested for significance using Wilcoxon signed-rank tests and those with FDR-corrected $p < 0.05$ were considered brain

regions with significant evoked responses. Out of 611 channels sampled, 235 demonstrated significant evoked responses. Brain regions with the greatest proportion of channels achieving extraction significance included the: postcentral gyrus (N = 24, proportion significant = 100%), orbitofrontal gyrus (N = 4, proportion significant = 100%), supramarginal gyrus (N = 4, proportion significant = 100%), Rolandic operculum (N = 14, proportion significant = 93.3%), middle cingulate gyrus (N = 20, proportion significant = 91%), precentral gyrus (N = 35, proportion significant = 89.7%), caudate (N = 12, proportion significant = 70.6%), superior temporal lobe (N = 11, proportion significant = 64.7%), and insula (N = 12, proportion significant = 60.0%). These results provide the first evidence of CM stimulation directly engaging distributed, large-scale networks including orbitofrontal, Rolandic, cingulate, and insular brain regions. Our findings provide new insight into potential mechanisms-of-action underlying CM-DBS and can inform ongoing efforts to optimize post-surgical outcomes through targeted engagement of specific brain networks.

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Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.12/B46

Topic: B.07. Network Interactions

Support: AMED 23dm0207084
JST CREST JPMJCR21E2
JSPS KAKENHI 19H03336, 21H00217, 23H04689, 24H02314

Title: Microcircuits in the marmoset prefrontal cortex with a large volume electron microscopy

Authors: *Y. KUBOTA[1,2,3], T. MIYAZAKI[1,3], N. L. KAMIJI[1,4], A. WATAKABE[4], M. SUGA[5,3], Y. KAWAGUCHI[6,3];
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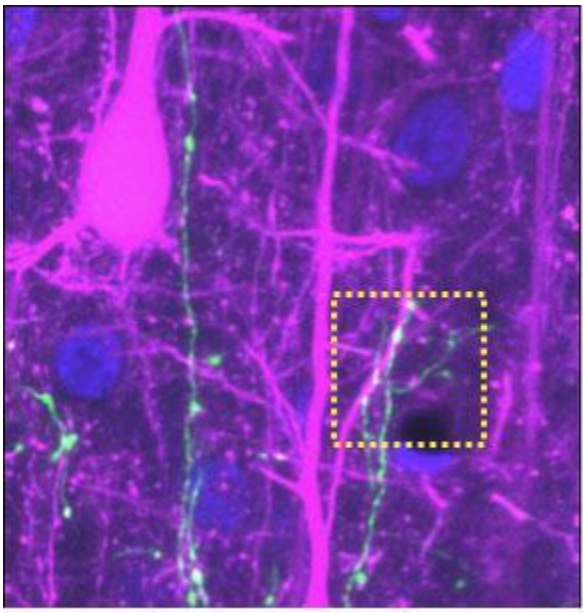
Abstract: In recent decades brain connectomics using large volume electron microscopy (vEM) has been introduced in several neuroscience laboratories. This methodology allows us to elucidate synaptic connections on a large scale such as cortical columnar wiring. To implement vEM we used a modified automated tape collecting ultramicrotome (ATUM) to collect large

numbers of serial ultrathin sections and a high-throughput EM imaging system (Blade, Voxa, Seattle). Firstly, we remodeled the original ATUM (RMC Boeckeler, Tuson) to control the timing of cutting to achieve reliable collection of serial ultrathin sections on individual slots of a grid tape. We succeeded in collecting more than 1000 serial ultrathin sections. These sections were placed securely and fairly reliably at similar locations within each grid slot. Secondly a transmission EM (TEM) equipped with Blade was used for imaging the ultrathin sections on the grid tape. The Blade-TEM system captured high-resolution (3.2 nm/pixel) images of an area 1.1 x 1.6 mm in size and a vEM dataset from the ~1000 serial ultrathin sections (50-nm thick sections) in about a month. Processing imaging data of such a huge size can be challenging. However, we developed a new high-throughput EM pipeline and we have been analyzing the circuit architecture of a marmoset prefrontal cortex (PFC). The PFC has dramatically expanded in primates. However, their connectivity at synaptic levels remains unclear. We co-injected antero- and retrograde viral tracers (in different colors) into the A10 area to visualize reciprocal projections between columnar patches in the A9 area in the marmoset PFC (Watakabe et al., Neuron. 2023; 111:2258-2273). To unravel the cortico-cortical circuit between the A10 and A9 areas we used confocal laser scanning microscopy of PFC sections in which both axons and dendrites were labeled anterogradely and retrogradely and acquired a large TEM dataset of the A9 PFC area. The results with this correlative light and EM using vEM will be reported.



Projected LSM image

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Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.13/B47

Topic: B.07. Network Interactions

Support: FG24932

Title: Yellow Fever Virus-Based Anterograde Transneuronal Tracing of the Locus Coeruleus Projection Network

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Abstract: Yellow Fever Virus-Based Anterograde Transneuronal Tracing of the Locus Coeruleus Projection Network
Matthew Rivera[1], Pan Gao[1], Wei Xu[2], Xiangmin Xu[1,31]Department of Anatomy and Neurobiology, School of Medicine, University of California, Irvine, California 92697, [2]Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA. [3]Department of Biomedical Engineering, University of California, Irvine, California 92697, Center for Neural Circuit Mapping, University of California, Irvine, California 92697,
The locus coeruleus (LC), located within the brainstem, is the primary synthesizer of the neurotransmitter noradrenaline/norepinephrine, which plays a pivotal role in regulating arousal, memory, attention, and mood. Noradrenaline is distributed by the LC across a broad network that includes the brainstem, cerebellum, forebrain, and spinal cord. Given that LC dysfunction has significant implications in various neurological disorders such as Alzheimer's disease (AD) and mood disorders, an improved understanding of the pathways of the LC-noradrenaline system is crucial. Recently, the Wei Xu lab developed an anterograde viral system based on a live attenuated vaccine for yellow fever (YFV-17D). Replication- or packaging-deficient mutants of YFV-17D can be transcomplemented in the brain, leading to efficient synapse-specific and anterograde-only transneuronal spreading. We administered a combination of engineered yellow fever virus (YFV-ΔCME-mVenus) and helper AAVs (AAVDJ-DIO-tTA and AAVDJ-TRE-CME-T2A-mRuby3) into the LC of DBH-Cre mice that express Cre recombinase in noradrenergic neurons. This approach enabled anterograde transneuronal tracing and the selective expression of mVenus in postsynaptic neurons directly connected to the LC noradrenergic neurons, thus facilitating detailed mapping of the LC-noradrenaline system's circuitry. Based on our successful mapping cases, we localized ~10 starter neurons that are co-labeled by mRuby3 and mVenus and found brain-wide projection labels with estimated ~3000 cells (up to hundreds and thousands) in ~84 brain regions. Our data, for the first time, provides direct visualization of the whole-brain-wide LC direct projection networks.

Disclosures: M. Rivera: None. **P. Gao:** None. **W. Xu:** None. **X. Xu:** None.

Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.14/B48

Topic: B.07. Network Interactions

Title: Live neuron morphological tracing in-vitro and in-vivo

Authors: *C. ZHOU[1], S. SEMAN[1], S. CARMODY[1], M. PREISEGGER[2], T. KUNKHYEN[2], J. GREGORY[3], C. E. CHEETHAM[4], M. S. GOLD[2], O. A. SHEMESH[5];
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Abstract: Neuronal morphology is key to understanding information processing in neural circuits, since the morphological diversity of axons and dendrites provides essential information about synaptic integration, signal transmission and network connectivity. To image the morphology of live neurons, we have created a live-cell morphological tracing tool. In comparison to BrainBow, which enabled tracing the morphology of fixed neurons through fluorescent proteins, our new molecular tool can be used for tracing densely packed, live neurons in real-time both in-vitro and in-vivo. We also traced densely packed neurons in conjunction with their spiking activity using calcium sensors, thereby linking morphology with the physiology of neural networks. This technique will enable a better understanding of neural connectivity and physiology, as well as the detection of neurodevelopmental defects and compromised physiology.

Disclosures: C. Zhou: None. **S. Seman:** None. **S. Carmody:** None. **M. Preisegger:** None. **T. Kunkhyen:** None. **J. Gregory:** None. **C.E. Cheetham:** None. **M.S. Gold:** None. **O.A. Shemesh:** None.

Poster

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Title: Neurd: automated proofreading and feature extraction for connectomics

Authors: *B. A CELII[1], S. PAPADOPOULOS[2], Z. DING[3], E. Y. WANG[3], P. FAHEY[2], C. PAPADOPOULOS[4], S. S. PATEL[3], A. BAE[5], A. L. BODOR[6], J. BUCHANAN[7], D. J. BUMBARGER[7], S. DORKENWALD[8], L. ELABBADY[6], T. MACRINA[8], G. MAHALINGAM[6], D. XENES[9], P. K. RIVLIN[10], C. A. BISHOP[9], B. A. WESTER[11], E. FROUDARAKIS[12], E. Y. WALKER[4], F. H. SINZ[13], F. C. COLLMAN[6], N. M. DA COSTA[6], X. PITKOW[14], A. S. TOLIAS[3], J. REIMER[3];
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Abstract: We are now in the era of millimeter-scale electron microscopy (EM) volumes collected at nanometer resolution (Shapson-Coe et al., 2021; Consortium et al., 2021). Automated segmentation methods can now yield exceptionally accurate reconstructions of cells (3-D meshes), but despite this accuracy, laborious post-hoc proofreading is still required to generate large connectomes free of merge and split errors. Therefore, extracting the detailed information (from the diameter, shape, and branching patterns of axons and dendrites, down to the fine-scale structure of dendritic spines) requires substantial effort to piece together existing tools into custom workflows. Building on existing open-source software for mesh manipulation, here we present "NEURD", a software package that decomposes each meshed neuron into a compact and extensively-annotated graph representation. With these feature-rich graphs, we implement workflows to automate a variety of tasks that would otherwise require extensive manual effort. Specifically, the package implements the following tasks and was validated on both the MICrONS and H01 datasets (with some of the MICrONS test set validation indicated): glia removal, soma segmentation, spine segmentation (f1 = 0.96, n = 18030 spines), exc / inh cell typing (acc = 0.98, n = 4024 exc cells, 961 inh cells), automatic proofreading (exc axon - prec = 0.87, recall = 0.61; exc dendrite - prec = 0.99, recall = 0.99; inh axon - prec = 0.91, recall = 0.57; inh dendrite - prec = 1, recall = 0.99; n=122 exc cells, 75 inh cells), cell typing for subclasses, and compartment segmentation. With the resultant cleaned meshes and comprehensive annotations and features, a wide variety of questions could be asked quickly, allowing our study to replicate, extend and explore new results. For instance, we replicated the known high correlation (0.762) between spine head volume and synaptic volume in both datasets (n = 222009 exc spines, 18237 inh spines), but then extended this observation, showing different linear relationships for different cell types. Additionally, we discovered that neurons with four or more synapses had significantly higher response correlations. With many more questions to ask of a

variety of scientific topics, NEURD makes these new massive and complex datasets more accessible to neuroscience researchers.

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Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.16/B50

Topic: B.07. Network Interactions

Support: U19MH114830

Title: Refining cortical-cortical and cortical-subcortical connectomes of the cortex: insights from molecularly-morphologically defined single full morph cell types

Authors: *Y. WANG[1], H.-C. KUO[1], S. YAO[1], X. KUANG[2], L. NG[1], P. LESNAR[1], Y. LI[2], L. EL-HIFNAWI[1], N. CHEN[1], R. DALLEY[1], G. WILLIAMS[1], J. ANDRADE WILSON[1], J. V. CHANDRASHEKAR[1], C. FARRELL[1], T. KARLSSON[1], S. WALLING-BELL[1], A. LI[3], H. GONG[3], Q. LUO[3], S. A. SORENSEN[1], H. ZENG[1];
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Abstract: Recent advances in neuroscience have brought to a deeper understanding of neuronal types molecularly defined, particularly those named as intratelencephalic (IT) and extratelencephalic (ET) cells, etc. Alongside, classical morphological classifications of cortical neurons (such as tufted TPC, small tufted SPC, stellate SSC, etc.) based on histochemical and Golgi staining techniques, have provided a foundational understanding of cortical circuitry for more than a century. The delineation of these cell types has relied on distinct somatodendritic and local axonal structures, particularly on the apical dendrite's morphology. Our study aims to bridge these two perspectives by integrating molecularly defined cell types with classical morphological types. For this study, neurons were sparsely labeled using tamoxifen-inducible Cre driver lines crossed to a bright GFP reporter. Single full morph cells were reconstructed by

using Vaa3D-TeraVR, based on a whole brain image stack acquired with a two-photon fluorescence micro-optical sectioning tomography system (2p-fMOST). Utilizing ~1.4k full morph cells from ML & CEBSIT public sources, we delineate ten molecular-morph combined cell types through comprehensive reconstructions of more than 1.2k single full morph cells across ~15 cortical regions spanning six functional areas, including L2/3IT_TPC, L4IT_SSC, L4IT_UPC L4IT_TPC, L5IT_SPC, L5ET_TPC, L5/6IT_IPC, L6CT_ NPC, L6IT_car3PC, L6IT_PC. Quantitative analysis of these integrated cell types across regions delves into their morphological features, convergent and topographic constructions, and connectomes, contrasting them with bulk injection-derived connectomes. Multiple organizational principles were uncovered in governing neuronal networks across the whole brain, particularly shedding light on cortical-cortical (C-C) and cortical-subcortical (C-subC) connectomes. Moreover, limitations of bulk injections were highlighted in detecting projection targets, which may have implications for future research. This integrated approach not only refines our understanding of cortical circuitry but also sets a precedent for future investigations into the intricate interplay between molecular and morphological aspects of neuronal diversity.

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Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.17/B51

Topic: B.07. Network Interactions

Title: Electrophysiological assay system based on iPSC-derived astrocytes generated by a rapid differentiation method

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Abstract: Astrocytes are involved in the regulation of various activities in the central nervous system. Recent studies have revealed that astrocytes also play pivotal roles in nervous system diseases. The neuron/astrocyte co-culture system is a tool that mimics living neural tissues and is an attractive test bed for drug screenings, toxicity assays, and disease research. The Quick-Tissue™ technology (Elixirgen Scientific, Inc.) is a transcription factor-based method for rapid differentiation of induced pluripotent stem cells (iPSCs) into desired cell types. To investigate whether astrocytes generated with the technology (“Quick-Glia™ Astrocyte” or “eSci

Astrocytes") are suited for pharmacological studies, we analyzed their neuron-supporting functions contributing to the establishment of robust neuron/astrocyte co-cultures. We co-cultured eSci Astrocytes with excitatory neurons generated with the method ("Quick-Neuron™ Excitatory) and compared them to neuronal monocultures. The cells were cultured on multi-electrode arrays (MEAs) and pharmacological analyses were conducted in the 6th week of culture. In neuronal monocultures, neurons tend to aggregate and exhibited sporadic firings, making long-term cultures unstable. In contrast, neurons co-cultured with eSci Astrocytes exhibited robust neurite extension and network formation and showed stable neuronal firing. In the co-cultures, an antagonist of NMDA-type glutamate receptors decreased neuronal firing, while an antagonist of GABA receptors exhibited no effect. Similar trends were confirmed across multiple cell batches. In addition, the results were consistent with those obtained for co-cultures of excitatory neurons and primary astrocytes. These results suggest that the eSci astrocytes facilitate the formation of excitatory synapses and that the co-culture system is applicable for drug response evaluations. Therefore, MEA plate assays based on astrocytes and excitatory neurons generated by the Quick-Tissue™ technology are useful evaluation systems for disease analyses and drug discovery.

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Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

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Title: Cerebral organoids reciprocally connected with a bundle of axons for modeling interregional circuits

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Abstract: An inter-regional cortical tract is one of the most fundamental architectural motifs that integrates neural circuits to orchestrate and generate complex functions of the human brain. To model inter-regional projections on development of neural circuits, we connected two cerebral organoids with a bundle of reciprocally extended axons. The connected organoids produced more complex and intense oscillatory activity than conventional or directly fused cerebral organoids, suggesting the inter-organoid axonal connections enhance the complex network activity. In addition, optogenetic stimulation of the inter-organoid axon bundles could entrain the activity of the organoids and induce short-term plasticity. These results demonstrated that the projection axons could serve as a structural hub that boosts functionality of the organoid-circuits. We will discuss how this model could contribute to further investigation on development and functions of macroscopic neuronal circuits in vitro.

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Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

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Title: Protosequences in brain organoids model intrinsic states in the developing cortex

Authors: T. VAN DER MOLEN[1], A. SPAETH[2], M. CHINI[3], S. HERNANDEZ[4], G. KAURALA[2], H. SCHWEIGER[5], J. BARTRAM[6], K. BHASKARAN-NAIR[7], L. BLAUVELT[2], L. PETZOLD[8], P. HANSMA[9], M. TEODORESCU[2], A. HIERLEMANN[10], K. B. HENGEN[11], I. L. HANGANU-OPATZ[12], K. S. KOSIK[13], ***T. SHARF**[2];
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Abstract: The theory of Neural Darwinism (Edelman 1987) states that, similar to evolutionary biology, neuronal function is governed by natural selection, and should favor pre-configured networks that are built bottom-up. However, technology limits have prevented direct evaluation of this theory - it has been impossible to meaningfully separate complex networks from the effects of experience. Human brain organoids are proving to be a valuable tool in neuroscience and are enabling the research community to model human brain development from the bottom-up (Sharf 2022). However, efforts to understand network-scale neurophysiology within organoids has been limited. Here, we demonstrate a major advance in understanding organoid neuronal dynamics and their utility as a tool to study the establishment of pre-configured networks in the human brain using state-of-the-art high-density microelectrode arrays (Molen 2024). We reveal that brain organoids of mouse and human origin can support spontaneous activity patterns that draw from log-normal firing rate and connectivity distributions. These features reflect underlying network constraints that are conserved across brain regions and phylogeny, and are key features of neural coding and information processing (Buzsaki 2014). Second, we discovered that embedded within spontaneous activity are a subset of neurons capable of temporally structured activity that establish sequences that arise devoid of sensory experience. This observation is important because experience-dependent pre-play of sequences has been shown to emerge from spontaneous patterned activity (Dragoi 2023); however, there has yet to be any evidence supporting that they are generated before eye opening and navigation occur. Third, we show that sequence and non-sequence generating neurons form low and high-dimensional latent subspaces, which give rise to non-random probabilistic state transitions. Finally, we describe the presence of non-random, sequence generating neuronal firing patters that are also present in neonatal slices of the mouse somatosensory cortex, which at this developmental stage are largely devoid of sensory input. Notably, these results provide the earliest experimental evidence of sequential pattern generation in the developing mouse brain. Importantly, these findings were not present in 2D primary cortical cultures with a random network architecture. These results highlight the potential for brain organoids to further explore how exogenous inputs can be used to refine neuronal circuits and enable new studies into the genetic mechanisms that govern assembly of functional circuitry during early human brain development.

Disclosures: T. van der Molen: None. **A. Spaeth:** None. **M. Chini:** None. **S. hernandez:** None. **G. Kaurala:** None. **H. Schweiger:** None. **J. Bartram:** None. **K. Bhaskaran-Nair:** None. **L. Blauvelt:** None. **L. Petzold:** None. **P. Hansma:** None. **M. Teodorescu:** None. **A. Hierlemann:** None. **K.B. Hengen:** None. **I.L. Hanganu-Opatz:** None. **K.S. Kosik:** None. **T. Sharf:** None.

Poster

PSTR151: New Approaches To Probing Circuit Interactionsand Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR151.20/B54

Topic: B.07. Network Interactions

Support: JSPS KAKENHI Grant JP22K06475

Title: The red nucleus and its networks

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Abstract: The red nucleus is traditionally divided into the magnocellular and the parvocellular part, where existing neurons have been thought to project to the spinal cord (SC) and inferior olive nucleus (IO), respectively. However, neurons of varying size are found in each part, and further the rubrospinal pathway were shown to include outputs not only from magnocellular but also from parvocellular neurons. This indicates that the morphological division of the red nucleus does not by itself completely define the component and functional parts of this nucleus.In this study, we classified the cells of red nucleus into three groups by projection targets: rubrospinal (RS), rubro-olivary (RO), and rubro-thalamic (RT) neurons. We accomplished this classification by retrograde labeling of their respective targets, namely SC, IO, and posterior thalamic nucleus (PT). We compared the cell characteristics, sizes, distributions within the nucleus, and the neurotransmitter phenotypes of axons.Initially, we retrogradely labeled RS, RO, and RT neurons using retrograde virus tracers, AAVretro-fluorescent proteins. These tracers were injected into SC, IO, or PT. We found that each neuron groups exhibited a range of cell size, including small and large cells, and were widely located along the rostro-caudal axis.Secondly, we performed anterograde labeling of RO and RT axon terminals by injecting biotin dextran amine (BDA) into the red nucleus. This allowed us to investigate the transmitter phenotypes associated with each axon. We employed antibodies against vesicular glutamate transporter (VGluT1and T2) and vesicular GABA transporter (VGAT) to identify excitatory and inhibitory terminals, respectively. In contrast to widely studied RS terminals, which were almost all VGluT2-positive, there has been no study of RO or RT terminals. We found that RO terminals contained mostly VGluT2 with few VGAT, while those of RT terminals contained mostly VGAT with few VGluT2. There was no terminal contained VGluT1. These results indicate that RO and RT neurons are mainly excitatory and inhibitory, respectivelyNext, we studied their cell characteristics by whole cell recordings with optogenetic stimulation. We expressed ChR2 in the cortico-rubal axons by the injection of AAV1-ChR2 into sensorimotor cortex and recorded cortico-rubal EPSCs by stimulating cortico-rubal axons with blue light. The differences in electrophysiological characteristics and synaptic plasticity were found between RO and RS neurons, while those of RO and RT neurons were similar.

Disclosures: T. Ohno: None. **N. Murabe:** None. **S. Fukuda:** None. **H. Miyake:** None. **T. Hayashi:** None. **T. Miyashita:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.01/B55

Topic: B.08. Epilepsy

Support: IBRO early career grant
ISN CAEN 1B grant

Title: Ptz kindling model of mood disorders associated epilepsy: an assessment of comorbidities progression versus exposure to seizures

Authors: *G. NGOUPAYE;
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Abstract: About 30-40% of patients affected by epilepsy have an associated psychiatric disorder. The present study was designed to characterise the sequence of occurrence of the two most prevalent comorbidity of epilepsy, anxiety and depression following kindling. Animals were divided in groups as follow, control groups, a group subjected to kindling (CKEOD+0), a group subjected to kindling which received 7 extra injections following kindled state (CKEOD+7), and a group subjected to kindling which received 14 extra injections following the kindling sate (CKEOD+14). Animals were then subjected to EPM, OFT to evaluate anxiety, to SPT and FST to evaluate depression. 24h following the last behavioural test, animals were decapitated and their blood, hippocampi, prefrontal cortices (PFC) were collected to assess oxidative stress (GSH, CAT, MDA), inhibitory signalisation (GABA and GABA-T), excitatory signalisation (glutamate and EAAT-2), neuroinflammatory signalisation (IL-1β, TNF-α and TGF-1β), and HPA-axis (corticosterone and CRH) and histological changes were also assessed. Results revealed that anxiety manifests before depression. The expression of the anxious phenotype is maximum and optimum in CKEOD+7, and depressive phenotype was maximum and optimum in CKEOD+14. Biochemical analysis demonstrated that oxidative stress was enhanced in all kindled groups both in the hippocampus and the PFC. Depressed inhibitory signalling coupled to an increased excitatory signalling pathway as well as increased CRF in hippocampi of both anxious and depressed animals and increased plasma corticosterone levels in depressed animals was shown. In addition, there was an enhancement of pro-inflammatory and a dampening of anti-inflammatory mechanisms in the hippocampus of kindled animals. Taken together these results demonstrate that on a sequential basis, anxiety occurs before depression, thus preclinical screening of anxiolytic and anti-depressive drugs in epilepsy animal models should consider this time scale for optimum response.

Disclosures: G. Ngoupaye: None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.02/B56

Topic: B.08. Epilepsy

Support: PROSNI 2023, CVM/LMC
Fortalecimiento 2023, LMC
Fodecijal 10229-2022, LMC

Title: Behavioral, Electrophysiological and Morphological Characterization of a Novel Epilepsy Model Induced by 4-Aminopyridine in Adult Male Rats

Authors: *O. VARGAS[1], C. VENTURA[2], L. G. MEDINA-CEJA[3];
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Abstract: Electrophysiological, Morphological and Behavioral Characterization of a Novel Epilepsy Model Induced by 4-Aminopyridine in Adult Male Rats Oreth E. Vargas-López, Consuelo Ventura-Mejía*, Laura Medina-Ceja. Epilepsy is a neurological disorder characterized by sustained and synchronized discharge of a cluster of brain neurons with a significant challenge in treatment due its resistant to conventional drugs, and animal models are employed to investigate the mechanisms of induction. 4-Aminopyridine (4AP) is a drug known for its ability to enhance synaptic transmission, which when administered intracerebrally induce seizures across various animal species. However, its potential for inducing spontaneous and recurrent seizures remains understudied.
In this study, we employed intracerebroventricular administration of 4-AP to assess its potential to induce spontaneous seizures in male Wistar rats. Animals were divided into control (0.9% NaCl) and experimental groups (4-AP, 20 mM). Convulsive behavior was evaluated post-administration (Racine scale, rating of 3, 4/5 considered as indicative) and subsequent monitoring via video (24 hours/7 days up to 6 months). Rats exhibiting spontaneous seizures underwent implantation of superficial electrodes for EEG recordings. Finally, was evaluated the morphology in the hippocampus and entorhinal cortex (hematoxilina-eosina) and performed cell counting.
Thirteen out of 16 animals showed spontaneous seizures (56% with a 4/5 Racine scale), lasting on average 104 seconds. The latency of spontaneous seizures was 128 days, resulting in a mortality rate of 19% (3 out of 16 rats). EEG analysis over three consecutive days revealed an average amplitude of 515.280μv ± 475.401μv (n=6). Morphology in the hippocampus demonstrates a reduction in the number of cells present in different areas of the brain compared to that shown in the control group control (n=1) (CA1 96.5 ± 32.745, CA2 83.6 ± 15.214, CA3 88.8 ± 9.731, Cortex 79.25 ± 5.737) experimental (n=2) (CA1 58.3 ± 17, CA2 61.416 ± 10.518, CA3 56.03 ± 5.759, Cortex 64.923 ± 19.435) these preliminary results show the decrease or neurons in the damaged tissue, however, analysis continues to complete the number of rats. These findings demonstrate that 4-AP has the ability to induce spontaneous seizures characterized by convulsive behavior and EEG abnormalities. Furthermore, status epilepticus post-4-AP administration persisted unabated by any drug intervention, and no additional care for

the animals was required. Support by University of Guadalajara (PROSNI 2023, CVM/LMC, Fortalecimiento 2023, LMC) and CoecytJal (Fodecijal 10229-2022, LMC).

Disclosures: O. Vargas: None. **C. Ventura:** None. **L.G. Medina-Ceja:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.03/B57

Topic: B.08. Epilepsy

Support: PE501082200-2023-PROCIENCIA

Title: Behavioral and Time-Dependent Molecular Characterization of the Pentylenetetrazol Model in Rats: Insights into Endocannabinoid-related Changes

Authors: C. MEDINA-SALDIVAR[1,2], B. GUZMAN PARO[1], C. BECERRA FLORES[1], G. E. PARDO[1,2], *L. PACHECO[1,2];
[1]Univ. Andina Del Cusco, Cusco, Peru; [2]Inst. Cientifico, Cusco, Peru

Abstract: Impaired seizure control can lead to long-lasting sequelae, such as refractory temporal lobe epilepsy and impaired cognitive functions. To address this, current efforts are directed toward the search for new and effective anticonvulsant drugs that can control and reduce the progression of epilepsy. Research suggests that activation of the endocannabinoid system may have therapeutic value due to its ability to reduce excessive excitatory signaling. To investigate this possibility, it is imperative to understand how epileptogenesis progresses and interacts with the response and adaptation of the endocannabinoid system. Our goal was to describe PTZ-induced epileptogenesis over time in both behavioral phenotype and molecular changes in endocannabinoid-related genes and proteins in the CA3 region of the hippocampus. First, 1-month-old Sprague-Dawley rats (n = 15) received an initial dose (70 mg/kg i.p.) and then a subconvulsant dose (35 mg/kg i.p.) of pentylenetetrazole (PTZ) or alternate-day saline (EOD), followed by 30 min of videotaping for 35 days. Latency, frequency and severity of epileptiform behavior were assessed using a modified Racine scale. Subsequently, PTZ was administered to another group of Sprague-Dawley rats (n = 35) in a manner similar to the first experiment. After 24 hours and 7, 14, 21, 21, 28 or 35 days of PTZ administration, brains were collected and CB1, FAAH and NAPE-PLD mRNA and protein expression were assessed in comparison with a control group (PND 65). We observed that 100% of PTZ-exposed rats reached a kindled state (Racine scale 4 or 5 for at least two continuous sessions). Catalepsy and neck jerks were the only epileptiform behaviors that showed time-dependent changes in both latency and frequency during the 35 days of the first experiment. When gene expression was analyzed, an increase in Faah and Cnr1 mRNA expression was observed on day 7, followed by a decrease on day 14, and then a slight increase in expression on the following days of PTZ administration. Napepld mRNA expression remained unaltered during the protocol (KW, p = 0.025). As for protein

expression, despite having the same trend as mRNA expression, CB1r, and FAAH expression remained unaltered throughout the experiment (KW, $p = 0.12$ and $p = 0.34$, respectively). This study provides evidence that catalepsy and neck jerks can be used to assess kindling status in the PTZ protocol. In addition, endocannabinoid-related gene expression, such as Faah and Cnr1, changes in the first weeks but tends to normalize by the end of the protocol. Nevertheless, despite the time-dependent gene changes, protein expression of endocannabinoid proteins such as CB1 and FAAH remained unchanged.

Disclosures: C. Medina-Saldivar: None. **B. Guzman Paro:** None. **C. Becerra Flores:** None. **G.E. Pardo:** None. **L. Pacheco:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.04/B58

Topic: B.08. Epilepsy

Support: ADRD Supplement R01NS095872-05S1 (GFB)
NIH/NINDS R01NS1129722-01S1 Diversity Supplement (GFB/BLK)
NIH/NIGMS T32GM139776 (University of Iowa MSTP; BLK and MJEL)

Title: Nighttime tendency of spontaneous spike-and-wave discharges and seizure-associated death in young APP/PS1 mice

Authors: *B. L. KREITLOW[1], M. J. E. LARSON[2], A. R. JONES[1], H. CUI[1], G. F. BUCHANAN[1];
[1]Neurol., [2]Univ. of Iowa Carver Col. of Med., Iowa City, IA

Abstract: *Background:* Alzheimer's Disease (AD) is a neurodegenerative disease hallmarked by neurofibrillary tangles and senile plaques composed of amyloid-β peptide (Aβ). Clinical and pre-clinical research have primarily focused on cortical pathology. Current AD therapies primarily aim to reduce symptom burden, and disease-modifying therapies have demonstrated limited efficacy in individuals with profound neuropathological changes. Studies have shown Aβ deposition in the brainstem before cortical pathology and prior to overt symptom onset. A bidirectional relationship has been suggested between AD and epilepsy, a neurological disease characterized by unprovoked seizures. Generalized seizures have been observed in a subset of AD patients. Late-onset epilepsy patients have demonstrated changes of cerebrospinal fluid Aβ42. *Rationale:* Multiple pre-clinical models of AD have epileptiform activity after the onset of cortical pathology. Previous studies have demonstrated frequent epileptiform discharges, uncommon generalized tonic clonic seizures (GTCS), and premature mortality of uncertain origin in the APP/PS1 mouse model of amyloidopathy. Preliminary evidence from our lab suggest that spontaneous seizures begin much earlier in life and likely preclude pathological

changes in the cortex. *Methods:* APP/PS1 mice express human mutant amyloid precursor protein (Mo/HuAPP695swe, APP) and a mutant human presenilin 1 (PS1-dE9, PS1). Young (8 - 16-week-old) animals were instrumented with hardware for electroencephalography (EEG) and electromyography for continuous video-EEG. *Results:* Continuous video-EEG revealed young APP/PS1 mice experienced 108 spike-and-wave discharges (SWDs) per day (95% CI: 78.67 - 137.1, N = 10). The mean SWD was 939 ms in duration (95% CI: 0.9116 - 0.9664, N = 1,019), but differed between individuals ($p<0.0001$, ordinary one-way ANOVA with multiple comparisons, N = 3). SWDs were approximately 3.2-times more likely to occur during the nighttime ($p<0.0001$, two-tailed paired t-test, N = 10). Two fatal seizures were captured with video-EEG. Both deaths were preceded by a GTCS (36 - 39 seconds in duration) with full hind-limb extension and occurred during the nighttime. *Conclusions:* The APP/PS1 mouse model of amyloidopathy experiences spontaneous epileptiform activity and seizure-associated death prior to evident cortical pathology. Better understanding the relationship between covert AD-associated pathology in the brainstem, AD-related epileptogenesis, and seizure-related death in this model may help us better understand the relationship between AD pathogenesis, epilepsy, and sudden unexpected death in epilepsy.

Disclosures: B.L. Kreitlow: None. **M.J.E. Larson:** None. **A.R. Jones:** None. **H. Cui:** None. **G.F. Buchanan:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.05/B59

Topic: B.08. Epilepsy

Support: R01NS129722 (GFB)
The Joanna Sophia Grant form CURE Epilepsy (GFB)

Title: Time-of-day-dependent seizure mortality in a mouse model of spontaneous epilepsy is greatest during the subjective dusk and night.

Authors: *A. NOVELLA MACIEL[1], B. KREITLOW[2], G. F. BUCHANAN[3];
[1]Univ. of Iowa, Iowa City, IA; [2]Neurosci., Univ. of Iowa, Iowa City, IA; [3]Neurol., Univ. of Iowa, Iowa City, IA

Abstract: *Rationale:* Epilepsy is a common neurological disease characterized by spontaneous seizures. Sudden unexpected death in epilepsy (SUDEP) is the leading cause of death in patients with medically refractory epilepsy. Dravet Syndrome is a genetic form of epilepsy that is associated with frequent seizures and a high rate of SUDEP. Seizure-associated death occurs more commonly at night, which is often attributed to seizures occurring during sleep. This is also true in multiple mouse models of epilepsy, including the $Scn1a^{R1407X/+}$ mouse model of Dravet Syndrome, when mice are housed in a standard 12:12 light-dark cycle. Our lab has shown that

the nighttime tendency persists in constant darkness following induced seizures in other mouse models. However, this has not been demonstrated in a genetic mouse model of epilepsy. *Methods:* In this project, young Scn1a$^{R1407X/+}$ mice (PND 18-21) were placed into constant darkness in individual cages with pyroelectric infrared motion sensors for long-term locomotor activity monitoring. This was used to determine their free running period, which was used to identify the endogenous circadian time of day when fatal seizures occurred. Additionally, some mice were under constant observation through cameras so that their seizure activity could later be analyzed. Mice were monitored in this manner until experiencing a fatal seizure, determined by the mice being found dead in pen with full hind limb extension, or until post-natal day 90. *Results:* In alignment with previous findings from our lab, the results demonstrated that *Scn1a*$^{R1407X/+}$ mice are more likely to die following spontaneous seizures during the subjective night. Additionally, there was a spike in mortality around the subjective dusk, which corresponds with the light to dark transition in a 12:12 light-dark cycle. *Conclusions:* Our lab has demonstrated that both induced and spontaneous models of seizure-associated death are time-of-day-dependent and persist in constant darkness, suggesting that an endogenous circadian rhythm may mediate the nighttime susceptibility of seizure-associated death.

Disclosures: A. Novella Maciel: None. **B. Kreitlow:** None. **G.F. Buchanan:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.06/B60

Topic: B.08. Epilepsy

Support: R01NS129722 (GFB)
Undergraduate Fellowship from the UI office for Undergraduate Research (EEH)

Title: Investigating a Role of Time of Day and Serotonin On The Effects of Seizures On CO2 Arousal in Amygdala Kindled Mice

Authors: *E. HERMAN[1], B. KREITLOW[2], G. F. BUCHANAN[3], A. JONES[1];
[1]Univ. of Iowa, Iowa City, IA; [2]Neurosci., Univ. of Iowa, Iowa City, IA; [3]Neurol., Univ. of Iowa, Iowa City, IA

Abstract: Rationale: Epilepsy is a neurological disorder characterized by recurrent spontaneous seizures. Anti-seizure drugs fail in 1⁄3 of people with epilepsy. These patients are at higher risk of sudden unexpected death in epilepsy (SUDEP). SUDEP is more common at night in both humans and rodent models. Respiratory failure is a potential mechanism for SUDEP and the neurotransmitter serotonin (5-HT) is implicated due to its time-of-day modulation and role in epilepsy, sleep, and breathing. Several etiologies have been proposed for SUDEP, including impaired arousal. CO2 is a potent arousal stimulus. Preliminary studies from our lab suggest that

seizures impair CO2 arousal. We hypothesized that CO2 arousal is impaired to a greater extent by seizures occurring during the night, which may contribute to increased nighttime mortality. Methods: Serotonin-neuron deficient (Lmx1bf/f/p) mice and wildtype (Lmx1bf/f) littermates were implanted with hardware to monitor EEG and EMG for sleep-wake state and seizure recording and with a bipolar electrode in the basolateral amygdala (BLA; AP= -1.34, ML= -2.80, DV= -4.70, mm from bregma) to induce epileptogenesis via amygdala kindling. The BLA rendered hyperexcitable by increasing current stimulation (60 Hz, biphasic square waves; 1 second duration; 20 µA step; 2 minute inter-stimuli interval) until mice experienced an epileptiform discharge. This afterdischarge threshold was repeated twice daily until three consecutive bilateral tonic-clonic seizures were elicited. After amygdala kindling, mice were exposed to 7% CO2 (21% O2, balanced N2) or room air (21% O2, balanced N2) before and after seizures at different times of day (Zeitgeber Time 6 and 18 +/- 1 hour). Mice were exposed to CO2 after falling asleep (based on EEG and EMG characteristics of NREM sleep) until awakening. Results: During the daytime, arousal latency following seizure induction was significantly increased in wild-type controls (2.11-fold increase; $p = 0.0094$, paired t-test) and to a lesser extent in 5-HT knockout animals (1.66-fold increase; $p = 0.0277$, paired t-test). Interestingly, seizures induced during the nighttime resulted in profoundly impaired CO2 arousal (14.81-fold increase; $p = 0.0268$, paired t-test). Conclusion: The inability to be awakened when exposed to CO2 postictally is a risk factor for SUDEP. This arousal mechanism being blunted at night may contribute to the nighttime mortality trend observed in SUDEP.

Disclosures: E. Herman: None. **B. Kreitlow:** None. **G.F. Buchanan:** None. **A. Jones:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.07/B61

Topic: B.08. Epilepsy

Support: DFG: HE8155/1-2 (FOR-2715)
Johannes Dichgans doctoral scholarship from Hertie Institute for Clinical Brain Research

Title: Characterization of a Kcna2 loss-of-function mouse model

Authors: *H. CALAP[1], P. MUELLER[1], A. ELTOKHI[3], T. OTT[2,4], H. LERCHE[1], T. V. WUTTKE[1,5], U. B. HEDRICH[1];
[1]Dept. of Neurol. and Epileptology, [2]Hertie Inst. for Clin. Brain Research, Univ. of Tuebingen, Tuebingen, Germany; [3]Dept. of Biomed. Sci., Mercer Univ. Sch. of Med., Columbus, GA; [4]Max Planck Inst. for Biol. Cybernetics, Tuebingen, Germany; [5]Dept. of Neurosurg., Univ. of Tuebingen, Tuebingen, Germany

Abstract: Developmental and epileptic encephalopathies (DEEs) represent rare but severe neurological conditions that place immense burdens on affected patients and their families. In 2015, we identified DEE patients with variants in *KCNA*2, a gene that encodes the voltage-gated potassium channel subunit $K_V1.2$.[1] DEE patients with *KCNA*2 loss-of-function (LOF) variants predominantly experience focal epileptic seizures.[1] We recently generated a *Kcna2*$^{+/P405L}$ knock-in mouse model to understand both epileptogenic and compensatory effects for this LOF variant. We performed metabolic and behavioral phenotyping, intracranial video EEG monitoring, as well as patch clamp recordings from acute slices. Brains of mice aged P62-112 that previously underwent EEG were cut in coronar slices and stained against $K_V1.2$ and Caspr to identify the paranodal region of nodes of Ranvier and c-Fos and NeuN to detect the unknown origin of focal seizures caused by this variant. Furthermore, we performed Golgi stainings for spine morphology.
All *Kcna2*$^{+/P405L}$ mice with C57Bl/6 background died prematurely between one and two months of age, while roughly half of Swiss *Kcna2*$^{+/P405L}$ mice showed normal survival. In general, *Kcna2*$^{+/P405L}$ mice showed a slight hyperactivity, with males exhibiting underweight tendencies. Notably, during EEG recordings heterozygous *Kcna2*$^{+/P405L}$ mice exhibited focal and bilateral tonic-clonic seizures. We found significantly increased c-Fos expression in primary motor and somatosensory cortices and the hippocampus in brains of mutant animals. The distribution of channels including $K_V1.2$ subunits at nodes of Ranvier was not different for *Kcna2*$^{+/P405L}$ animals compared to their wildtype littermates. Firing frequency of cortical pyramidal cells was similar to wildtype and the only difference in intrinsic properties was a significant increase in the afterhyperpolarization amplitude.
To conclude, *Kcna2*$^{+/P405L}$ mice presented focal seizures and increased mortality. The origin of these seizures might lie in the cortex, since seizures often evolved from motor to bilateral tonic-clonic ones but is likely not driven by increased excitability of pyramidal neurons. In addition, *Kcna2*$^{+/P405L}$ mice were hyperactive, and males displayed underweight.
[1] Syrbe S, Hedrich UBS et al. (2015). De novo loss- or gain-of-function mutations in *KCNA2* cause epileptic encephalopathy. Nature genetics.

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Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.08/B62

Topic: B.08. Epilepsy

Support: NIH Grant NS084959

Title: Seizure-induced alterations of gene expression profiles within the dentate gyrus of Scn1a$^{+/-}$ Dravet syndrome mice

Authors: T. N. THENSTEDT, D. M. ECHEVARRIA-COOPER, N. A. HAWKINS, *J. A. KEARNEY;
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Abstract: *Scn1a*$^{+/-}$ mice recapitulate key features of Dravet syndrome, an epileptic encephalopathy characterized by epilepsy, developmental delay, and high mortality risk. Phenotype penetrance and severity vary depending on genetic background. *Scn1a*$^{+/-}$ mice on a 129S6/SvEvTac strain (S6.*Scn1a*$^{+/-}$) have no overt phenotype, while *Scn1a*$^{+/-}$ mice on a [S6 x C57BL/6J]F1 strain (F1.*Scn1a*$^{+/-}$) exhibit seizures and early lethality. We used single nucleus RNA sequencing (snRNAseq) to profile gene expression at the single cell level. Prior to seizure onset, there were few differentially expressed genes (DEGs) between Scn1a$^{+/-}$ and WT on either strain. Following seizure onset, F1.*Scn1a*$^{+/-}$ mice had many DEGs, particularly within dentate granule cells (DGCs). In this study, we examined DGC subclusters unique to F1.*Scn1a*$^{+/-}$ mice with seizures, performing DEG and gene ontology (GO) analysis, and RNAscope in situ hybridization to localize top DEGs within the dentate gyrus. Female and male S6.*Scn1a*$^{+/-}$ and F1.*Scn1a*$^{+/-}$ mice and wild-type (WT) littermates (S6.WT or F1.WT) were used for snRNAseq. *Scn1a*$^{+/-}$ mice were video-monitored for seizures at P22-P24 and stratified by presence or absence of seizures prior to hippocampi collection at P24. Single nuclei were isolated and 3' v3 libraries were prepared, sequenced, and analyzed using Cell Ranger (7.0.1), Seurat (4.3.0), DeSeq2 (1.34.0) and SynGO. A separate cohort of F1.*Scn1a*$^{+/-}$ mice were perfused at P24 and processed for RNAscope to determine localization of two representative seizure-upregulated genes within the dentate gyrus. In the absence of seizures, there were no DEGs in the DGC cluster between genotypes. However, F1.*Scn1a*$^{+/-}$ mice with seizures had unique DGC subclusters distinguished by activity-dependent gene profiles. Enrichment analysis indicated overrepresentation of “synapse” and “process in the synapse” GO terms. *Penk* (proenkephalin) and *Sorcs1* (Sortilin-Related VPS10 Domain Containing Receptor) were among the most highly seizure-upregulated genes. We investigated expression patterns of *Penk*, *Sorcs1* and the DGC marker *Prox1* comparing F1.*Scn1a*$^{+/-}$ mice with or without seizures. *Penk* and *Sorcs1* expression was upregulated in the DGC layer of mice with seizures compared to no seizures. Mice with more frequent seizures within six hours of tissue collection had greater upregulation than those with fewer seizures. *Penk* expression was present throughout the DGC layer, while *Sorcs1* expression exhibited a medial bias with the DGC layer, suggesting upregulation in newer DGCs. Overall, our results show that F1.Scn1a$^{+/-}$ mice with seizures have altered DGC gene expression profiles suggestive of altered synaptic activity.

Disclosures: T.N. Thenstedt: None. **D.M. Echevarria-Cooper:** None. **N.A. Hawkins:** F. Consulting Fees (e.g., advisory boards); Takeda Pharmaceuticals. **J.A. Kearney:** 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.; Praxis, Neurocrine. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Novartis, Stoke Therapeutics, Emugen, Regel Therapeutics, Biogen, Encoded Genomics, GlaxoSmithKline, GW Pharma, Iama Therapeutics, Pfizer, Regulus, Sangamo, Tevard. F. Consulting Fees (e.g., advisory boards); Dravet Syndrome Foundation, FamilieSCN2a.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.09/B63

Topic: B.08. Epilepsy

Support: R01NS129722 (GFB)
The Joanna Sophia Grant form CURE Epilepsy (GFB)

Title: The role of serotonin in spontaneous time-of-day-dependent seizure mortality in the Scn1a$^{R1407X/+}$ mouse model of Dravet Syndrome

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Abstract: *Background:* Sudden unexpected death in epilepsy (SUDEP) is the leading cause of death in individuals with medically refractory epilepsy. The mechanisms leading to SUDEP are poorly understood, but the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) has been implicated due to its role in the regulation of breathing, arousal, and influence on seizure severity and mortality. SUDEP occurs more frequently during the nighttime, and levels are lowest during the night in both humans and nocturnal rodents. The *Scn1a*$^{R1407X/+}$ mouse model of Dravet Syndrome experiences spontaneous seizures early in life and has a high rate of seizure-associated mortality. Fatal seizures occur more often during the nighttime, consistent with human SUDEP observations and other nocturnal mouse models of seizure-associated death. Preliminary evidence from our lab has shown that mice lacking central nervous system 5-HT neurons have high seizure-induced mortality regardless of time of day. *Rationale:* Oscillating levels of 5-HT throughout the twenty-four-hour day may influence risk of seizure-associated death. The aim of this study is to determine the role of 5-HT in time-of-day-dependent seizure mortality in a mouse model of spontaneous seizure-associated death. *Methods:* *Scn1a*$^{R1407X/+}$ mice (PND 18 - 21) were individually housed in cages outfitted with motion detectors and video monitored chronic monitoring to identify spontaneous seizure-associated death. Mice received an 800 mg/kg dose of para-chlorophenylalanine (PCPA) or volume-matched saline injection intraperitoneally for five consecutive days or until experiencing a fatal seizure. PCPA is an irreversible tryptophan hydroxylase inhibitor, significantly reducing 5-HT after repeated injections. *Results:* Treatment with PCPA significantly blunted weight gain over the course of injections (slope = -0.43%, R^2 = 0.0090, simple linear regression) compared to saline-treated controls (slope = 7.55%, R^2 = 0.7150). Consistent with previous findings, saline-treated controls were more likely to die during the dark-phase (5/7) with a peak mortality early in the dark-phase (Zeitgeber Time (ZT) 17). Interestingly, PCPA-treated animals died more frequently during the light phase (6/7), with peak mortality just prior to the light-dark transition (ZT 10). *Conclusions:* Preliminary evidence suggests that 5-HT is involved in the nighttime risk following both induced and spontaneous seizures. Understanding how low levels nighttime 5-HT increase risk of seizure-associated death

could set the stage for novel chronotherapeutic strategies to reduce risk of SUDEP for people living with epilepsy.

Disclosures: S. Ryan: None. **B. Kreitlow:** None. **A. Jones:** None. **A. Novella Maciel:** None. **M. Summerfield:** None. **G.F. Buchanan:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

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Topic: B.08. Epilepsy

Support: CCHMC Department of Anesthesia
NIH NS065020
NIH NS062806

Title: Focal post-natal deletion of TSC2 causes epilepsy

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Abstract: Epilepsy is one of the most common neurological symptoms in patients with tuberous sclerosis complex, occurring in approximately 80 percent of affected children. This disorder frequently occurs from two-hit germline + somatic mutations to either the TSC1 or TSC2 genes, causing hyperactivation of the mTOR pathway. Most animal models use germline or cell type specific mutations, which do not recapitulate the focal nature of patient brain lesions. We aimed to model a focal loss of Tsc2 from cortical excitatory neurons, which will allow investigators to better study the process in which these lesion cause and maintain spontaneous seizures. Here, we removed Tsc2 from cortical neurons by injecting a small volume (4 x 50 nl) of AAV9-CaMKII-Cre-mCherry into the cortex of $Tsc2^{fl/fl}$ (n=6M; 4F) and $Tsc2^{wt/wt}$ (n=6M; 4F) pups at postnatal day 2. At 8-12 weeks old, mice were implanted with cortical electrodes, and underwent at least one week of 24/7 video-EEG monitoring, before tissue was collected. All focal $Tsc2^{fl/fl}$ knockout (fTsc2 KO) mice had seizures. Mice averaged 3.95 +/- 0.49 (mean +/- SEM) seizures per day, whereas littermate $Tsc2^{wt/wt}$ (control) mice had no seizures ($p<0.001$). Seizures lasted on average 40.29 +/- 2.89 seconds. Histological analyses showed mCherry labeled cells in fTsc2 KO mice were larger in diameter compared to those in control mice (371 ± 25.6 μm^2 and 221 ± 20.6 μm^2, respectively, $p<0.001$) and were more likely to express pS6, a measure of mTOR activation, compared to control mice (93.36% ± 1.64 and 52.60% ± 6.39, respectively, $p<0.001$). fTsc2 KO mice also showed decreased parvalbumin (PV) and somatostatin (SST) cell density compared to control mice (PV: KO, 4609 ± 625 and control, 7347 ± 570 per mm^3, $p<0.01$; SST: KO, 4988 ±

550 and control, 6706 ± 504 per mm^3, $p<0.05$). In conclusion, we describe a simple and robust approach to introduce focal loss of Tsc2 in mouse brain. The neonatal AAV-Cre cortical injection approach reliably produced a focal area of viral infected cells without disrupting animal development and led to a robust epilepsy syndrome. Histological analyses confirmed upregulation of mTOR in abnormally large, virally infected cells in fTsc2 KO mice, although increased pS6 may also be due to the seizure phenotype. Moreover, fTsc2 KO mice had decreased inhibitory neurons in the region surrounding the abnormal Tsc2 knockout cells, which likely promotes spontaneous seizures. Further histological analyses are ongoing to delineate changes in the cortex of fTsc2 KO mice that support seizure development and possible targets for therapeutic interventions.

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Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

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Program #/Poster #: PSTR152.11/B65

Topic: B.08. Epilepsy

Title: Hypomyelination and decreased number of oligodendrocytes in a loss-of-function Slc35a2 mouse model

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Abstract: Focal Cortical Dysplasias (FCDs) are the most common etiology of medically intractable epilepsies. One type of FCD, mild malformations of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE), is classified by increased clusters of oligodendrocytes in the white matter accompanied by hypomyelination and heterotopic neurons. Approximately half of diagnosed MOGHE patients carry somatic pathogenic SLC35A2 variants in resected brain tissue. SLC35A2 encodes uridine diphosphate galactose transporter (UGT), a transmembrane protein responsible for shuttling galactose from the cytosol into the Golgi apparatus for N-linked glycosylation. We hypothesized that deletion of *Slc35a2* in oligodendrocytes will result in hypomyelination in mice, resembling the MOGHE pathology in patients. To test our hypothesis, we utilized a conditional knockout (cKO) of *Slc35a2* in oligodendrocytes to achieve an oligodendrocyte-specific deletion of Slc35a2 using Cre-LoxP. Littermates without the Olig2-cre were considered as controls. All fourteen male/female were hetero/hemizygous for the Slc35a2-floxed allele, and approximately half of both litters were positive for the Olig2-cre (referred to as “cKO”). Six cKO mice (7.3 ± 0.79) were smaller than their eight littermate controls (9.8 ± 1.39) in weight (g). Immunohistochemistry revealed fewer

Olig2+ cells within the corpus callosum of cKO mice compared to controls. CNPase staining demonstrated patches of hypomyelination throughout the cortex in cKO mice in contrast to no lesions in controls. Investigating the understudied aspects of white matter pathology and the role of Slc35a2 in oligodendrocytes is necessary to probe the underlying mechanisms of MOGHE.

Disclosures: T. bartel: None. **A. Nemes-Baran:** None. **C. Ghosh:** None. **I.M. Najm:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

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Program #/Poster #: PSTR152.12/B66

Topic: B.08. Epilepsy

Support: 1R56NS123565-01A1

Title: Cellular, circuit and behavioral changes resulting from chronic developmental inhibition of $Na_v1.6$ underlie development of epilepsy in Xenopus laevis tadpoles

Authors: *A. C. THOMPSON, C. VADUVA, C. A. TORO CEPEDA, K. NEVAREZ, R. THOMPSON, D. MENDOZA, C. D. AIZENMAN;
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Abstract: $Na_v1.6$ channelopathies cause a debilitating childhood disorder characterized by intellectual disability and infantile seizures. To develop effective treatment strategies, we must first understand the cellular mechanisms by which neurons and circuits become abnormally excitable as a result of $Na_v1.6$ channel dysfunction. Seizures manifest in a newborn's first months, suggesting that neuronal and circuit changes that trigger seizures occur during later stages of perinatal development. As such, we need an experimentally tractable model system with levels of analysis ranging from single synapses to circuits and behavior, to study the molecular mechanisms of these events in embryonic development. We previously found that modulation of $Na_v1.6$ expression is a key regulator of excitability in neurons of the *Xenopus* optic tectum during circuit development and for visual stimulation-driven homeostatic changes in excitability. We extend on these findings to describe a model of developmental $Na_v1.6$ dysfunction induced by rearing *Xenopus* tadpoles in the specific $Na_v1.6$ channel inhibitor MV1312 and characterizing the molecular, biophysical, circuit and behavioral changes. Using 4hr and 24hr MV1312 exposures in later circuit development, we also show how cells and circuits of the tectum become highly excitable as a result of $Na_v1.6$ channel inhibition and how these changes compare to generalized Na_v channel inhibition using tetrodotoxin. Our whole-cell patch clamp electrophysiology and field potential recordings show that $Na_v1.6$ inhibition results in a maladaptive increase in the excitability of tectal neurons that is driven by increased Na^+ current amplitude, occuring together with an elevated E/I ratio and ictal-like discharges. To further assess how $Na_v1.6$ dysfunction affects neuronal connectivity, we measured dendritic morphology of tectal neurons following chronic $Na_v1.6$ channel inhibition. Our analysis showed

that developmental MV1312 exposure caused abnormal branching patterns with an increased number of branches formed closer to the soma. Next, we quantified behavior of freely-swimming tadpoles, showing that the altered biophysical properties and morphology of tectal neurons are associated with a spontaneous seizure phenotype. Finally, we used gene expression analysis to investigate the molecular mechanisms by which developmental MV1312 exposure results in increased Na^+ current amplitude and increased neuronal excitability. Our findings reveal cellular and molecular mechanisms for how chronic $Na_v1.6$ dysfunction affects neuronal and circuit development and function during a disease-relevant window of embryonic development.

Disclosures: A.C. Thompson: None. **C. Vaduva:** None. **C.A. Toro Cepeda:** None. **K. Nevarez:** None. **R. Thompson:** None. **D. Mendoza:** None. **C.D. Aizenman:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.13/B67

Topic: B.08. Epilepsy

Support: RO1NS131223

Title: A single-cell and spatially-resolved atlas of cell autonomous and non-autonomous effects of Nprl2 mutations in a mouse model of focal cortical dysplasia

Authors: *A. BISWAS[1], S. BRUCKMEIER[2], S. A. AMENT[3], P. H. IFFLAND, II[4];
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Abstract: Focal Cortical Dysplasia 2 (FCD2) is a malformation of cortical development leading to childhood-onset epilepsy, most commonly caused by loss-of-function mutations in genes that regulate the mammalian target of rapamycin (mTOR) pathway, including *NPRL2*, which encodes the nitrogen permease regulator like 2. Somatic *NPRL2* mutations in excitatory neural progenitors cause numerous developmental changes, including mTOR hyperactivation, neotony, migration deficits, and enlarged soma, but the molecular mechanisms are poorly understood. Here, we produced a single-cell and spatial transcriptomic atlas for the effects of *Nprl2* mutations in a mouse model of FCD2. *Nprl2* loss-of-function was induced in excitatory cortical progenitors via *in utero* electroporation with CRISPR/Cas9 constructs. Control animals were electroporated with non-targeting guide RNAs. An additional group received *Nprl2* knockout guide RNAs coupled with rapamycin treatment to test whether the effects of *Nprl2* knockout can be rescued by blocking mTOR hyperactivation. We characterized the electroporated regions with 10x Genomics single-nuclei RNA sequencing. We characterized the entire cortical hemisphere and adjacent subcortical regions using cellular-resolution spatial transcriptomics with Curio Seeker (Slide-seq). We are analyzing these data to identify differentially expressed genes

associated with *Nprl2* mutations and the effects of rapamycin, including both cell autonomous effects in excitatory neurons and cell non-autonomous effects on the surrounding regions.

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Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.14/B68

Topic: B.08. Epilepsy

Title: Audiogenic Seizure Susceptibility in Cntnap2 Knockout Mice

Authors: *L. STEWART, S. TOOMEY, M. LAMANNA, J. GREWAL, K. KOTTMANN, L. A. GABEL;
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Abstract: Contactin-associated protein-like 2 (Cntnap2) gene has been associated with a number of neurodevelopmental disorders, including cortical dysplasia-focal epilepsy (CDFE) syndrome. In humans, CNTNAP2 mutations are associated with focal or secondarily generalized seizures,which started around two years of age. Mice lacking the Cntnap2 gene experience spontaneous seizure behavior beginning between 4-6 months of age (Thomas et al. 2017; Peñagarikano et al., 2011), which is associated with a reduction in parvalbumin-positive interneurons in the hippocampus, and a reduction in the amplitude and frequency of inhibitory postsynapticpotentials onto pyramidal cells in CA1 of the hippocampus (Peñagarikano et al., 2011; Paterno et al., 2021). Interestingly, conflicting results regarding the presence of spontaneous seizure behavior in Cntnap2 knockout (KO) mice has been noted. In one study, 90% of Cntnap2 KO mice exhibited spontaneous seizure behavior, with 42% exhibiting clonic/tonic seizure with loss of balance (Peñagarikano et al., 2011), whereas another study recorded increased epileptiform activity between 4 - 8 months of age in CA1 of the hippocampus, but with no indication of motor seizures (Thomas et al., 2017). In this study we examined spontaneous seizure behavior,and auditory induced seizure behavior, in Cntnap2 KO and C57Bl6/J mice between 6 - 10 months of age. Cntnap2 KO mice have been shown to exhibit an increased sensitivity to auditory stimuli, whereas C57Bl6/J mice are resistant to audiogenic seizures. An 18 kHz tone was played at an intensity of either 100 or 110 dB, and presented continuously for a duration of 1-3 minutes. Seizure behavior was noted for 3 minutes prior to the presentation of the tone, during the tone, 2 minutes following the presentation of each tone, and for 3 minutes following the completion of the experiment. Using a modified Racine scale for mice to evaluate spontaneous motor seizures, Cntnap2 KO mice were regularly observed exhibiting stages 3 and 4 (bilateral forelimb clonus [stage 3] with rearing behavior [stage 4]) prior to the onset of the tone, with a few mice experiencing full loss of righting reflex or jumping [stage 5]. These behaviors continued through the presentation of the tone, however, the number of events significantly increased during the post-stimulus interval. It is unclear whether the enhanced seizure activity

observed in the Cntnap2 KO mice following the stimulus is the result of increased stress generated by the presentation of the stimulus. Future studies need to examine the relationship between susceptibility to stress and seizures in Cntnap2 KO mice.

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Poster

PSTR152: Epilepsy Mechanisms: Animal Models

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Topic: B.08. Epilepsy

Support: NIH Grant NS115049

Title: Interleukin-1 Type 1 Receptor (IL-1R1) in the hippocampal dentate gyrus is implicated in generalized tonic-clonic seizures in a transgenic mouse model of adult-onset epilepsy

Authors: T. DILLON[1], A. TRAN[2], ***C. ISGOR**[3];
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Abstract: Dysregulations in hippocampal dentate granule neuron (DGN) gating properties may create a permissive synaptic environment that spreads hyperexcitability in epileptogenic forebrain circuits. The pro-inflammatory cytokine interleukin-1-type-1-receptor (IL-1R1) exhibits elevated levels in clinical models of epilepsy, implicating several inflammatory pathways in pro-convulsive synaptic plasticity. IL-1R1 is abundantly present in DGNs. We use a transgenic mouse that overexpresses the brain derived neurotrophic factor under the CAM kinase II alpha promoter in the forebrain as a model of adult-onset epilepsy (termed TgBDNF mice). TgBDNF mice develop generalized tonic/clonic seizures (GTCSs) at ~3 months of age in response to tail lifting/ cage agitation, which progressively intensify evidenced by prolonged durations of post-ictal generalized cortical suppression (PGES) with successive GTCSs, indicative of increased risk of death. In advanced stages, mice expire following a very prolonged PGES. In this study we hypothesized that IL-1R1 is a pro-epileptogenic modulator of dentate synaptic circuits in the TgBDNF mouse model of adult-onset epilepsy. We bred the TgBDNF mice with a mouse that had selective expression of IL-1R1 in the dentate gyrus, primarily in DGNs (termed IL-1R1 RESTORE). IL-1R1 is globally knocked out by disruptions in gene sequence with targeted mutations (termed IL-1R1 KO). Mating the global KO mouse with a region specific CreERT allowed restoration of the IL-1R1 in the dentate. Conditional activation of RESTORE is accomplished by tamoxifen injections (i.p., 5 days, 75 mg tamoxifen / kg body weight) on postnatal days 29-33. TgBDNF/IL-1R1 RESTORE, TgBDNF/IL1R1 KO, and TgBDNF without alterations in IL1R1 were implanted with skull EEG arrays to monitor epileptogenesis. Preliminary data showed that 50% of the TgBDNF mice with IL-1R1 KO did

not develop GTCSs with at least 12 weeks of seizure assessment. The remaining mice that did develop GTCSs had milder seizures without progressive increase in the duration of the PGES compared IL-1R1 intact TgBDNF mice. Preliminary findings also showed that the TgBDNF mice with restored IL-1R1 in dentate gyrus had indistinguishable progression to epilepsy when compared to EEG data from control TgBDNF mice. Further data will be collected to compare the patterns of temporal ontogeny to epilepsy and severity of GTCSs with successive seizure events between TgBDNF mice with intact IL-1R1 versus TgBDNF mice with restored IL-1R1 specifically in the dentate gyrus. Findings from this study will establish the critical role of IL-1R1 signaling particularly in DGNs in the emergence and severity of epilepsy.

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Poster

PSTR152: Epilepsy Mechanisms: Animal Models

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Program #/Poster #: PSTR152.16/B70

Topic: B.08. Epilepsy

Title: Gut microbiota plays a critical role in the physiological neuronal activation in the brain

Authors: *S. KAI[1], T. MATSUDA[1], T. KITAMURA[2], K. NAKASHIMA[1];
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Abstract: Bidirectional communication between the gut and the brain affects health and disease. Various environmental and/or peripheral factors influence gut-brain interactions, including intestinal microbiota. Epileptic patients often have gastrointestinal symptoms, while patients with inflammatory bowel disease have a higher susceptibility to epilepsy. The ketogenic diet (KD) has long been used as a non-drug therapy with good curative effects in patients with drug-resistant epilepsy, especially children when surgery is not medically appropriate. These clinical phenomena could be caused by the alteration of the composite of microbiota in response to KD. A recent study has reported that KD suppressed seizures by altering pathology-specific gut microbiota in the epileptic model mice (Olson et al. Cell 2018). However, it remains unknown whether the gut microbiota, which is endemic under normal conditions, regulates neuronal activity and/or seizure susceptibility in the adult brain. Here, we show that the microbiota residing in the physiological condition exaggerates neuronal activity-induced seizure in the adult mouse brain. The glutamate receptor stimulant, convulsant-inducing kainic acid (KA), was administered to 12-week-old sterile (GF) and specific pathogen-free (SPF) mice, and convulsion scores were evaluated. We found that GF mice had significantly lower seizure scores than SPF mice, indicating that GF mice are less likely to cause convulsions than SFP mice. We then immune-stained brain sections of these mice with a marker for neuronal activity, cFos, 1 hour after KA challenge. Many cFos-positive cells were observed in SPF mice treated with KA, but few were observed in GF mice, consistent with their low seizure susceptibility. When KA was

administered to 3Abx mice that received three mixed antibiotics (ampicillin, neomycin, and vancomycin) treatment from 8 to 12 weeks of age, the mice had significantly lower seizure scores than control SPF mice. These data suggest that microbiota present in normal SPF mice impairs neuronal function, aggravating seizure susceptibility. To clarify whether these changes in seizure susceptibility were mediated by metabolites released from bacteria, aerobic and anaerobic culture supernatants of mouse or human feces were administered to 3Abx mice. We observed that aerobic culture supernatant increased seizure susceptibility to KA in 3Abx mice. Overall, these results suggest that aerobic bacterial metabolites in mouse and human feces disturb neuronal function and intensify seizure susceptibility.

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Poster

PSTR152: Epilepsy Mechanisms: Animal Models

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Program #/Poster #: PSTR152.17/B71

Topic: B.08. Epilepsy

Support: NINDS K08NS118107 (CMM)

Title: Whole brain activity mapping to dissect the molecular pathogenesis of brain disorders in larval zebrafish

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Abstract: Genetic brain disorders such as epilepsy often lack validated molecular mechanisms to enable precision pharmacotherapy. Although some developmental epileptic encephalopathies (DEE) affect genes with clear synaptic function (e.g. SLC6A1, encoding GABA Transporter 1 (GAT1); SCN1A, encoding alpha subunit of voltage-gated sodium channel Nav1.1), many DEE are caused by pathogenic variants in genes with many functions, no direct synaptic function, or unknown functions (e.g. CDKL5, cyclin-dependent kinase-like 5). Meanwhile, the use of reduced preparations such as acute slice physiology may be of limited utility to explain the systems-level perturbations that occur in response to the combination of acute and chronic molecular deficits associated with gene loss-of-function. We propose to integrate existing techniques to assess whole brain activity from calcium fluorescence in larval zebrafish expressing genetically encoded calcium indicator (elavl3::GCaMP6s) in the setting of defined molecular perturbations using a combination of pharmacological and genetic approaches.

Disclosures: C.M. McGraw: None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

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Topic: B.08. Epilepsy

Support: NIH Grant R01NS111122

Title: Pnpo deficiency induced developmental impairment and lethality in drosophila and mice

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Abstract: Pyridox(am)ine 5'-phosphate oxidase (PNPO) converts dietary vitamin B6 (VB6) to the active form, pyridoxal 5'-phosphate (PLP), a coenzyme required for the syntheses of GABA. Mutations in *PNPO* have been found in neonatal epileptic encephalopathy patients, characterized by severe seizures in newborns and developmental delay. We previously discovered a fly strain with a hypomorphic point mutation in *PNPO* (*sgll*^{95}). We also generated knock-in strains by replacing the fly *PNPO* with human *PNPO* (*hPNPO*) found in epilepsy patients (h^{WT}, h^{R116Q}, h^{D33V} and h^{R95H}, with h^{R95H} being the most severe). We investigated specific developmental stages in which PNPO deficiency leads to developmental impairments and lethality and contributions by specific cell types. With normal fly diet, no homozygous h^{R95H} flies were generated. We supplemented heterozygous h^{R95H} breeders with PLP. We found a dose-dependent rescue of homozygous h^{R95H} with complete rescue by 40 μg/mL. And fewer homozygous *sgll*^{95} adult flies were generated under normal fly diet. We treated w^{1118} and *sgll*^{95} flies with a chemically defined synthetic diet without VB6. If eggs were transferred from grape juice agar plate to diet lacking VB6, both strains didn't grow much bigger and didn't molt, *sgll*^{95} larvae were much smaller than w^{1118} larvae, all of them died before day 10. If adult flies were transferred to diet lacking VB6, *sgll*^{95} flies didn't survive more than one week, while w^{1118} flies had normal survival, indicating that PLP is necessary for larvae development and adult survival. To examine which specific cell type is most responsible for lethality, we expressed *hPNPO* in specific cell types on the *sgll*^{95} background. Survival of adult flies were tested on sugar-only (lack of VB6) diet. We found that survival was rescued by about 50% and 20% when *hPNPO* was expressed in GABAergic neurons and pan-glia, respectively; no rescue was observed when *hPNPO* was expressed in glutamatergic or cholinergic neurons, suggesting GABAergic neurons contribute most to lethality. We also generated knock-in mouse models by introducing the R116Q and D33V point mutations into the mouse *PNPO*. Homozygous D33V mice could not survive in embryonic development without PLP supplementation. With PLP supplementation, they were born and developed severe seizures after P15. By using a GABA sensor and *in vivo* fiber photometry recording, we found reduced GABA release in these mice compared to controls. We are currently characterizing their inhibitory postsynaptic responses.

Disclosures: W. Fu: None. **B. Wang:** None. **W. Chi:** None. **X. Zhuang:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.19/B73

Topic: B.08. Epilepsy

Support: EVMS Incentive Research

Title: Planarian model for experimental seizures

Authors: J. VU[1], T. MILLER[1], C. TRAN[1], K. HAQ[1], B. MUSTO[1], A. RITVIK[2], ***A. MUSTO**[1]; [1]Eastern Virginia Med. Sch., Norfolk, VA; [2]Pathology and Anat., Eastern Virginia Med. Sch., Norfolk, VA

Abstract: Epilepsy, a central nervous system disease affecting 50 million people globally, is characterized by spontaneous recurrent seizures which are mostly controlled by anti-seizure medication (ASM). However, there remains an unfulfilled medical need to find an ASM that effectively prevents seizures with minimum adverse effects. *Dugesia dorotocephala*, or planarian worms, present as a possible invertebrate model for experimental seizures due to their increased affordability, vertebrate-like neurons, and quantifiable behaviors, setting them apart from other invertebrates or larger animals. The goal was to evaluate seizure phenotype of *planarian* as a model of acute seizures. Planarians were exposed to different concentrations of pilocarpine solutions to investigate their behavioral response. Video tracking software was used to analyze pilocarpine-induced behaviors in areas consisting of center and perimeter zones. Behaviors were quantified by the following responses: in- zone frequency, in- zone cumulative duration, clockwise rotation, and counterclockwise rotation. Track pathway visualizations were obtained. After observing the planarian's behavior in pilocarpine, seizure phenotypes were identified through video analysis. Six seizure phenotypes in planarian were observed: oscillating dorsal expansion (ODE), head and tail dorsal expansion, C-shape, head and tail flick. Thereafter, planarians were exposed to pilocarpine and increasing concentrations of lamotrigine to investigate the effects of the anti-seizure medication. The morphology of the nerve fibers were then analyzed using the Golgi and immunohistofluorescence staining**.** Six seizure phenotypes in planarian were observed: oscillating dorsal expansion (ODE), head and tail dorsal expansion, C-shape, head and tail flick after pilocarpine. . Analysis of Variance (ANOVA) tests revealed statistically significant differences in average ODE frequencies between the experimental concentrations and the control concentrations for both pilocarpine and lamotrigine exposure. As the pilocarpine concentration increased, ODE frequency increased but was attenuated by lamotrigine. Moreover, preliminary data indicates that pilocarpine triggers seizures, disrupts the overall behavior of the planarian and induces nerve damage. These preliminary results support the use of planarians as models in epilepsy research. Future research would involve conducting histological studies, specifically synaptic analysis, and possibly genetic studies to further assess planarians as a model in experimental epilepsy. This is especially relevant for screening a potential ASM.

Disclosures: J. Vu: None. **T. Miller:** None. **C. Tran:** None. **K. Haq:** None. **B. Musto:** None. **A. Ritvik:** None. **A. Musto:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.20/B74

Topic: B.08. Epilepsy

Title: Seizures in a bang-sensitive epilepsy model impact associative learning in Drosophila larva

Authors: K. GAMMONS, S. CHO, *E. REYNOLDS;
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Abstract: The seizures associated with epilepsy may impact learning and memory and are associated with early cognitive decline, however, the mechanism of how the seizures themselves lead to these deficients is not clear. Drosophila mutant flies that have seizure phenotypes has been used effectively as a model for human epilepsy. In this study, we tested whether seizures induced in Drosophila *para*^{bss} mutants, a mutation in the fly sodium channel gene, affect learning. We used both rewarding and aversive stimuli to test associative learning in *Drosophila* larvae, similar to the methods developed by Apostolopoulou et al. (2010) and Pauls et al. (2010). Third instar larva were collected from CS (wildtype) and *para*^{bss} lightly laid bottles. For the rewarding stimulus task, larva were trained 3x by pairing an odor (octanol or amyl acetate) with a reward (sugar). They were then tested for movement towards the odor they were trained on in a choice assay. For the aversive learning task, a brief shock was paired with the odor as training and then movement away from the trained odor was determined. We found that these odors were not neutral as described in the literature. In control experiments, octanol was repulsive in our assays while amyl acetate was attractive. And so, octanol was used to train in a positive associate learning task and amyl acetate was used in aversive assays. We could demonstrate associative learning using larva in assays with both odors and that *para*^{bss} learned well as compared to wildtype using both rewarding and aversive stimulus tasks. To test the impact of seizure, larva were exposed to the cold between the training and testing trials, which cause the *para*^{bss} mutant larva to seize. Seizure in the mutant larva disrupted the rewarding and aversive associative learning. Similar to results we obtained previously in an adult courtship assay, seizures in *para*^{bss} mutants can disrupt learning.

Disclosures: K. Gammons: None. **S. Cho:** None. **E. Reynolds:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.21/B75

Topic: B.08. Epilepsy

Support: NSF 2052764

Title: Wireless recording of neural activity in two novel chronic epilepsy models in swine

Authors: *S. P. DUARTE[1], M. GUTIERREZ-HERNANDEZ[2], J. VOLAKIS[2], J. J. RIERA[1]; [1]Biomed. Engin., [2]ECE Dept., Florida Intl. Univ., Miami, FL

Abstract: Our team is currently developing a battery-less, wireless neurosensing system (mWiNS) that can be used to record and map epileptiform activity in drug resistant epilepsy patients facing surgical resection. The device consists of an implant connected to standard, FDA-approved electrodes and an interrogator that sits outside the scalp (Figure 1A, B). Although mWiNS has been validated in rats (Moncion et al., 2022), it needs to be tested in an animal model that is comparable in size and neural complexity to humans. Pigs are increasingly being used to study human neurological diseases such as Alzheimer's disease and traumatic brain injury (Hoffe & Holahan 2019), not only due to their larger brain size, but also for their comparable neuroanatomical structure. However, this shift towards using swine as a translational model is still in its infancy. While rodent models of chronic epilepsy are readily available, these have not yet been established in swine. Here we establish two novel models of chronic epilepsy by injecting an epilepsy-inducing agent into either the hippocampus (Figure 1C, D) or neocortex of pigs, before implanting depth (Figure 1E) or surface electrodes connected to the mWiNS implant. Our preliminary data recorded from an awake pig (Figure 1F) demonstrates that we can elicit and record seizure behaviors such as repetitive head bobbing, twitching and lip smacking (Figure 1G, top). We are also able to record neural activity from the pig while at rest, and demonstrate the natural cycling between high frequency activity interspersed with slow wave epochs. Therefore, the mWiNS device will allow us to wirelessly record subclinical epileptiform activity and acute seizure activity from the temporal lobe or neocortex of awake, behaving, epileptic swine.

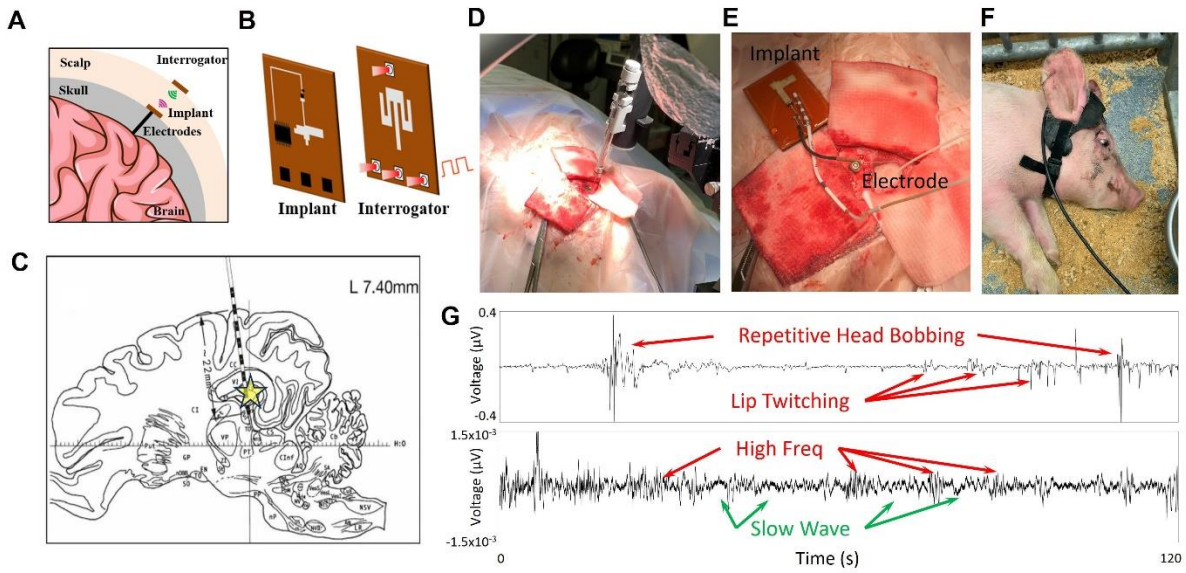

Figure 1: A) Schematic of wireless neural implant beneath scalp connected to standard, FDA approved electrodes, with interrogator outside scalp. B) Image of implant and interrogator. C) Pig brain atlas diagram showing penetrating electrode targeting hippocampus (yellow star). D) Micromanipuator with micro-injector for injecting kainic acid into hippocampus. E) Penetrating electrode placed in brain and connected to implant, ready for placement in subcutaneous pocket. F) Post-operative pig with implant and wearing helmet to hold interrogator to scalp during a wired recording session. G) Two minute recording from single channel implant while pig exhibits seizure behaviors such as repetitive head bobbing, twitching and lip smacking (top). Bottom panel shows another trial where the pig is resting quietly, where neural activity cycles between high frequency and slow wave epochs.

Disclosures: S.P. Duarte: None. **M. Gutierrez-Hernandez:** None. **J. Volakis:** None. **J.J. Riera:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.22/B76

Topic: B.08. Epilepsy

Title: Automated detection of spike-wave discharges in WAG/Rij rats: age-progression and response to chronic valproate

Authors: *J. R. HUXTER[1], P. PATRA[1], S. THEVARKUNNEL[2], L. GIGGINS[1], G. A. HIGGINS[2];
[1]Transpharmation Ltd. UK, London, United Kingdom; [2]Transpharmation Canada, Fergus, ON, Canada

Abstract: Absence seizures are characterised by sudden, frequent episodes of unconsciousness, often accompanied by automatic movements. Approximately 10% of childhood epilepsy cases involve typical absence seizures, with genetic factors believed to be the primary cause. It occurs at a rate of 6-8 per 100,000 children aged 0 to 15 years. To understand absence seizures, we employed the WAG/Rij rat model coupled with EEG in 6 rats, using a new automated detection

method to distinguish the spike-wave discharges (SWDs) associated with absence seizures from sleep-spindles. Over a period from 14 to 20 weeks of age, we conducted four EEG recordings (24 hours recording/session) at one-week intervals to monitor the emergence of SWDs, alongside locomotion, body temperature and wake/sleep architecture in animals implanted with DSI telemetry. The WAG/Rij rats showed an age-related increase in the number of SWDs ($F(2.20,19.78)=29.79;p<.001$), with a significant effect of time-of day ($F(1.89,17.00)= 28.54$, $p<.001$): most SWDs occurred during wakefulness in the second half of the dark phase (14WKS:4.00±0.91, 16WKS:7.00±1.42, 18WKS:11.25±1.87, 20WKS:19.86±1.96). There was also an age-related decrease in locomotor activity ($F(3, 27)=27.02;p<.001$) and core body temperature ($F(3,27)=26.46;p<.001$) in this strain, which was most evident during lights-off. Following these tests, we evaluated the effect of acute (250 mg/kg, i.p.) and sub-chronic (250 mg/kg BID, i.p.) valproate administration on these parameters. Valproate significantly reduced the number of SWDs relative to baseline ($F(4,24)=5.25;p=0.004$) with effects most evident during wakefulness in the first half of the dark cycle, and returning to supra-baseline levels after a 7-day washout (BASELINE:23.00±3.78, ACUTE:10.17±3.44,CHRONIC:4.67±3.49,WASHOUT:31.33±10.34). Notably, both acute and sub-chronic valproate treatments significantly mitigated spike-wave discharges, indicating an anticonvulsant effect. Our study thus provides comprehensive insights into the vigilance status and SWD patterns of WAG/Rij rats, while validating a fully automated SWD-detection algorithm using the known efficacy of valproate in reducing SWDs in this model. These findings thus contribute to our understanding of absence seizures, and enable higher-throughput screening of novel compounds for therapeutic intervention in epilepsy management.

Disclosures: J.R. Huxter: None. **P. Patra:** None. **S. Thevarkunnel:** None. **L. Giggins:** None. **G.A. Higgins:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.23/B77

Topic: B.08. Epilepsy

Support: NIH Grant R42NS107148

Title: Performance assessments of semi-automated noninvasive seizure detection in Scn8a mice

Authors: K. D. DONOHUE[1], D. HUFFMAN[2], T. L. CAMACHO[3], C. KRZYZANIAK[4], *B. F. O'HARA[6], *B. F. O'HARA[6,1], M. F. HAMMER[5], S. SUNDERAM[7];
[1]Signal Solutions, LLC, Lexington, KY; [2]Signal Solutions, LLC USA, Lexington, KY; [3]Univ. of Arizona, Tucson, AZ; [4]Univ. of Arizona, Tuscon, AZ; [5]BIO5 Inst., Univ. of Arizona, Tucson, AZ; [7]Dept. of Biomed. Engin., [6]Univ. of Kentucky, Lexington, KY

Abstract: Preclinical research into epilepsy and other seizure disorders often requires methods to assess changes in seizure events and their frequency. This is primarily accomplished through manual review of electroencephalographic (EEG) and/or video recordings, which is time- and resource-intensive. Thus, experimental tools for identifying seizures automatically and without the need for EEG could be of great utility in this field. This work presents performance results of an automated system that detects events of interest using piezoelectric motion sensors and applies machine learning techniques to distinguish seizures from arousals and other short-term changes in activity patterns. Ten Scn8a^{N1768D} mice (5m/5f; 1-3 months aged) were transferred to cages equipped to monitor motion via piezoelectric sensors and continuously monitored until SUDEP occurred. An accompanying video record was manually screened to identify a total of 227 seizure events, which were used to train and assess the performance of the automated system under test. An initial set of candidate events were detected by processing piezoelectric signals to compute a line length signal feature in 1-second intervals and identifying the top 800 peaks in the feature time series per day. Additional features were extracted around each candidate peak based on signal energy and coherence to fill out each event bout. Then a set of 16 descriptors from each event bout associated with envelope shape, amplitude statistics, and contrast with neighboring intervals were extracted and evaluated using a minimum-redundancy maximum-relevance criterion, and the top 8 features were fed into an ensemble decision learning algorithm. A bootstrapping resampling method was applied to randomly extract 160 seizure events and 160 non-seizure events from the candidate set. An ensemble decision tree regression algorithm was applied to each bootstrapped sample using a 5-fold cross validation train-and-test cycle to produce a seizure likelihood value, and thresholds were applied to results with a 90% recall rate, and the 95% confidence ranges for precision were computed over 25 bootstrapped tests. For a 90% recall seizures rate, the 95% confidence range for precision was 88% to 91%. Similarly for an increased 95% recall rate, the precision ranged from 81% to 86%. Overall, these early results indicate the potential utility of this approach as a pre-screening method to identify regions with high probability of seizure and greatly reduce the time required to assess seizure yield in mouse models of seizure disorders.

Disclosures: K.D. Donohue: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions, LLC. **D. Huffman:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. **T.L. Camacho:** None. **C. Krzyzaniak:** None. **B.F. O'Hara:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions, LLC. **B.F. O'Hara:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions, LLC. **M.F. Hammer:** None. **S. Sunderam:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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Topic: B.08. Epilepsy

Support: MRTS-CCTST Pilot Award (D.T)
NIH grants R01NS107453
Postdoctoral fellowship from the American Epilepsy Society (D.T)

Title: From autism to epilepsy network: Age-dependent home cage behavior and reactivity in a Cntnap2 mouse model

Authors: M. SHERIDAN[1], M. RICE[2], R. MAHADESHWAR[1], C. SUNIL[1], C. GROSS[2,3], ***D. TIWARI**[4,5];
[1]Neurol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Neurol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [3]Department of Pediatrics, University of Cincinnati, Cincinnati, OH; [4]Div. of Neurol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [5]Neurology, Department of Pediatrics, University of Cincinnati, Cincinnati, OH

Abstract: Contactin-associated protein-like 2 (CNTNAP2) is a transmembrane protein that mediates neuron glia interaction and regulates dendritic spine growth and neuronal migration. Mutations in the *Cntnap2* gene are linked to autism and epilepsy. Younger *Cntnap2* KO mice mimic autism phenotypes, while older mice are a model for epilepsy. Thus, comparing behavioral phenotypes across different ages is needed to better understand the age dependent development of disordered brain networks caused due to lack of *Cntnap2* expression. Male and female *Cntnap2* KO and WT controls were tested across different age groups (4, 5, 7, 9, and ~11 months) using digging, stimulus (reactivity), and nesting assays. Older *Cntnap2* KO mice (7, 9, and ~11 months) showed a significant increase in home cage reactivity (stimulus) assay compared to younger mice at 4 and 5 months of age ($p<0.05$). Similar trends were observed in male and female *Cntnap2* KO mice. No significant differences were observed in the WT controls. A significant difference in digging assay was observed in KO female mice between younger (4 month) and older mice post nest removal ($p=0.03$). An age-dependent significant reduction in nesting behavior was observed in female KO mice ($p=0.006$), however no difference was observed in the WT controls. Ongoing analysis is looking at the age dependent intraneuronal network in the *Cntnap2* KO mice using parvalbumin immunostaining. Our findings suggest disruption in home cage behavior and reactivity in older epileptic *Cntnap2* KO mice indicating an age-dependent network alteration and behavior deficits.

Disclosures: M. Sheridan: None. **M. Rice:** None. **R. Mahadeshwar:** None. **C. Sunil:** None. **C. Gross:** None. **D. Tiwari:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.25/B79

Topic: B.08. Epilepsy

Support: NIH NINDS R01-NS126247
VA I01-BX004938

Title: Altered neuronal activation in the hippocampus and cerebellum of seizure-prone CACNA2D2 knockout mice

Authors: *A. GALLAGHER[1], A. ANDERSON[1], A. DANIS[1], E. SCHNELL[1,3,2];
[2]Neurosci. Grad. Program, [1]Oregon Hlth. & Sci. Univ., Portland, OR; [3]Portland VA Healthcare Syst., Portland, OR

Abstract: The voltage-gated calcium channel subunit gene CACNA2D2 controls calcium-dependent signaling in neurons, and loss of this subunit causes epilepsy and ataxia in both mice and humans. This gene is primarily associated with parvalbumin-neuron cell function in the cerebellum and hippocampus. Homozygous CACNA2D2 mutant mice manifest electroencephalographic spike-wave discharges (SWDs) as well as generalized tonic-clonic seizures. SWDs are typically associated with aberrant thalamocortical activation, but due to the existence of generalized seizure events, we had sought to determine whether these mice manifested signs of altered neuronal activation in the hippocampus and cerebellum. We measured expression of the activity-dependent c-fos and ΔFosB in juvenile (21-28 do) CACNA2D2 wildtype (WT) and knockout (KO) mice, using immunohistochemical staining and confocal microscopy. Both genotypes demonstrated similarly sparse c-fos and ΔFosB expression within the hippocampal dentate granule cell layer (GCL) at baseline, consistent with no difference in basal activity of granule cells between genotypes. Surprisingly, when mice were assayed 1 hour after handling-associated convulsions, KO mice had fewer c-fos-positive cells but dramatically increased ΔFosB expression in the dentate gyrus compared with WT mice. After administration of a subthreshold pentylenetetrazol dose, however, KO mice dentate had significantly more c-fos expression compared to WT. In contrast to the lack of difference in activity of WT and KO mice at baseline in the hippocampus, KO animals at baseline had increased ΔFosB expression across several lobes of the cerebellum, suggesting differential activation of cerebellar circuits between genotypes. Together, our work suggests differences in the basal and seizure-associated activation of the dentate gyrus and cerebellum in the absence of α2δ-2 protein, which may relate to altered functional properties of their corresponding neuronal components.

Disclosures: A. Gallagher: None. **A. Anderson:** None. **A. Danis:** None. **E. Schnell:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.26/

Topic: B.08. Epilepsy

Support: CAPES
CNPq Grant 306981/2023-8
FAPESP

Title: Impairments of neocortical synaptic inhibition and glucose metabolism in a two-hit model of epilepsy

Authors: *F. DOS SANTOS[1,2], T. MARTINS[3], G. LAZZAROTTO[4], G. TERIBELE VENTURIN[5], J. C. DA COSTA[5], E. R. ZIMMER[4], N. GARCIA-CAIRASCO[6], M. CALCAGNOTTO[3];
[1]Univ. Federal do Rio Grande do Sul, Porto Alegre, Brazil; [2]Biochemistry, NNNESP Lab-UFRGS, Porto Alegre, Brazil; [3]Biochem., NNNESP Lab-UFRGS, Porto Alegre, Brazil; [4]Biochem., Univ. Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil; [5]Inst. Do Cerebro (insCer) - Brain Inst., Porto Alegre, Brazil; [6]Ribeirao Preto Sch. Med., Ribeirao Preto, Brazil

Abstract: Deficits in GABAergic system and disruption in glucose metabolism may contribute to brain hyperexcitability in refractory epilepsies, such as those associated with developmental cortical malformations (DCM). Here we evaluated the spontaneous inhibitory postsynaptic currents (sIPSC) into cortical pyramidal neurons and the brain glucose metabolism in a two-hit animal model of epilepsy (Wistar Audiogenic Rat [WAR] with DCM). DCM was induced in the right somatosensory cortex (SSC) of newborn Wistar (WIS) and WAR animals by freeze-lesion (FL) (WIS-SHAM, WIS-DCM, WAR-SHAM, WAR-DCM). The sIPSC were recorded in the layer III cortical pyramidal cells using the whole-cell patch-clamp technique and brain glucose metabolism was measured by [^{18}F] FDG uptake in Positron Emission Tomography (microPET) scans at P35-45. The sIPSC were recorded in pyramidal cells of paramicrogyral cortex (right) and contralateral SSC (left). The sIPSC parameters were analyzed with MiniAnalysis 6.0.7 software. The microPET images were captured (n=4-5 animals/group) and acquired in a rat magnetic resonance template. Standardized [^{18}F] FDG uptake value ratio (SUVr) was calculated using the pons as a reference. The sIPSC and microPET data were statistically analyzed by General Linear Models followed by Bonferroni (Jamovi 2.3.28 software, $p \leq 0.05$). The voxel levels were analyzed by Student´s *t* test-statistics (RMINC), $p<0.05$ ($t>2$) (Ethical committee approval # 40836). Neurons from contralateral SSC of WAR-DCM (n=5 cells/5 animals) had faster sIPSC rise-time than WAR-SHAM (p=0.002, n=8 cells/5 animals), WIS-SHAM ($p<0.001$, n=7 cells/4 animals) and WIS-DCM ($p<0.001$, n=11 cells/8 animals) in the same hemisphere. The right temporoparietal cortex of WAR-DCM had lower [^{18}F] FDG uptake than WIS-SHAM (p=0.05). Thalamus of WAR-MDC and WAR-SHAM had lower [^{18}F] FDG uptake than WIS-SHAM, regardless of DCM and hemisphere (p=0.010, p=0.042, respectively). Hippocampi of WAR-DCM also had lower [^{18}F] FDG uptake than WIS-SHAM (p=0.05) regardless of the hemisphere. A voxel Student´s *t* test-statistics showed hypometabolic regions in detail, mainly in WAR-DCM. These preliminary findings from cortical pyramidal cells in WAR-DCM, demonstrating faster sIPSC kinetics, suggest possible changes in cortical inhibition. The observed glucose hypometabolism may reflect a metabolic disruption within the reorganized network. Together, these factors could potentially play a role in the refractory epileptogenesis.

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Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.27/B80

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

Support: NINDS NS121817
NIMH T32MH065215-19

Title: Altered GABAergic Neurotransmission in mouse model of slc6a1^{s295l}developmental and epileptic encephalopathy

Authors: *K. ZAVALIN[1], K. RANDHAVE[2], J.-Q. KANG[3];
[1]Vanderbilt Univ. Med. Ctr., Nashville, TN; [2]Neurol., Vanderbilt Univ. Med. Ctr., Nashville, TN; [3]Dept Neurol., Vanderbilt Univ., Nashville, TN

Abstract: We have a long history of studying neurologic disorders caused by variants of *SLC6A1*, coding for ɣ-aminobutyric acid (GABA) transporter 1 (GAT-1) that is the primary means of extracellular uptake of inhibitory neurotransmitter GABA. The most severe are developmental and epileptic encephalopathies with interrelated phenotypes of epilepsy and developmental delay, for which pathophysiologies are often poorly defined, and treatment options are lacking, requiring urgent investigation of both. In the past, we found that chemical chaperone 4-phenylbutyrate restored pathologies in GAT-1 expression and GABA uptake for multiple *SLC6A1* variants *in vitro* and *in vivo*, and significantly reduced seizures in knock-in mice expressing the *SLC6A1*(S295L) variant. Our research prompted an ongoing clinical trial (NCT04937062) that is showing very high promise in treating patients with *SLC6A1* disorders. However, treatment development is limited by our knowledge gaps in pathological mechanisms of this disorder, particularly how GABAergic neurotransmission is impacted by loss of GAT-1 in these disorders. Here, we address these gaps by investigating synaptic and extrasynaptic GABAergic neurotransmission, and the effects of 4-phenylbutyrate treatment in the *Slc6a1*$^{+/S295L}$ mouse model.

Disclosures: K. Zavalin: None. **K. Randhave:** None. **J. Kang:** None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.28/B81

Topic: B.08. Epilepsy

Title: Closed-loop optogenetic control of spontaneous & hyperventilation-provoked absence seizures

Authors: *S. HE, S. KILIANSKI, E. WU, A. CARNS, E. DULKO, G. NAIK, M. P. BEENHAKKER;
Univ. of Virginia, Charlottesville, VA

Abstract: Absence seizures primarily affect children between 4 and 14 and are characterized by abrupt, short breaks in consciousness. The hallmark of childhood absence seizures (CAE) is the bi-synchronous, symmetric spike-wave discharge (SWD) of 2.5-4 Hz, as measured by electroencephalographic (EEG) recordings. Notably, among all the seizure-triggering events, hyperventilation stands out as the most predictive in CAE, capable of provoking SWD in nearly all patients. In this study, we aim to compare the seizure generation circuits between spontaneous and hyperventilation-provoked SWD by testing their susceptibility to neuromodulation. We employed the C3H/HeJ mice model, which harbors the Gria4 mutation leading to spontaneous seizures a few weeks after birth. We induced hyperventilation in mice by adjusting the gas component in the plethysmography chamber, permitting electrocorticography (ECoG)/electromyography (EMG) recordings. We delivered halorhodopsin (HR) or Channelrhodopsin-2 (ChR2) to either the reticular thalamic nucleus (RT) or the centromedian nucleus (CM), two thalamic nuclei, to manipulate the seizure generation bidirectionally. Our closed-loop seizure detection algorithm is voltage amplitude-based and monitors the ECoG signal from the primary somatosensory cortex (S1); the delay between event detection and light stimulation is 2ms +- 0.66ms. We show that unilateral tonic inhibition of the RT aborts seizures in both hemispheres. The average duration from LED illumination to seizure cessation is 0.45s +-0.06s, whereas the average, unmanipulated seizure duration is 5.38s +-0.41s. One-third of the manipulated seizures are followed by another seizure within 1s of light termination, suggesting the burst firing of RT mediated by the hyperpolarizing deinactivation of T-type Ca2+ current. Our findings verify the rapid crosstalk mediated by the intrathalamic commissural fibers from the rostral RT to the contralateral RT and intralaminar nuclei. The variance in susceptibility to optogenetic stimulation between spontaneous and hyperventilation-provoked absence seizures helps elucidate the mechanism of SWD generation, contributing to clinical research and drug development.

Disclosures: S. He: None.

Poster

PSTR152: Epilepsy Mechanisms: Animal Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR152.29/B82

Topic: B.08. Epilepsy

Title: Mesoscale excitatory and inhibitory mapping of node recruitment in focal neocortical epilepsy

Authors: *J. E. NIEMEYER, F. ZHAN, H. MA, T. H. SCHWARTZ;
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Abstract: Focal neocortical epilepsy forms a network of synaptically connected nodes and exhibits drug resistance in about 25% of patients, of which only about 50% achieve seizure-freedom with surgery. To better understand how seizures initiate and spread in recruited cortical networks, we developed an experimental paradigm that permits simultaneous electrographic and mesoscopic calcium imaging of Thy1 excitatory or PV inhibitory neurons across a defined neocortical network. We injected 4-Aminopyridine (4-AP), a chemoconvulsant, into primary somatosensory cortex (S1), which has known connections with ipsilateral secondary motor cortex (iM2) and contralateral S1 (cS1). Following injection of 4-AP (2 mM), we observed robust focal seizures at S1 that exhibited variable but non-stochastic recruitment of mono-synaptically connected nodes (n=12 mice). Despite strong anatomical connection between S1 and cS1, seizures never propagated to cS1 without first recruiting iM2, and rarely propagated to cS1 without first recruiting the mirror node monosynaptically connected to iM2, a site with little connection to S1. To test a mechanism of this propagation pattern, we used electrical microstimulation to probe the difference in excitatory and inhibitory activity using mice (n=10). We find that cross-callosal excitation/inhibition balance varies between the nodes of the S1-M2 network. This data demonstrates that propagation patterns of focal to bilateral neocortical seizures are guided by excitatory/inhibitory balance, which varies across the corpus callosum. Our data also highlight that specific network nodes outside of the seizure focus, in this case iM2, may serve as targets to control seizure propagation. These findings will guide subsequent studies examining how different cell types and brain regions can be manipulated to constrain or prevent focal neocortical seizures.

Disclosures: J.E. Niemeyer: None. **F. Zhan:** None. **H. Ma:** None. **T.H. Schwartz:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.01/B83

Topic: B.09. Glial Mechanisms

Title: In vitro and ex vivo models of OPC maturation and myelination for drug discovery

Authors: B. HALL, *T. MODEBADZE, M. PATERSON, H. SCOTT, R. BURGOYNE, L. NIXON, M. BARBOUR, E. MALAVASI;
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Abstract: Oligodendrocytes are the myelin-producing cells of the Central Nervous System (CNS). Intact myelin is not only essential for the fast conduction of electrical signals along axons, but it also provides protection and trophic support to neurons. Several neurodegenerative diseases, including Multiple Sclerosis, Alzheimer’s disease, Amyotrophic Lateral Sclerosis, and

stroke, are associated with loss of myelin (demyelination) in the CNS, which leads to neuronal death. Myelinating oligodendrocytes originate from Oligodendrocyte Precursor Cells (OPCs), a pool of immature cells with proliferative capacity that persist in the adult CNS. OPCs are normally quiescent and respond to damage by proliferating and migrating to lesion sites, where they differentiate into oligodendrocytes, leading to the deposition of new myelin. However, in several neurodegenerative diseases this process either fails or becomes inefficient. Therefore, the development of novel OPC-targeted therapeutics that can boost or restore the proliferation, migration and/or maturation of these cells into oligodendrocytes is the focus of considerable research efforts. As a result, robust translational in vitro assays that enable accurate and reproducible quantification of functional phenotypes in OPCs are needed to support and accelerate drug discovery in neuroregeneration. Here, we present data demonstrating how purified OPCs can be used to model key functional cellular phenotypes in vitro, which can be predictably and reproducibly modified using control compounds. OPCs are immunopurified from the brains of postnatal rats, then induced to proliferate in vitro before being exposed to either Vehicle or substances known to promote OPC maturation to oligodendrocytes, such as Triiodothyronine (T3) or Leukaemia Inhibitory Factor (LIF), for up to 4 days. The effect of drug treatments is then quantified by measuring expression levels of Myelin Basic Protein (MBP) by immunostaining or qPCR, or by quantifying changes in the levels of key maturation-associated metabolites, by LC-MS/MS. In addition, we share data accrued during the development and characterisation of an ex-vivo 3D myelination model based on organotypic brain slices, which recapitulates key aspects of in vivo myelination. Finally, we discuss how these therapeutic modality-agnostic and versatile models and assays can be utilised at multiple stages of the drug discovery process, from target validation through to candidate selection.

Disclosures: B. Hall: A. Employment/Salary (full or part-time):; Concept Life Sciences Ltd. **T. Modebadze:** A. Employment/Salary (full or part-time):; Concept Life Sciences Ltd. **M. Paterson:** A. Employment/Salary (full or part-time):; Concept Life Sciences Ltd. **H. Scott:** A. Employment/Salary (full or part-time):; Concept Life Sciences Ltd. **R. Burgoyne:** A. Employment/Salary (full or part-time):; Concept Life Sciences Ltd. **L. Nixon:** A. Employment/Salary (full or part-time):; Concept Life Sciences Ltd. **M. Barbour:** A. Employment/Salary (full or part-time):; Concept Life Sciences Ltd. **E. Malavasi:** A. Employment/Salary (full or part-time):; Concept Life Sciences Ltd.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.02/B84

Topic: B.09. Glial Mechanisms

Support: R01NS131486
McKnight Scholar Award
Rita Allen Scholar Award

Klingenstein-Simons Fellowship Award in Neuroscience
DP2MH132943

Title: Identifying the molecular mechanisms underlying microglial regulation of oligodendrocyte precursor cells (OPCs) and their function in the adult brain

Authors: *J. A. KAHNG[1,2], A. FERRO[1], L. CHEADLE[1,3];
[1]Neurosci., Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]School of Biological Sciences at Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; [3]Howard Hughes Medical Institute, Cold Spring Harbor, NY

Abstract: The establishment and maintenance of neuronal circuits in the brain is required for neurological functions, including behavior in adult animals. While historically research has focused on identifying the neuronal intrinsic mechanisms regulating these processes, lately there has been an increased interest in identifying the roles of glia, the non-neuronal cells of the brain, in circuit development and maintenance. We have recently identified a novel role for oligodendrocyte precursor cells (OPCs), the progenitor cells of myelinating oligodendrocytes, in synaptic refinement. We demonstrated that OPCs engulf thalamocortical inputs in the primary visual cortex (V1) both during development and in adulthood. This function, when paired with the ability of OPCs to receive *bona fide* synaptic inputs from neurons, poses an interesting role for OPCs in monitoring and pruning synapses based on activity. However, the mechanisms underlying the ability of OPCs to recognize and remove synaptic material remain to be elucidated. Previously, we found that pharmacologically depleting microglia, the brain's resident immune cells, using a CSFR1 inhibitor (Plexxikon 5622) during postnatal development dampened the OPC-mediated engulfment of synaptic material, suggesting that microglia are necessary for the engulfment of synapses by OPCs. Yet, little is understood about how OPCs and microglia interact in the brain. To address this gap in knowledge at the molecular level, we utilized two proteomic approaches to characterize the impact of microglial depletion on the proteomic composition of OPCs. In the first method, cortical OPC whole-cell proteomes were characterized and compared between OPCs in the presence or absence of microglia. In the second method, an *in vivo,* virally induced proximity-based labeling approach (AAV-hSyn-TurboID) was used to tag and identify proteins at the interaction sites between OPCs and cortical neurons also in the presence or absence of microglia. These two approaches allowed us to address how OPCs change as a result of loss of microglia, both at the whole-cell protein expression level and also at the synapse-OPC interactome level. These characterizations provide critical information about how microglia regulate the OPC-mediated engulfment of synapses, and also deliver specific insight into the molecular mechanisms governing synapse-OPC communication.

Disclosures: J.A. Kahng: None. **A. Ferro:** None. **L. Cheadle:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.03/B85

Topic: B.09. Glial Mechanisms

Support: Telethon Foundation Multiround 21-24 – Round 1 2022 Track Basic (ID: GMR22T1066
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Title: Unraveling the roles of oligodendrocyte progenitor cells in the development of the cortical inhibitory system

Authors: *M. KHASTKHODAEI ARDAKANI[1,2], M. BONATO[1,2], A. INCERTI TINTERRI[1,2], N. DI CINTIO[1,2], M. FELLINE[1,2], F. FERRINI[3], A. BUFFO[1,2], E. BODA[1,2]; [1]Dept. of Neuroscience, Univ. of Turin, Turin, Italy; [2]Neurosci. Inst. Cavalieri Ottolenghi, Orbassano, Italy; [3]Dept. of Vet. Sciences, Univ. of Turin, Grugliasco, Italy

Abstract: Recent studies have revealed an unexpected contribution of oligodendroglial cells to inhibitory circuit establishment and function. Yet, the entire spectrum of oligodendroglia-interneuron interactions as well as the impact of oligodendroglia loss or dysfunction on the development of the inhibitory system, remain unresolved. We exploited two mouse models (Cit-k KO or Sox10Cre::Cit-k fl/fl animals) in which the deletion of citron kinase, a kinase involved in DNA repair and cytoskeletal dynamics, either at the germinal or oligodendroglia-specific level, leads to the selective ablation of cortical oligodendrocyte progenitor cells (OPCs) in the first two postnatal weeks. Cit-k KO mice display impaired activity-dependent inhibition of cortical pyramidal neurons as assessed by patch-clamp recordings and a shorter lifespan due to lethal seizures. Although Sox10Cre::Cit-k fl/fl mice do not show a spontaneous epileptic phenotype, they display increased vulnerability to the epileptogenic drug pentylenetetrazol compared to wild-type littermates, indicating that OPC loss is associated with an altered excitation/inhibition balance in both models. In Cit-k KO mice, the pharmacological recovery of cortical OPCs - although not restoring myelination - resulted in a significant rescue of cortical inhibitory neurotransmission and a reduced epileptic phenotype. This positive outcome was amplified when OPC repopulation was achieved by wild-type OPC transplantation in the brain of Cit-k KO mice, leading to the abrogation of susceptibility to epileptogenic drug and a remarkable increase in mouse lifespan. Mechanistically, OPC rescue/graft was associated with significant alterations in microglia phenotype and number along with an upregulation of interneuron maturation markers. Investigation of the role of microglia as a possible mediator of the effects of OPC rescue in our mutants is ongoing. Overall, our data suggest the loss of OPCs at neonatal stages is associated with alterations in the cortical inhibitory system that can be restored by oligodendroglia restoration.

Disclosures: M. Khastkhodaei Ardakani: None. **M. Bonato:** None. **A. Incerti Tinterri:** None. **N. Di Cintio:** None. **M. Felline:** None. **F. Ferrini:** None. **A. Buffo:** None. **E. Boda:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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Topic: B.09. Glial Mechanisms

Support: NIH K12NS079414 (CME)
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Boston Children's Hospital Department of Neurology (CME)
Child Neurology Society Dodge Young Investigator Award (CME)

Title: Developmental regulation of zinc homeostasis in differentiating oligodendrocytes

Authors: *C. M. ELITT[1,2], M. ROSS[3], J. WANG[3], C. FAHRNI[4], P. A. ROSENBERG[1,2];
[1]Neurol., Boston Children's Hosp., Boston, MA; [2]Harvard Medical Schoool, Boston, MA;
[3]Boston Children's Hosp., Boston, MA; [4]Georgia Inst. of Technol., Atlanta, GA

Abstract: Zinc is abundant in the central nervous system (CNS) and required for the function of 10% of all proteins, including many enzymes and transcription factors. Several of these are important for myelination. Increases in free ionic zinc (Zn^{2+}) play a critical role in oligodendrocyte (OL) injury, but less is known about zinc homeostasis during OL development. OLs progress through sequential developmental stages, and understanding pathways regulating their differentiation remains an important area of investigation. Previous studies using the ratiometric zinc sensor chromis-1 demonstrated a reduction in intracellular free zinc concentrations in mature MBP+ OLs compared with earlier stages. The objective of the current study was to understand the temporal course of zinc homeostasis across development using stage-specific rat OL cultures and to test the hypothesis that changes in expression of zinc storage proteins and zinc transporters contribute to developmental changes in zinc homeostasis. Using chromis-1, we found a transient increase in free zinc after O4+, O1- pre-OLs 24 hours after OLs were switched from proliferation medium into terminal differentiation medium. qPCR was used to evaluate mRNA expression of metallothioneins (MTs), the major zinc storage proteins, and metal regulatory transcription factor 1 (MTF-1), which controls expression of MTs. MT-1, MT-2 and MTF1 mRNAs were increased 5-10 fold in mature OLs compared to OLs in proliferation medium, supporting the hypothesis that as OLs differentiate, there is increased mobilization of free zinc. Expression or function of zinc transporters (ZnTs) or Zrt-and-Irt-like proteins (ZIPs) may underlie changes in zinc homeostasis. Based on published RNAseq data, we selected a sample of the 24 known zinc transporters for qPCR. We found 3-4 fold increases in ZIP9 and ZnT1 after transfer to differentiation medium. ZIP1 was unchanged until the most mature OL stage, when it increased 4 fold. To assess the depth of the zinc buffer in OLs and its change over development, we assayed zinc release from intracellular stores using the thiol-selective oxidant 2,2’-dithiodipyridine (DTDP). Exposure to DTDP resulted in ~100% increase in free zinc in pre-OLs but, paradoxically, a more modest ~60% increase in mature OLs despite increased expression of MTs. These results suggest that zinc homeostasis is regulated and may be important during OL development; furthermore OLs may be a useful model for studying zinc

homeostasis in the CNS. Disruption of zinc homeostasis in OLs may be important in white matter injury and repair in multiple sclerosis, the encephalopathy of prematurity, brain trauma, and white matter stroke.

Disclosures: C.M. Elitt: F. Consulting Fees (e.g., advisory boards); Akebia Therapeutics. **M. Ross:** None. **J. Wang:** None. **C. Fahrni:** None. **P.A. Rosenberg:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.05/B87

Topic: B.09. Glial Mechanisms

Support: NIH 1R01NS107525
Owens Family Foundation

Title: JAM proteins mediate oligodendrocyte myelin targeting during zebrafish neurodevelopment

Authors: *A. T. PERL[1,2], A. J. LATIMER[3,2], S. C. KUCENAS[3,2];
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Abstract: In vertebrates, nerve impulse transmission often depends on the myelination of axons by specialized glial cells. Glial ensheathment by myelin simultaneously insulates and nourishes the axon, and can increase the speed of neurotransmission by orders of magnitude. In the central nervous system (CNS), oligodendrocytes (OLs) myelinate only select axons in the brain and spinal cord under physiological conditions. Why they don't myelinate other structures, including neuronal cell bodies, is not known. To probe the cellular and molecular mechanisms underlying this specificity of myelin targeting during development, we analyzed our own and publicly available single-cell RNA sequencing (scRNA-seq) datasets covering oligodendroglial lineage cells and neurons within the period of zebrafish, mouse and human neurodevelopment. The criteria for identifying target genes were: a) they encoded transmembrane or cell adhesion proteins in OLs or neurons, and b) they were highly expressed in myelinating OLs relative to OL progenitor cells, or in neurons during the period of active myelination in CNS development. Among the genes which met these criteria were junctional adhesion molecules (*JAMs*) *2* & *3* and their orthologs (*jams 2a*, *2b*, *3a* and *3b* in zebrafish). JAMs are transmembrane proteins expressed by endothelial and epithelial cells, leukocytes, and others, and are involved in tight junction formation and maintenance, leukocyte migration, and cell polarity, but the role these proteins play in the CNS is largely unexplored. Using CRISPR/Cas9 genome modification, our group found that zebrafish larvae harboring mutations in each of these four *jam* genes have inappropriate myelin targeting. Using these mutant lines, we are leveraging the power of the zebrafish model system to investigate the role of JAM proteins in myelin targeting during CNS

development, and elucidating the molecular mechanisms that drive this unique cell-cell interaction. We are exploring cellular/molecular phenotypes in developing and adult fish using *in vivo* time-lapse imaging, RNAscope, and electron microscopy, and phenotypes at the level of the whole organism by applying a battery of behavioral tests. To identify binding partners of JAMs in the CNS, we are using TurboID, which has the advantage of working *in vivo*. When complete, our studies will provide a fuller understanding of the mechanisms that drive developmental myelin targeting in the CNS. This will be instrumental in establishing treatments for disorders where myelin targeting is abnormal, and in devising effective remyelination therapies for demyelinating and dysmyelinating diseases like multiple sclerosis and leukodystrophies.

Disclosures: A.T. Perl: None. **A.J. Latimer:** None. **S.C. Kucenas:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.06/B88

Topic: B.09. Glial Mechanisms

Support: NIH R01NS128117
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Broad Institute of MIT and Harvard
Stanley Center for Psychiatric Research
Charles A. King Trust

Title: Molecular mechanisms governing projection-neuron-specific myelination in the cortex

Authors: *N. DOMÍNGUEZ ITURZA[1,2], V. A. JOKHI[3,2], K. KIM[4,5,6], A. S. SHETTY[3,2], D. DI BELLA[3,2], C. ABBATE[3], P. OYLER-CASTRILLO[3], X. JIN[7,5,2], S. SIMMONS[8,2], J. Z. LEVIN[8,2], J. R. BROWN[3,2], P. ARLOTTA[3,2];
[1]Harvard Univ., Cambridge, MA; [2]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, MA; [3]Harvard Stem Cell and Regenerative Biol., Harvard Univ., Cambridge, MA; [4]Stanley Ctr. for Psychiatric Res., Broad Inst. of MIT and Harvard, Cambridge, MA; [5]Harvard Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA; [6]Klarman Cell Observatory, Broad Institute of MIT and Harvard, Cambridge, MA; [7]Neurosci., Scripps Res., La Jolla, CA; [8]Klarman Cell Observatory, Broad Inst. of MIT and Harvard, Cambridge, MA

Abstract: Myelin is a distinctive feature of the vertebrate central nervous system (CNS). During CNS development, oligodendrocytes (OLs) wrap their membrane around axons to create an insulating, lipid-rich structure called the myelin sheath. While defects in axonal myelination are associated with multiple neurological disorders, including autism spectrum disorders, schizophrenia, and multiple sclerosis, we are only starting to uncover the mechanisms that regulate myelin development, maintenance, and remyelination. Cortical myelination is highly

heterogeneous and follows a gradient distribution, with deep-layer projection neurons (PNs) being uniformly extensively myelinated, while upper-layer PNs have more diverse patterns and are more sparsely myelinated. OLs are a heterogenous population of cells with remarkable target specificity *in vivo*. However, the mechanisms underlying oligodendrocyte target selection are still unknown. Here, we applied single-cell molecular profiling of OLs across different cortical layers and across a postnatal time course to understand layer-specific differences in PN myelination. We found that while all cortical layers have a similar compendium of OL states, mature OLs are preferentially located in deep layers. To investigate if PN subtypes can guide oligodendrocyte maturation and myelination, we generated a predicted ligand-receptor interaction map between PNs subtypes and oligodendrocyte states across cortical layers and time. *In vivo* testing of candidate modulators of layer-specific myelination identified *Fgf18* and *Ncam1* as novel myelin-promoting molecules. Our results indicate that neuron-class-specific molecular signals can guide differential myelination across cortical layers. This knowledge is fundamental to understanding the development and regeneration of myelin in the mammalian CNS.

Disclosures: N. Domínguez Iturza: None. **V.A. Jokhi:** None. **K. Kim:** None. **A.S. Shetty:** None. **D. Di Bella:** None. **C. Abbate:** None. **P. Oyler-Castrillo:** None. **X. Jin:** None. **S. Simmons:** None. **J.Z. Levin:** None. **J.R. Brown:** None. **P. Arlotta:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder and SAB member of Vesalius Therapeutics. F. Consulting Fees (e.g., advisory boards); SAB member at Foresite Labs, SAB member at CNSII.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.07/B89

Topic: B.09. Glial Mechanisms

Support: JSPS KAKENHI Grant (20H05894)

Title: Newly translated of myelin basic protein modulates nerual activity and is essential for motor learning

Authors: *S. SUGIO[1,2], H. WAKE[1,2];
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Abstract: Oligodendrocytes (OCs) are myelin-forming glia in the central nervous system and regulated axon conduction. Recent studies have suggested that OCs are responded to the changes in neural activities and altered their myelin morphology to regulate the temporal patterns of neural activities, which are important for the motor skill learning. Although, the physiological significances of OCs on motor skill learning have been known in last decade, while the effects of

OCs on the axon conduction in learning processes have not been cleared. Here, we performed a motor skill training (lever-pull task) on mice and evaluated whether the OCs modulate axon conduction on training processes. We found that the lever-pull task induced *Mpb* mRNA (a myelin gene) and local MBP translation, which changes in the length of node of Ranvier (NOR) in the task associated neural tract (ventroanteriol/ventrorateral thalamic nuclei to motor cortices tract: VA/VL-motor tract). Here, we expressed channelrhodopsin2 (ChR2) into VA/VL neurons in mice and measured ChR2-evoked spikes that elicited by blue light illumination into motor cortex then antidromically arriving to VA/VL nuclei, and found that the timing of antidromic spikes arriving to VA/VL nuclei through each axons were synchronized by lever-pull task. The increment of success trial of the task was highly correlated with the spike synchrony arriving VA/VL nuclei. To further evaluate whether upregulation of *Mbp* mRNA and MBP protein was associated with the changes in axon conduction, we further established a method to suppress newly translation of MBP protein induced through lever-pull task. For the neural activity-dependent translation of MBP protein, the formation of RNA granule composed of *Mbp* mRNA and heterogeneous nuclear ribonucleoprotein 2A (hnRNP A2) is required. The hnRNP A2 is bound to a specific sequence on 3' untranslated region of *Mbp* mRNA (*Mbp* 3'UTR) and induced RNA granule formation. Once OCs received neural cues from neighboring neurons or axons, *Mbp* mRNA is released form the granule and locally translated by free ribosomes. Here we constructed a vector encoded *Mbp*3'UTR lacking protein coding site (CDS-lacking Mbp 3'UTR) and overexpressed to OCs, and found that the CDS-lacking *Mbp* 3'UTR was inhibited neural activity-dependent translation of MBP protein, suppressed changes in NOR, and disturbed lever-pull learning. These results suggested the oligodendrocyte responded to the changes in neuronal activity and adapt their morphology and myelination to regulate the temporal activity of neural populations via synchronizing axon conduction, which are important for the motor skill learning.

Disclosures: S. Sugio: None. **H. Wake:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

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Topic: B.09. Glial Mechanisms

Support: This work was supported by the PRE2020-093827 and PID2022-136882NB-I00 Spanish grants, funded by MICIU/AEI/ 10.13039/501100011033

Title: The Functional Significance of NG2 Cells in the Mouse Olfactory Bulb

Authors: *S. BARRIOLA, M. FIGUERES OÑATE, C. PERNÍA-SOLANILLA, R. FERNÁNDEZ ARIAS, L. M. LOPEZ-MASCARAQUE;
Cajal Inst., Madrid, Spain

Abstract: NG2 glia, also referred to as oligodendrocyte precursor cells (OPCs), are widely distributed throughout the adult brain in a grid-like pattern. These cells play diverse functions in the central nervous system (CNS) under both normal and pathological conditions. Besides their established role as oligodendrocyte precursors (OPCs), they persist in the adult brain as a resident, self-renewing population with significant progenitor potential. Unlike other glial cell types, NG2 glia interact bidirectionally at the synaptic level with neurons, actively participating in neuronal circuitries. In this work we have used different viral monosynaptic tracers to show the NG2 glia-to-neuron connectome in the olfactory pathway. By enhancing our understanding of these interactions, our findings will advance the development of more accurate models of olfactory neural networks, providing insights into the broader olfactory bulb circuitry and the role of diverse glial cells in shaping postnatal olfactory circuits' plasticity.

Disclosures: S. Barriola: None. **M. Figueres Oñate:** None. **C. Pernía-Solanilla:** None. **R. Fernández Arias:** None. **L.M. Lopez-Mascaraque:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.09/B91

Topic: B.09. Glial Mechanisms

Support: Korea Health Industry Development Institute (KHIDI) / M.D.-Ph.D./Medical Scientist Training Program

Title: Cryopreservation of primary-cultured oligodendrocytes for in vitro studies

Authors: *H. KIM[1], B. KIM[2], S. KOH[3], H. CHO[4], J. CHOI[5];
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Abstract: Oligodendrocytes (OLs) are the myelinating cells in the central nervous system (CNS). The adequate myelination of neurons is necessary for their saltatory conduction and metabolic support. The death and dysfunction of OLs lead to the demyelination of the neurons they ensheath, culminating in neuronal death. Demyelination and the succeeding neurodegeneration can be observed in various CNS disorders, such as multiple sclerosis, ischemic stroke, traumatic brain injury, and neurodegenerative diseases. The lack of an immortalized cell line that fully reproduces the various stages of the OL lineage, spanning from OL progenitor cells (OPCs) to mature myelinating OLs, makes the in vitro research of OLs dependent on primary OL culture. Primary cultured OLs start as OPCs, and through the induction of differentiation, usually with thyroid hormone T3, they mature into myelinating OLs which can enwrap neurons in vitro. However, the small yield of conventional primary OL culture

methods and the inability of cryopreservation make the application of methods, especially bulky experiments such as high throughput screening difficult and costly. In the present study, we demonstrate an undemanding method of primary OL culture, along with a protocol to cryopreserve OPCs for later use. OPCs were isolated from neonatal Sprague-Dawley rat brains through a simple two-step differential centrifugation, and after 5 days of proliferation with OPC proliferation media containing platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF), highly pure OPCs could be obtained after passaging with Accutase®. For cryopreservation, passaged OPCs were resuspended in Cellbanker-2^{TM}, a serum-free cryopreservation media, and frozen at -80°C in freezing containers using isopropyl alcohol for cooling rate control. OPCs that had; 1. Not been frozen, 2. Frozen for 1-2 months, 3. Frozen for 3-6 months did not differ in their proliferation capacity measured by the ratio of EdU uptake (N=4). After maturation for 4 days with 40ng/mL T3, OPCs from all three groups successfully differentiated into myelin basic protein (MBP) positive myelinating OLs, showing a comparable proportion of MBP+ OLs (N=4), morphological complexity (N=4), and capacity to myelinate nanofibers replicating the physical structure of axonal processes. Thus, we provide a primary OL culture system that is both simple and cost-efficient, along with a protocol for the cryopreservation of cultured OPCs, which collectively allows a great advance in the accessibility of in vitro OLs for bulky experiments.

Disclosures: H. Kim: None. **B. Kim:** None. **S. Koh:** None. **H. Cho:** None. **J. Choi:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.10/B92

Topic: B.09. Glial Mechanisms

Title: Rapid generation of $O4^{+}$ oligodendrocyte progenitor cells from human induced pluripotent stem cells for myelination studies

Authors: *C. FLIGOR, K. JANKE, D. HELD, H. RUETH, W. LI, K.-D. CHOI;
BrainXell, Madison, WI

Abstract: The intricate functionality of the central nervous system (CNS) is contingent on the cooperation of neurons and glial cells such as astrocytes and oligodendrocytes (OL). Oligodendrocyte progenitor cells (OPCs), precursors to OLs comprising 5% of the adult brain, retain their ability to differentiate into mature oligodendrocytes. Oligodendrocytes appear late during development, providing essential trophic and metabolic support to neurons. Mature oligodendrocytes produce myelin sheaths which wrap around neuronal axons, providing electrical insulation. Myelination increases the conduction velocity of action potentials and the speed of neural processing. Without sufficient myelination devastating effects occur in the body. Despite demyelination contributing to multiple CNS disorders, the failure rate of candidate drugs in clinical trials is very high. Using a human induced pluripotent stem cell (hiPSC) neuron-OL

platform would enable screening and testing of therapeutic approaches to create in vitro myelination models specific to a patient population. Early approaches to generate OLs from human iPSCs used growth factors and small molecules to guide differentiation but provided low efficiency and required extensive production times. OL differentiation is a lengthy process, requiring approximately three months to differentiate hiPSCs to OPCs and three additional months to mature into myelin-producing oligodendrocytes. We recognize that there is a critical need for highly enriched, functionally mature OPCs with consistent quality. Therefore, BrainXell developed an efficient protocol that accelerates the OPC differentiation timeline from three months to less than one month. Resulting cell populations were highly pure for OPC-specific marker O4 (>93% O4+ via flow cytometry) and displayed bipolar or tripolar morphology typical of proliferating OPCs. O4+ OPCs were further analyzed by qPCR, revealing an upregulation in oligodendrocyte progenitor markers (CNP, CSPG4 and OLIG2) as well as myelin-associated markers (PLP1 and MBP). Readily available human oligodendrocytes will bring new understanding to oligodendrocyte-related neurodegenerative disease mechanisms and allow for accelerated development of high-throughput screening platforms for new drug discovery.

Disclosures: C. Fligor: None. **K. Janke:** None. **D. Held:** None. **H. Rueth:** None. **W. Li:** None. **K. Choi:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.11/B93

Topic: B.09. Glial Mechanisms

Support: NIH Grant 1SC2NS125021

Title: Oligodendrocyte Precursor Cells in the Mouse Prefrontal Cortex Express Dopamine Receptor Transcripts

Authors: *M. CALDWELL[1], L. YETNIKOFF[2];
[1]City Univ. of New York CUNY Neurosci. Collaborative, Staten Island, NY; [2]Psychology, City Univ. of New York CUNY Neurosci. Collaborative, Staten Island, NY

Abstract: Oligodendrocyte precursor cells (OPCs), self-renewing cells that differentiate into mature oligodendrocytes, are critical for experience-dependent myelination. OPCs express glutamate, GABA, and kappa receptors, and the cellular activity of OPCs can be regulated by glutamatergic, GABAergic and dynorphin neurons. We recently demonstrated in the adult mouse that, in addition to innervation of the anterior corpus callosum by dopamine terminals, ~40% of OPCs in this region express dopamine d1 receptor (*Drd1*) and dopamine d2 receptor (*Drd2*) transcripts, indicating a possible role for dopamine in myelin plasticity. Research has shown that OPCs are heterogeneous, with different populations of OPCs expressing different profiles of receptors and ion channels depending on the brain region the cells reside in. Here, we asked

whether OPCs in the adult medial prefrontal cortex (mPFC), which is more densely innervated by dopamine terminals than the corpus callosum, also express dopamine receptor transcripts. Single plex RNAscope for *Drd1* or *Drd2* with co-detection immunohistochemistry for GFP and tyrosine hydroxylase (TH) was conducted on brain sections containing the mPFC obtained from PDGFRα^{EGFP} reporter mice. Preliminary analyses indicate that ~60% of OPCs in the mPFC express *Drd1* and *Drd2* transcripts. Furthermore, we show that TH+ axons make perisomatic terminations with ~75% of OPCs expressing dopamine receptor transcripts. These results indicate that a role for dopamine in myelin plasticity may not be restricted to the anterior corpus callosum and suggest dopamine may be involved in the regulation of the proliferation and/or differentiation of OPCs in the mPFC. This work furthers our understanding of neuron-glia interactions with important implications for myelin plasticity by identifying dopamine as a potential regulator of mPFC oligodendrocyte lineage cells.

Disclosures: M. caldwell: None. **L. Yetnikoff:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.12/B94

Topic: B.09. Glial Mechanisms

Support: 1SC2NS125021

Title: The effects of social isolation during adolescence on dopamine axon terminal density in the anterior corpus callosum

Authors: *C. ALARCON[1], M. CALDWELL[2], X. ZHU JIANG[3], L. YETNIKOFF[4];
[1]Psychology, Col. of Staten Island, Staten Island, NY; [2]City Univ. of New York CUNY Neurosci. Collaborative, Staten Island, NY; [3]CUNY Col. of Staten Island, Staten Island, NY; [4]Psychology, City Univ. of New York CUNY Neurosci. Collaborative, Staten Island, NY

Abstract: The corpus callosum is the largest myelinated tract of the brain, carrying contralaterally-projecting cortical axons of varying myelinated states ranging from unmyelinated to densely myelinated axons. This wide variability in myelination renders the corpus callosum particularly sensitive to experience-induced myelin plasticity. While glutamatergic synapses between axons and oligodendrocyte precursor cells (OPCs) in the corpus callosum were identified at the ultrastructural level nearly two decades ago, we recently demonstrated in the adult mouse that midbrain dopamine neurons send projections into the anterior corpus callosum and make perisomatic terminations with OPCs. More recently, we demonstrated that there is a greater density of dopamine axon terminals in the anterior corpus callosum during early adolescence (P21) compared to adulthood (P90). Here, we asked whether social isolation experienced during the time between early adolescence and adulthood would alter the architecture of dopamine terminal density in the anterior corpus callosum given that it is a

behavioral manipulation known to induce corpus callosal myelin plasticity and regulate midbrain dopamine neuron activity. Male and female wild-type mice were either socially isolated (n = 1 per homecage) from P21 – P60 or housed in groups (n = 2 – 5 per homecage). At P60, all mice were perfused, and their brains prepared for immunohistochemical analysis. Dopamine axons in the anterior corpus callosum were labeled using tyrosine hydroxylase immunofluorescence, imaged with a Leica DM6 THUNDER microscope at 100x, and quantified with IMARIS microscopy software by students blind to experimental condition. Preliminary analyses demonstrate that mice that lived in social isolation throughout adolescence exhibit a greater density of corpus callosal dopamine terminals in adulthood compared to mice that lived in group housing throughout adolescence. This suggests that social isolation during adolescence arrests the normal developmental processes that regulate dopamine terminal innervation of the anterior corpus callosum. A similar approach is being used to investigate the effects of social isolation during adulthood on corpus callosal dopamine terminal architecture as well as on dopamine receptor transcript expression by oligodendrocyte lineage cells in order to identify the relationship between midbrain dopamine neuron function and experience-dependent myelin plasticity of the corpus callosum.

Disclosures: C. Alarcon: None. **M. caldwell:** None. **X. Zhu Jiang:** None. **L. Yetnikoff:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.13/B95

Topic: B.09. Glial Mechanisms

Support: NIH R01MH118441
PSC-CUNY
ASRC Seed Grant

Title: Satellite oligodendrocyte lineage cell interactions with parvalbumin interneurons and perineuronal nets of the infralimbic cortex promote anxiolytic effects of safety learning.

Authors: *E. DENHOLTZ[1,2], E. LIKHTIK[3];
[1]Biol., City Univ. of New York, NY, NY; [2]Biology, Hunter College, New York, NY; [3]Biol., Hunter Col., CUNY, New York, NY

Abstract: Stress and anxiety disorders such as post-traumatic stress disorder (PTSD) are characterized by poor fear suppression, which is linked to lower medial prefrontal cortex (mPFC) volume, and white matter loss in tracts connecting the mPFC with other regions. One promising approach to mitigate excessive fear expression is safety learning, which establishes an explicit association between a cue and safety and decreases overgeneralized fear. Parvalbumin interneuron (PV IN) signaling in the mPFC is important for modulating fear behavior in the presence of a learned safety cue, and PV INs are known to communicate with oligodendrocyte

lineage cells. However, it's unclear whether safety learning engages PV IN-glial interactions in the mPFC to promote the therapeutic effect of safety learning. Here, we demonstrate a link between PV IN activity in the infralimbic cortex (IL) of the mPFC during safety learning with the recruitment of satellite oligodendrocyte lineage cells (satOLCs) to PV INs, as well as the breakdown of the perineuronal net (PNN) surrounding the PV IN. To this end, we first show that in male mice, a learned safety signal suppresses fear to the context during recent (next day) and remote (21 days) retrieval. Next, we show that PV IN activity during safety learning is associated with 1) satOLC recruitment, 2) satOLC maturation, and 3) a decrease in PNNs surrounding PV INs and satOLCs in the IL during remote retrieval. Then, we demonstrate that optogenetic inhibition of PV INs in the IL during safety learning improves next-day safety retrieval but prevents the safety-cue mediated reduction of contextual fear at remote retrieval. Furthermore, inhibiting PV IN activity in the IL during safety learning prevents satOLC recruitment to PV INs, satOLC maturation, and digestion of PNNs surrounding the PV INs at remote retrieval. We propose that during safety learning, PV IN activity in the IL recruits satOLCs, which help digest the PNN through signaling via digestive enzymes, thus revealing a non-myelinating function for satOLCs that promotes plasticity of PV INs in the IL. These extracellular modifications could give the PV INs a more flexible activity range, which is critical for learning.

Disclosures: E. Denholtz: None. **E. Likhtik:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.14/B96

Topic: B.09. Glial Mechanisms

Support: NIH R01NS117407
NYSCF Druckenmiller Fellowship

Title: Investigating the surprising potential of GDF11 to improve myelination in aging and disease.

Authors: *K. M. WELLS[1], K. HOLTON[2], H. BENTZ[2], A. TYAGI[1], A. KOLAJ[1], L. L. RUBIN[1];
[1]Stem Cell and Regenerative Biol., [2]Harvard Univ., Cambridge, MA

Abstract: Aging and aging-related neurodegenerative diseases are a growing concern as the average life expectancy steadily increases. Despite this, there are currently no therapeutics capable of reversing aging-associated cognitive decline. Recently, researchers have demonstrated that factors circulating in young animals can reverse some deleterious effects of aging and disease. One such circulating factor, Growth Differentiation Factor 11 (GDF11), improves vascularization, neurogenesis, and cognitive function when administered systemically to aged mice. These promising results counter what is known about GDF11's function within the brain

where it plays a repressive role on neurogenesis. A better understanding of its functions and mechanisms in the brain is necessary to fully comprehend its clinical potential. Through a transcriptomic screen using an inducible genetic knockout mouse line, we endeavor to improve our understanding of the role of endogenous GDF11. Surprisingly, in addition to the expected impact on neurogenesis, the most down-regulated pathways were associated with myelin and myelin-producing oligodendrocytes. Myelin, a lipid-rich coating around axonal projections in the central nervous system, provides insulation and protection and influences neural connectivity and circuits. While myelin levels continue to increase after birth, there is a dramatic decrease with old age, and this is exacerbated in certain neurodegenerative diseases such as Multiple Sclerosis and Alzheimer's Disease. Thus, finding therapeutics capable of improving remyelination would be beneficial in treating general aging and demyelinating diseases. Using the in vivo inducible GDF11 knockout mouse model and immunofluorescent staining of brain sections, we have confirmed that GDF11 knockout results in decreased levels of myelination and reduced size of the major white matter tract, the corpus callosum. Furthermore, GDF11 regulates the number of myelin-producing oligodendrocytes both in vivo and in vitro. Current studies are elucidating the pathway of this regulation and determining its impact on neural activity and cognitive function. These critical experiments will provide insight into GDF11's potential as a therapeutic in a broad range of diseases.

Disclosures: K.M. Wells: None. **K. Holton:** None. **H. Bentz:** None. **A. Tyagi:** None. **A. Kolaj:** None. **L.L. Rubin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vesalius Therapeutics. F. Consulting Fees (e.g., advisory boards); ProjenX, Corsalex, Vesalius Therapeutics. Other; Alkahest.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.15/B97

Topic: B.09. Glial Mechanisms

Support: NIH R01 DC018797

Title: Impact of Enriched Sound Experience on Myelin and Synaptic Plasticity in the Juvenile Mouse Auditory Brainstem

Authors: *S. YOON, W.-C. WU, J. H. KIM;
Kresge Hearing Res. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: Auditory input is fundamental in shaping auditory neurocircuitry during postnatal development. However, the mechanisms by which sound experience influences auditory circuitry in the critical period remain largely unexplored. This study aims to assess the effects of enriched sound experience on myelin and synaptic plasticity within the auditory brainstem of juvenile

mice. We employed a mouse model (C57/BL6J) that was exposed to additional sound stimulation (sound enrichment), consisting of 80 dB broad-band noise centered at 16 kHz, administered for three hours daily from P13 to P19 within a sound attenuation chamber. Our studies focused on highly myelinated Calyx terminals and MNTB neuron synapses to elucidate the impact of sound stimulation on myelination and synaptic plasticity. The results demonstrated a significant enhancement in oligodendrocyte lineage cell development, marked by increased proliferation and differentiation of oligodendrocyte progenitor cells (OPCs). The number of Pdgfra+ OPCs and BCAS1+ newly formed oligodendrocytes was increased in the MNTB of sound-enriched mice. Western blot analysis revealed a notable increase in myelin basic protein (MBP) levels, and electron microscopy confirmed a corresponding increase in myelin thickness in the sound enrichment group, indicating experience-dependent myelin plasticity. Furthermore, synaptic transmission and plasticity at the calyx of Held synapses in the MNTB were assessed using whole-cell patch clamp recordings. The recordings of excitatory postsynaptic currents (EPSCs) showed significantly increased release probability and short-term depression in the sound-enriched group. These findings suggest that sound enrichment not only enhances myelination but also modifies short-term synaptic plasticity in the auditory brainstem. Overall, the study demonstrates that myelin plasticity and short-term synaptic adaptations are responsive to unique auditory experiences during early postnatal development, offering new insights into the mechanisms of experience-driven myelin and synaptic plasticity in the auditory nervous system.

Disclosures: S. Yoon: None. **W. Wu:** None. **J.H. Kim:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.16/B98

Topic: B.09. Glial Mechanisms

Support: NIH Grant AG078565

Title: Myelin status on dopaminergic neuron axons from the ventral tegmental area to the nucleus accumbens in the mouse brain

Authors: J. COAKLEY[1], E. IKPEME[3], H. KIM[1], Y.-J. SON[1], ***S. KANG**[2];
[1]Neural Sci., Temple Univ. Sch. of Med., Philadelphia, PA; [2]Neural Sci., Temple Univ. Sch. of Med., Philadelphia, MD; [3]Biol., Temple Univ. Col. of Sci. and Technol., Philadelphia, PA

Abstract: Myelin, an insulating sheath crucial for rapid axonal conduction, also enables efficient neural connectivity and provides metabolic support for most projection neurons. However, different neuronal circuits have varying myelin status, which can be affected by developmental stage, neuronal activity, or disease. In the brain, dopamine (DA) neurons located in the ventral tegmental area (VTA) project axons to the nucleus accumbens (NAc), forming the key neural circuit responsible for reward processing and motivational behavior. Although the VTA-NAc

DA neuron connectivity is a substrate of neural plasticity, its myelin status has not been well studied. Using transgenic approaches and AAV-mediated cell labeling tools for DA neurons as well as myelin-forming oligodendrocytes, we found that the density of oligodendrocytes remains unaltered around the VTA-NAc pathway from the initial rise before P15 to at least P90. We also observed that there is no or little association between MBP or $EGFP^+$ oligodendrocyte processes and DA neuron axons, as well as between markers for the nodes of Ranvier and DA neuron axons. VTA-originated DA neuron axons terminated in different brain areas did not overlap with MBP^+ myelin. In contrast, $NF200^+$ projection neuron axons in the same brain area were highly overlapped with oligodendrocyte processes and the nodes of Ranvier maker. Moreover, AAV-mediated chemogenetic stimulation of DA neurons did not increase the oligodendrocyte processes near DA neurons. These findings suggest that the VTA-NAc DA neuron axons are not myelinated in the young mouse brain, and their short-term enhanced activity does not involve new myelination as a neural plasticity mechanism.

Disclosures: J. Coakley: None. **E. Ikpeme:** None. **H. Kim:** None. **Y. Son:** None. **S. Kang:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.17/B99

Topic: B.09. Glial Mechanisms

Support: K. Lisa Yang and Hock E. Tan Center for Molecular Therapeutics in Neuroscience

Title: Ablation of Mical3 Disrupts Oligodendrocyte Development in Mice

Authors: *S. MERROW, C. LI, L. JOO, G. FENG;
McGovern Inst. for Brain Res., MIT Brain and Cognitive Sci., Cambridge, MA

Abstract: Demyelinating diseases such as multiple sclerosis (MS) cause debilitating symptoms that severely impact the quality of life of patients. MS is a pressing public health issue with more than 2.3 million people diagnosed worldwide. Despite its prevalence, treatment for demyelination is limited. Investigating the development of oligodendrocytes (OLs) provides possible mechanisms to target in therapeutic strategies of demyelinating diseases. Our initial in vitro OPC screen found gene microtubule-associated monooxygenase (Mical3) affects OL differentiation. In this study, we aim to elucidate the significance of Mical3 in myelination. To accomplish this, we created a Mical3fl/fl GPR17-Cre conditional knockout line to ablate the Mical3 function. Immunofluorescence staining revealed a significant reduction in myelin basic protein (MBP) expression, a hallmark of myelination, in Mical3fl/fl GPR17-Cre mice compared to littermate wildtype controls. RNA-fluorescence in situ hybridization (FISH) further demonstrated diminished expression with proteolipid protein (plp1), a marker for mature Ols, in

Mical3fl/fl GPR17-Cre. We also performed stereotactic injections of lysophosphatidylcholine (LPC) in the corpus callosum to induce demyelination. Following recovery, the area of injection increased the expression of Mical3 compared to the non-injected hemisphere suggesting the recruitment of Mical3 may be necessary for remyelinating mechanisms. Given these results, Mical3 plays a relevant role in myelination and highlights its potential as a therapeutic target to treat demyelinating diseases.

Disclosures: S. Merrow: None. **C. Li:** None. **L. Joo:** None. **G. Feng:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.18/B100

Topic: B.09. Glial Mechanisms

Support: NINDS R01NS110776
Adelson Medical Research Foundation

Title: Transplanted human CD44-sorted glial progenitor cells manifest phenotypic plasticity with superior engraftment and myelination than PDGFRA/CD140a-defined oligodendrocyte progenitors

Authors: *C. C. LONG[1], S. J. SCHANZ[2], D. M. CHANDLER-MILITELLO[3], J. N. MARIANI[4], S. A. GOLDMAN[5];
[1]Univ. of Rochester Med. Ctr., Rochester, NY; [2]Ctr. for Translational Neuromedicine, Univ. of Rochester, Rochester, NY; [3]Neurol. and the Ctr. for Translational Neuromedicine, Med. Ctr., Rochester, NY; [4]Ctr. for Translational Neuromedicine and the Dept. of Neurol., Univ. of Rochester Med. Ctr., Rochester, NY; [5]Dept of Neurol., Univ. Rochester Med. Ctr., Rochester, NY

Abstract: Human glial progenitor cells (GPCs) exhibit stage-dependent heterogeneity in both their gene expression and fate potential; this diversity of phenotype may need to be considered in the design of cell therapeutics for glial replacement. Here we focused on two populations of GPCs, respectively defined by their expression of either CD44, the hyaluronic acid receptor, and CD140a, the PDGFalpha receptor. We first characterized the composition of three populations of progenitors derived from 20-22 week gestational age human fetal brain, by using FACS to isolate CD140a+/CD44-, CD140a-/CD44+, and CD140a+/CD44+ cells, which were then subjected to single cell RNA-sequencing (scRNA-seq, 10X Genomics). We found little overlap in cell type composition between the three sorts. Most CD140a+/CD44- cells was contained in a cluster expressing PDGFRA/CD140a, SOX10, and NKX2-2, suggesting oligodendrocyte lineage, while a minority still expressed ELAVL4, TUBB3, DLX2 and DCX, suggesting admixture with a less differentiated neural progenitor. The CD44+/CD140a fraction was largely comprised of a cluster - including 94% of CD44+/CD140a- cells - that expressed astrocytic

lineage markers including SOX9, GFAP, and AQP4. The relatively few CD140+/CD44+ cells consisted primarily of ITGAM and P2RY12 expressing microglia, suggesting their identity as microglia. Given the apparent dichotomization of CD44- and CD140-defined pools, we asked if they might retain astrocytic vs. oligodendrocytic biases upon transplantation into hypomyelinated mice. Either CD140a+ or CD44+ progenitors were transplanted neonatally into the presumptive corpus callosa (CC) of immunodeficient and dysmyelinated shiverer mice, which were then killed at 19 weeks. Transplanted CD44+ cells yielded significantly higher callosal engraftment densities for OLIG2+ and/or GSTpi+ oligodendrocyte lineage cells than did CD140a+ progenitors ($9.6x10^4$ vs $6.3x10^4$ cells/mm^3 respectively, p=0.02). CD44+ cells proved superior to CD140a+ cells at engraftment and colonization, likely owing to their receptive relationship with the hyaluronic acid matrix of the developing brain. The myelinogenic competence of CD44+ cells proved as high as that of CD140a+ cells, such that neonatal transplant of CD44+ cells significantly extended the survival of shiverer mice, which otherwise died by 20 weeks; 35% of this cohort survived out to 72 weeks, when they were killed, revealing extensively myelinated brains. Thus CD44+ fetal human GPCs, are highly plastic in their phenotypic potential, colonize host brain effectively, and differentiate readily as oligodendrocytes in vivo when faced with a hypomyelinated environment.

Disclosures: C.C. Long: None. **S.J. Schanz:** None. **D.M. Chandler-Militello:** F. Consulting Fees (e.g., advisory boards); Sana Biotechnology. **J.N. Mariani:** None. **S.A. Goldman:** A. Employment/Salary (full or part-time):; Sana Biotechnology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sana Biotechnology.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.19/B101

Topic: B.09. Glial Mechanisms

Support: NIH grant R01NS122800
AHA grant 23PRE1018862
Esther A. & Joseph Klingenstein Fund

Title: Mitochondrial network reorganization and transient expansion during oligodendrocyte generation

Authors: *X. BAME, R. A. HILL;
Biol. Sci., Dartmouth Col., Hanover, NH

Abstract: Oligodendrocyte precursor cells (OPCs) give rise to myelinating oligodendrocytes of the central nervous system. This process persists throughout life and is essential for recovery from neurodegeneration. To better understand the cellular checkpoints that occur during

oligodendrogenesis, we determined the mitochondrial distribution and morphometrics across the oligodendrocyte lineage in mouse and human cerebral cortex. During oligodendrocyte generation, mitochondrial content expanded concurrently with a change in subcellular partitioning towards the distal processes. These changes were followed by an abrupt loss of mitochondria in the oligodendrocyte processes and myelin, coinciding with sheath compaction. This reorganization and extensive expansion and depletion took 3 days. Oligodendrocyte mitochondria were stationary over days while OPC mitochondrial motility was modulated by animal arousal state within minutes. Aged OPCs also displayed decreased mitochondrial size, content, and motility. Thus, mitochondrial dynamics are linked to oligodendrocyte generation, dynamically modified by their local microenvironment, and altered in the aging brain.

Disclosures: X. Bame: None. **R.A. Hill:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.20/B102

Topic: B.09. Glial Mechanisms

Title: Transfer of nuclear and ribosomal material from Sox10-lineage cells to neurons in the mouse brain

Authors: *O. CHECHNEVA[1], F. MAYRHOFER[2], C. SADEGH[3], M. F. NAVEDO[4], A. SOULIKA[5];
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Abstract: Material transfer is an essential form of intercellular communication to exchange information and resources between cells. Material transfer between neurons and from glia to neurons has been demonstrated to support neuronal survival and activity. Understanding the extent of material transfer in the healthy nervous system is limited. Here we report that in the mouse central nervous system (CNS), neurons receive nuclear and ribosomal material of Sox10-lineage cell (SOL) origin. We show that transfer of SOL-derived material to neurons is region dependent, establishes during postnatal brain maturation, and dynamically responds to LPS-induced neuroinflammation in the adult mouse brain. We identified satellite oligodendrocyte-neuron pairs with loss of plasma membrane integrity between nuclei, suggesting direct material transfer. Together, our findings provide evidence of regionally coordinated transfer of SOL-derived nuclear and ribosomal material to neurons in the mouse CNS, with potential implications for the understanding and modulation of neuronal function and treatment of neurological disorders.

Disclosures: O. Chechneva: None. **F. Mayrhofer:** None. **C. Sadegh:** None. **M.F. Navedo:** None. **A. Soulika:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.21/B103

Topic: B.09. Glial Mechanisms

Support: NIH Intramural Research Program

Title: Phosphatidylinositol 4-kinases PI4KA and PI4KB differentially regulate myelination and basal lamina development within the Peripheral Nervous System (PNS) in mice

Authors: *A. ALVAREZ-PRATS[1], Z. N. ALDWORTH[2], M. A. STOPFER[3], T. BALLA[4]; [1]Eunice Kennedy Shriver NICHD, NIH., Bethesda, MD; [3]NICHD, [2]NIH-NICHD, Bethesda, MD; [4]NIH, Bethesda, MD

Abstract: Myelin formation is a highly organized and dynamic process that requires well-orchestrated communication between the various participating cells. In the PNS, the myelinating Schwann cells (SCs) that surround the peripheral axons, synthesize and transport large amounts of membrane lipid components to form the myelin sheath. Two phosphatidylinositol 4-kinases (PI4Ks), namely PI4KA and PI4KB, produce most of the phosphatidylinositol 4-phosphate (PI4P), a regulatory lipid that controls both vesicular trafficking and non-vesicular lipid transport. PI4KA is localized at the plasma membrane (PM), where it produces PI4P that serves both as the precursor of $PI(4,5)P_2$ and as a driver of phosphatidylserine (PS) transport from the endoplasmic reticulum (ER) to the plasma membrane (PM). In contrast, PI4KB, primary located in the Golgi, controls post-Golgi vesicle transport as well as the delivery of cholesterol and ceramide from the ER to the Golgi. Remarkably, in peripheral nerves, PI4KB also localizes to the node of Ranvier, where along with basal lamina (BL) proteins like laminin-α2 and -α5, are necessary for proper myelination as well as clustering of sodium channels and hence proper nerve conductance. Here, by using two mouse models developed in our lab with targeted deletion of PI4KA (α-mice) or PI4KB (β-mice) specifically in their SCs, we show that both models display dramatic defects in peripheral myelination with striking phenotypic differences. The α-mice, more severely affected, display much more severe motor defects than the β-mice. Moreover, while both models show a reduction in the total lipid content of their sciatic nerves, this reduction is more prominent in the α-mice model and affects mostly PS while the β-mice show relatively greater reductions in phosphatidylethanolamine and sphingomyelin but not in PS. The laminin receptor integrin-α6β1 significantly decreases in the sciatic nerves of both models; however, the BL protein laminin-α2, shows accumulation and focal mislocalization in the sciatic nerves of α-mice, whereas a dramatic and homogenous decrease in the β-mice. This latter result is quite notable given our observation that the nerve pathology of the β-mice shares several features reported in patients with LAMA2 neuropathy, a demyelinating neuropathy caused by

loss of function mutations in the laminin-α2 gene. We conclude that basal lamina defects make important contributions to the distinct myelination defects in PI4KA and PI4KB-deficient SCs. Current efforts are focused on elucidating the underlying molecular mechanisms involved in the basal lamina pathologies observed in the two animal models.

Disclosures: A. Alvarez-Prats: A. Employment/Salary (full or part-time):; NICHD, NIH. **Z.N. Aldworth:** None. **M.A. Stopfer:** None. **T. Balla:** None.

Poster

PSTR153: Oligodendrocytes: Development, Function and Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR153.22/B104

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH R01HL146574
NIH RF1AG065345
NIH R01NS134134
NIH R21AG073862
NIH R21AG064422

Title: Oligodendrocyte-derived laminin regulates blood-brain barrier integrity and CNS myelination

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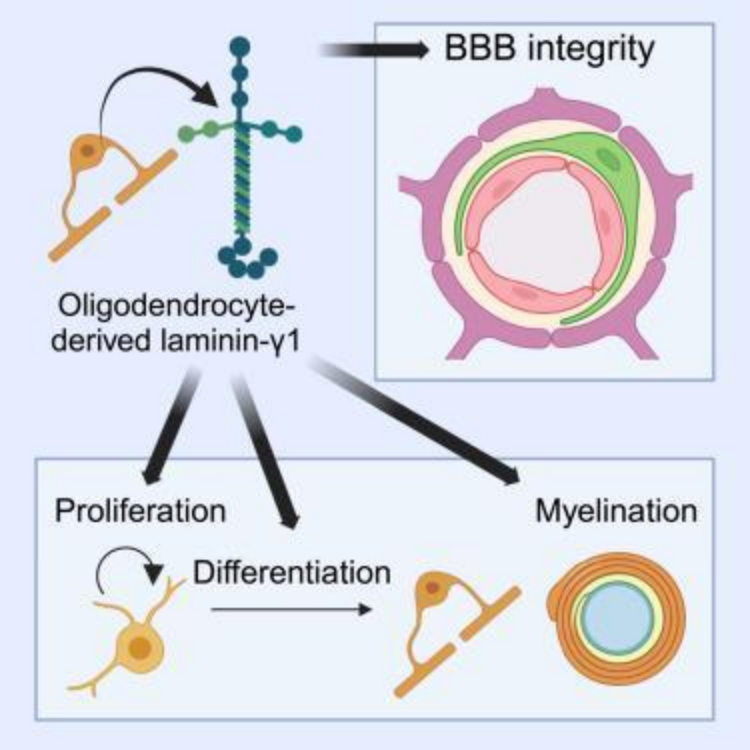
Abstract: Although oligodendrocytes (OLs) are able to synthesize laminin-gamma1, the most widely used gamma subunit, its functional significance in the CNS remains unknown. To answer this important question, we generated a conditional knockout mouse line with laminin-gamma1-deficiency in OL lineage cells (OKO). OKO mice exhibited muscle weakness/paralysis and all died by P33. In addition, OKO mice developed blood-brain barrier (BBB) disruption in both cortex and striatum. Subsequent studies revealed decreased Mfsd2a expression and increased endothelial caveolae vesicles, but unaltered tight junction protein expression and intact tight junction ultrastructure, indicating a transcellular rather than paracellular mechanism of BBB breakdown. Furthermore, significantly reduced OL lineage cells, OL precursor cells (OPCs), proliferating OPCs, and mature-OLs were observed in OKO brains in a region-specific manner. Consistent with this finding, various defects in myelination were detected in OKO brains at both biochemical and ultrastructural levels. Overall, these results highlight important roles of OL-derived laminin-gamma1 in BBB maintenance and OL biology (proliferation, differentiation, and myelination).



Disclosures: Y. Yao: None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.01/B105

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

Support: NIH Grant R03 AG072218
NIH Grant R01 NS129632

Title: Characterization of myelin and AD disease markers in a novel hypermyelinating App knock-in mouse

Authors: *T.-H. TUNG[1], P. BASA[2,3], M. ROMER[4], V. FIESLER[1], T. SAITO[5], T. SAIDO[6], X. TANG[1], J. B. GRINSPAN[7], T. D. KOZAI[1], F. CAMBI[1];
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Abstract: Oligodendrocyte (OL) dysfunction and myelin loss play critical roles in the pathogenesis of Alzheimer disease (AD). In this study, we sought to investigate whether enhancing myelin resilience attenuates disease progression in an AD mouse model. We have generated a novel hypermyelinating preclinical AD mouse model by crossing the hypermyelinating $Fus^{OL}cKO$ with the $App^{NL\text{-}G\text{-}F}$ (referred to as AppKI/FuscKO). In the AppKI, Aβ aggregation is fully developed at 6 months, but cognitive deficits are mild and manifest in >12-month-old (mo) AppKI mice. To investigate how hypermyelination influences cellular changes in the hippocampus (HC) and prefrontal cortex (PFC), we have measured myelin, Aβ plaques, and astroglial activation of the AppKI and AppKI/FuscKO mice at 6 months of age. Myelin oligodendrocyte glycoprotein (MOG), a marker of outer myelin and myelin basic protein (MBP), a marker of compact myelin were stained in brain cryosections, imaged by confocal microscopy and quantified by Image J. Greater density of MOG (5.43 ± 0.64 and 2.78 ± 0.27, $p = 0.03$) and MBP (6.34 ± 0.48 and 2.88 ± 0.79, $p=0.003$) was present in PFC layer 2-3 of the AppKI/FuscKO mice vs. the AppKI. Only MBP density was higher in the AppKI/FuscKO CA1 subregion of the HC (5.54 ± 0.15) vs. the AppKI (2.98 ± 0.22, $p = 0.0006$). Cholesterol, a major driver of myelin formation, was higher in AppKI/FuscKO (11.00 mg/g tissue ± 0.30) vs. AppKI HC (8.30 ± 0.18, $p < 0.0001$). In contrast, the cholesterol level was similar between genotypes in the PFC (9.74 ± 0.31 and 8.58 ± 0.33). No significant differences were detected in Aβ plaque number, density, area, and size in PFC and CA1 subregion. $GFAP^+$ and $IBA1^+$ densities, a measure of glial activation, were similar in PFC and CA1 of AppKI and AppKI/Fuscko. Given the significant increase in myelin and cholesterol in the AppKI/FuscKO, we next investigated how these cellular changes influenced memory using novel spatial recognition test. There was a slight but not significant trend towards greater time spent in the novel arm in 15-16 mo AppKI/FuscKO (36.17 ± 3.36%, n=6-8) vs. AppKI (32.07 ± 2.89%). The data show that Fus depleted AppKI OL support myelin density in a spatially defined manner in areas of AD neurodegeneration. Cholesterol is critical to maintain synaptic integrity, therefore, selective increase of cholesterol in CA1 suggests a role in preventing AD dependent synaptic loss. Studies are underway to characterize neurodegeneration, synaptic density and spatially restricted glial activation and define the underlying molecular mechanisms.

Disclosures: T. Tung: None. **P. Basa:** None. **M. Romer:** None. **V. Fiesler:** None. **T. Saito:** None. **T. Saido:** None. **X. Tang:** None. **J.B. Grinspan:** None. **T.D. Kozai:** None. **F. Cambi:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.02/B106

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

Support: NIH Grant AG027297
NIH Grant PO1AG078116
NIH Grant AG078116

Hazel Embry Research Trust
Sylvia Mansbach Endowment for AD Research

Title: The monoclonal antibody, 17E1, selectively labels glial fibrillary acid protein in 5xFAD mice

Authors: *S. KRANER[1], J. GOLLIHUE[1], B. E. WEISS[1], P. SOMPOL[2], C. M. NORRIS[3]; [1]Sanders Brown Ctr. on Aging, [2]Pharmacol. and Nutritional Sci., [3]Sanders-Brown Ctr. on Aging, Univ. of Kentucky, Lexington, KY

Abstract: Background: Our lab recently developed two mouse monoclonal antibodies that preferentially react with "distressed astrocytes". One monoclonal, 26A6, was found to react preferentially with a form of the Ca2+/calmodulin-dependent protein phosphatase, calcineurin (CN), that has been cleaved by calpain, to generate a 48 kDa CN fragment (ΔCN). We recently published a characterization of this antibody. Surprisingly, the second antibody (17E1), generated to the same antigen used to create 26A6, preferentially binds to glial fibrillary acid protein (GFAP) and is present at elevated levels in the 5xFAD mouse strain. **Methods:** A peptide encompassing the calpain sensitive region of the CN carboxyl terminus was used for mouse monoclonal antibody generation.**Results:** We identified two monoclonal antibodies: 26A6 and 17E1. The 26A6 antibody binds to a recombinant CN fragment (48 kDa) that is generated by calpain-mediated proteolysis. We've shown that this antibody selectively labels subsets of reactive astrocytes in human brain in conjunction with Alzheimer's and cerebrovascular pathology, but provides very little labeling in healthy control brain tissue. The 17E1 antibody also labels a 48 kDa protein, but does not label recombinant CN, nor does it label calpain generated CN fragments. Western blot analyses found that the 17E1 and 26A6 antibodies exhibit differential labeling of a 48 kDa protein found in 5xFAD mice. Specifically, 26A6 showed no labeling while 17E1 exhibited highly selective labeling. The 48 kDa band was also detected with a commonly used commercial CN antibody from EMD Millipore (07-1492) that labels full length CN and multiple C terminus truncation products of CN. To our surprise, immunoprecipitation experiments followed by Western and mass spectroscopy showed that the 48 kDa fragment bound by both 17E1 and 07-1492 was actually GFAP. Both the 17E1 and 07-1492 Abs label recombinant GFAP, but 26A6 did not. 26A6 also exhibited no substantial labeling in 5xFAD brain tissue, while 17E1 selectively labels astrocytes in 5X but not wt brain tissue. **Conclusion:** Both 26A6 and 17E1 preferentially interact with "distressed astrocytes". However, 26A6 labels proteolyzed CN, while 17E1 labels GFAP. The results emphasize the importance of using the correct antibodies to evaluate the role of CN in neurodegenerative disease. Further characterization of 17E1 in human brain tissue and rodent models of disease is underway.

Disclosures: S. Kraner: A. Employment/Salary (full or part-time):; University of Kentucky. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Kentucky. **J. Gollihue:** A. Employment/Salary (full or part-time):; University of Kentucky. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Kentucky. **B.E. Weiss:** A. Employment/Salary (full or part-time):; University of Kentucky. **P. Sompol:** A. Employment/Salary (full or part-time):; University of Kentucky. **C.M. Norris:** A. Employment/Salary (full or part-time):; University of Kentucky. E.

Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Kentucky.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.03/B107

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

Support: BrightFocus Foundation Grant A2020833S
Alzheimer's Association Grant AARG-18-52336
National Institutes of Health Grant R01AG066171
National Institutes of Health Grant R01AG077611
National Institutes of Health Grant 5R01AG054598

Title: Disruptions of astrocytic calcium transients in a mouse model of Alzheimer's disease: a compartment-specific analysis

Authors: *M. ABEDIN[1], Y. E. LEE[1], M. ZHANG[2], B. J. BACSKAI[1], K. V. KASTANENKA[1]; [1]Dept. of Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [2]Ctr. for Computat. Mol. Biol. at Brown Univ., Providence, RI

Abstract: Alzheimer's disease (AD) is the most common cause of dementia in the elderly with limited treatments. AD is characterized by deposition of extracellular plaques, intracellular tau tangles and significant neuronal cell death. In addition to neurons, astrocytes functionally modulate neuronal network activity through tripartite synapses. Astrocytic calcium signaling has been implicated in the pathological processes of AD, such as disrupting synaptic transmission, dysregulating glutamate homeostasis, reduction in vascular function through endfeet, and impairing sleep. Despite the neuronal amyloid hypothesis in the progression of AD, there is a lack of a systematic and comprehensive analysis of the astrocytic contribution to disease progression. We utilized in vivo multiphoton imaging of Yellow Cameleon 3.6, a genetically encoded ratiometric calcium sensor, targeted specifically to astrocytes under the GFAP promoter in a mouse model of AD. We monitored spontaneous calcium transients in cortical astrocytes of anesthetized APP/PS1 mice. To gain a complete understanding of astrocyte dynamics in AD, we comprehensively analyzed astrocyte calcium activity in non-transgenic (NTG) control and APP/PS1 mice at 4-6 months an age with significant amyloid production. We quantified the event rate, duration of the events, area under the curve (AUC), and peak amplitude of the events in 3 distinct astrocytic compartments: soma, processes, and microdomains. We report that the activity duration and peak amplitude was statistically higher in the soma of APP/PS1 mice than that in NTG controls. The event rate was significantly higher in the processes and microdomains of APP/PS1 mice. However, the activity duration, AUC, and peak amplitude were statistically lower than those in NTG controls. Thus, astrocytic processes and microdomains were hypoactive, whereas the somas were hyperactive in APP/PS1 mice. Our analysis included a

correlation study, which revealed that the correlated activity was greater in APP/PS1 mice compared to NTG controls. We observed an inverse relationship between pairwise correlation in astrocytic activity and cell-to-cell distance in NTG controls. However, this relationship was absent in APP/PS1 mice. This could be due to astrocytes being further apart in the presence of plaques and/or oligomeric Aβ. Our study provides systematic analyses of calcium transients in different astrocytic compartments and illustrates compartment-specific disruptions of calcium activity caused by amyloidosis. This work provides a strong foundation for future therapeutic development efforts aimed at restoring aberrant astrocytic activity.

Disclosures: M. Abedin: None. **Y.E. Lee:** None. **M. Zhang:** None. **B.J. Bacskai:** None. **K.V. Kastanenka:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.04/B108

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

Support: NIH/NIA 5K08AG064039
Alzheimer's Association
Massachusetts Life Science Center
The Karen Toffler Charitable Trust
The Jack Satter Foundation
Harrison Gardner, Jr. Innovation Award
Real Colegio Complutense at Harvard University
Prince Mahidol Youth Award
Martin L. and Sylvia Seevak-Hoffman Award for Alzheimer Research

Title: Amelioration of neuronal tauopathy by astrocyte GFAP upregulation involves multiple proteostasis-related GFAP interactors

Authors: C. MUÑOZ-CASTRO[1,2,3], M. A. HEALEY[4], M. JAISA-AAD[4,3], A. NOORI[4,5], R. JAYAKUMAR[4], E. ZANIEWSKI[4], R. MORRIS[4,3], M. CALVO-RODRIGUEZ[6], E. HUDRY[7], S. DAS[4,3], W. HAAS[4,3], B. T. HYMAN[4,3], *A. SERRANO-POZO[4,3];
[1]Univ. of Seville, Seville, Spain; [2]Massachusetts General Hospital, Boston, MA; [3]Harvard Medical School, Boston, MA; [4]Massachusetts Gen. Hosp., Boston, MA; [5]Harvard College, Boston, MA; [6]Discovery Neurosci., Foundational Neurosci. Ctr., AbbVie, Cambridge, MA; [7]Novartis Inst. for Biomed. Res., Cambridge, MA

Abstract: Background: Reactive astrogliosis is spatially associated with the amyloid-β plaques and phospho-tau (pTau) neurofibrillary tangles that define Alzheimer's disease (AD) but its impact on these AD neuropathological changes remains controversial. A long-known feature of reactive astrocytes is the upregulation of the glial fibrillary acidic protein (GFAP). While this is

mainly thought to represent a remodeling of the astrocyte intermediate filament cytoskeleton intended to both enhance motility and form the glial scar, GFAP has also been implicated in chaperone-mediated autophagy and synaptic function. Here we hypothesized that GFAP upregulation helps reactive astrocytes control the burden of pTau aggregates and/or protect synapses and neurons. **Methods:** Using a viral transfer strategy, we overexpressed Myc-tagged human wild-type GFAP ($GFAP^{WT}$) or the Alexander disease-linked R239H mutant ($GFAP^{R239H}$) in astrocytes of 4-month-old THY-Tau22 mice (Thy1.2-*MAPT*$^{G272V/P301S}$, 1N4R) and euthanized them 7 months later. Negative control groups consisted of THY-Tau22 littermates injected with either phosphate-buffered saline (PBS) or the same viral vector encoding the enhanced green fluorescent protein (EGFP). *Ex vivo* phenotyping included immunohistochemistry, biochemistry, bulk RNA-seq, and 18-plex tandem mass tag (TMT)-based mass spectrometry. **Results:** Both $GFAP^{WT}$ and $GFAP^{R239H}$ mice had reduced hippocampal AT8+ neurofibrillary tangles and AT8+ pTau levels and increased synaptophysin levels relative to EGFP and PBS groups. Cortical bulk RNA-seq followed by weighted gene correlation network analysis (WGCNA) and pathway enrichment analysis revealed a positive correlation of GFAP expression levels with gene modules enriched in extracellular matrix and the Rho GTPases involved in lamellipodia and filopodia formation. Analysis of the GFAP interactome by immunoprecipitation followed by TMT-based proteomics uncovered many novel GFAP interactors, including effectors of the endolysosomal and macropinocytosis pathways, and small stress chaperones. **Conclusions:** Our findings indicate that GFAP upregulation by astrocytes may attenuate neuronal pTau burden through several proteostasis mechanisms mediated by novel GFAP interactors. These data suggest a novel role of GFAP as orchestrator of astrocyte-extracellular matrix interactions, intracellular vesicle trafficking, and protein aggregate clearance. Ongoing work will further elucidate the intimate molecular mechanisms involved in GFAP-mediated proteostasis.

Disclosures: C. Muñoz-Castro: None. **M.A. Healey:** None. **M. Jaisa-aad:** None. **A. Noori:** None. **R. Jayakumar:** None. **E. Zaniewski:** None. **R. Morris:** None. **M. Calvo-Rodriguez:** A. Employment/Salary (full or part-time):; Current affiliation: AbbVie. **E. Hudry:** A. Employment/Salary (full or part-time):; Current affiliation: Novartis. **S. Das:** None. **W. Haas:** None. **B.T. Hyman:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; research grants from the National Institutes of Health, Cure Alzheimer's Fund, Tau Consortium, and the JPB Foundation – and sponsored research agreements from Abbvie, BMS, and Biogen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dewpoint, Novartis, and lattus. F. Consulting Fees (e.g., advisory boards); AbbVie, Ambagon, Aprinoia Therapeutics, Arvinas, Avrobio, AstraZenica, Biogen, BMS, Cure Alz Fund, Affiliation: Cell Signaling, Dewpoint, Latus, Novartis, Sofinnova, Vigil, Violet, Voyager, WaveBreak. **A. Serrano-Pozo:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ionis Phamaceuticals, Inc. (receipt of reagents).

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.05/B109

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

Support: R01NS131486
McKnight Scholar Award
Rita Allen Scholar Award
Klingenstein-Simons Fellowship Award in Neuroscience
DP2MH132943

Title: Exploring oligodendrocyte precursor cell-synapse interactions in Alzheimer's disease

Authors: *S. X. TANG[1,2], A. FERRO[1], L. CHEADLE[1,3];
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Abstract: Alzheimer’s disease (AD) is the most prevalent neurodegenerative disease, characterized by two major hallmarks: extracellular amyloid-β plaques and intracellular neurofibrillary tau tangles. The best correlate of AD progression is synaptic loss, which begins at a preclinical stage before cognitive decline is evident. Understanding the cellular and molecular changes that drive synaptic loss in the early stages of AD is crucial for developing interventions which may prevent or slow disease progression. Our recent findings reveal that oligodendrocyte precursor cells (OPCs) phagocytose presynaptic inputs in the developing and adult brain independent of their role in oligodendrogenesis. Additionally, OPCs are unique among glial cells in that they form functional synaptic contacts with neurons, thereby linking neuronal activity to OPC function. However, the role of OPCs in AD-related synaptic changes has not been well-studied. Using an immunohistological approach, we developed an optimized engulfment assay to investigate OPC-synapse interactions in the CA1 region of the hippocampus, a brain area that mediates learning and memory and is highly susceptible to AD. In the 3xTg-AD mouse model, we evaluated the contact and engulfment of synapses by OPCs at 3, 6 and 12 months of age, representing different stages of AD progression. At 6 months, OPCs in AD mice contacted and engulfed fewer intra-hippocampal, vesicular glutamate transporter 1 (VGluT1)-expressing inputs from CA3 while the engulfment of cortico-hippocampal, vesicular glutamate transporter 2 (VGluT2)-expressing inputs were unaffected. OPCs also exhibited increased cell volume compared to control mice at 6 months. However, no significant differences in OPC-synapse interactions were observed at 3 and 12 months. This suggests that, while OPC-synapse interactions remain largely stable in the earliest stages of AD, intra-hippocampal inputs are selectively spared from engulfment at 6 months, before the appearance of amyloid-β plaques. Furthermore, this reduction in OPC-mediated synapse engulfment appears to be temporally restricted as this pattern of engulfment is resolved at later stages of the disease. Interestingly, the trends in OPC engulfment were similar to those in OPC synaptic contacts across all timepoints, suggesting a potential link between the two. Our study demonstrates that disruptions in OPC-synapse interactions occur at a stage preceding the appearance of amyloid-β plaques and tau tangles. These findings underscore the significance of OPCs in the early stages of AD and may guide therapeutic strategies aimed at mitigating synaptic loss during disease progression.

Disclosures: S.X. Tang: None. **A. Ferro:** None. **L. Cheadle:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.06/B110

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

Support: R56 AG049870
R01 AG059028
P01 AG005138
P30 AG066514
R01 AG078755

Title: Astrocytic and vascular changes associated with neocortical vulnerability in Alzheimer's disease

Authors: *A. K. MCKENDELL[1], S. MAGALHAES[1], E. MCDONOUGH[2], L. LOWERY[2], D. MEYER[2], J. I. LUEBKE[3], P. R. HOF[1], M. VARGHESE[1];
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Abstract: Alzheimer's disease (AD) is the most common form of dementia, affecting an estimated 7 million people in the US alone. AD follows a regional pattern of pathology and neurodegeneration across the brain that affects cognition and memory. However, our understanding of what contributes to this regional vulnerability is limited. To address this, we performed highly multiplexed immunofluorescence staining (MxIF) on postmortem human samples for 26 markers relating to cell types, cell features, cell states, tissue architecture, and AD neuropathology. We compared an AD-susceptible brain area, the dorsolateral prefrontal cortex (DLPFC, Brodmann's areas 9, 10 or 46) to an AD-resistant brain area, the primary visual cortex (V1, area 17), and related our findings to specific metrics of AD in age-matched subjects representing both women and men. Subjects were grouped as follows: for clinical dementia rating (CDR) scores, AD (n = 3, CDR 3), mild cognitive impairment (n = 4, CDR 0.5) and healthy controls (n = 5, CDR 0); for Thal stage, AD (n = 2, stage 4), pre-AD (n = 8, stages 1-3), and control (n = 2, stage 0); for Braak stage, AD (n = 5, stages III-VI), control (n = 7, stages I-II). The MxIF images were stitched together, registered across rounds using cell nuclei as reference, and the autofluorescence background was subtracted. Using the open-source image analysis program QuPath, the images were segmented for aldehyde dehydrogenase (ubiquitous astrocyte marker), glial fibrillary protein (reactive astrocyte marker), collagen IV (vasculature), and amyloid β protein (Aβ). We found that reactive astrocytes increased in proximity to Aβ plaques with worsening CDR specifically in the V1. Interestingly, we did not find increased juxtavascular reactive astrocytes with CDR, but rather an upward trend with worsening Thal

stage, specific to layers 4 through 6 and the white matter of the DLPFC. Given a similar trend in increased overall astrocytic reactivity, analyzing whether astrocytic reactivity increases specifically around vessels laden with Aβ will help determine if this is also a regional response to cerebral amyloid angiopathy or a ubiquitous change with worsening Thal stage. These preliminary results suggest a heterogenous astrocytic response proximal to vasculature and pathological protein deposits between neocortical regions of varying vulnerability to AD. We are investigating markers for astrocyte morphology and cell state to assess how astrocyte populations vary across the neocortex with worsening cognition, and ultimately how this may contribute to selective neuronal vulnerability in AD.

Disclosures: A.K. McKendell: None. **S. Magalhaes:** None. **E. McDonough:** None. **L. Lowery:** None. **D. Meyer:** None. **J.I. Luebke:** None. **P.R. Hof:** None. **M. Varghese:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.07/B111

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

Support: ARUK-PPG2021B-012

Title: Investigating the role of Endothelin B receptors in modulating astrocyte activation and neuroinflammation in mouse and human models of Alzheimer's disease

Authors: *J. LI, C. SZETO, A. B. ALI;
Univ. Col. London, London, United Kingdom

Abstract: Alzheimer's disease (AD) is a neurodegenerative disease characterised by progressive memory loss and cognitive impairment. Typical hallmarks of the disease include the accumulation of toxic amyloid-β (Aβ) and increased reactive astrocytes, abundant glial cells that co-express the Endothelin B receptor (ET_BR), thought to mediate antiinflammatory responses in various neurodegenerative conditions. Astrocytes are essential for providing neuronal support via the ET_BR, and its peptide agonist endothelin-1(ET-1), increases in response to traumatic brain injury. Although ET-1 plays a role in the pathogenesis of brain disorders, especially AD, there is a missing gap in our knowledge about the involvement of ET_BRs and dementia. We therefore, explored changes in neuroinflammation associated with ET_BR expression during AD and the modulation impact on neuroinflammatory markers. Immunoperoxidase and immunofluorescence combined with confocal microscopy was performed using brain slices containing the CA1 region of the hippocampus of a APP^{NL_F/NL_F} mouse model of AD that harbors the mutant human amyloid precursor protein, age-matched to wild-type littermates (12-16 months), as well as an induced pluripotent stem cell hiPSCs model generated from familial AD patients carrying *APP* mutations and their isogenic controls containing co-culture of atsrocytes, excitatory and inhibitory neurons. There was a significant increase in the expression of ET_BRs that appeared as

clusters in both resting and reactive astrocytes, indicated by the expression of the transcription factor Sox-9 (SOX9) and Glial Fibrillary Acidic Protein (GFAP), respectively in *APP*$^{NL\_F/NL\_F}$ mice compared to wild-type (WT) mice.These findings were also observed in our hiPSC co-culture model, and human post-mortem brain tissue from both control and confirmed cases of AD patients. We then performed *in vivo* dosing using a selective ET_BR antagonist, IRL 2500 (10 mg/kg), or vehicle (5% DMSO) using cohorts of *APP*$^{NL\_F/NL\_F}$ mice age-matched to WT mice. There was a significant reduction of ET_BR expression within GFAP in the cohort treated with ET_BR antagonist IRL 2500 compared to the vehicle-treated. This was further explored by co-expression studies of C3d/ S100A10 as the marker of A1 (neurotoxic) and A2 (neuroprotective) subtypes of astrocytes, which showed an alternation in ratio of the A1:A2 astrocytes. In conclusion, our data suggest that expression of ET_BRs is associated with astrocytes and is significantly upregulated in various AD models. The antagonism of ET_BRs may therefore be a promising therapeutic strategy in modulating neuroinflammation in AD by "normalization" of ET_BR expression.

Disclosures: J. li: None. **C. Szeto:** None. **A.B. Ali:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.08/B112

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

Support: P01AG014449
P01AG025204
P30AG066468

Title: Characterization of [18F]SMBT-1 binding in relation to reactive astrogliosis in Alzheimer's disease

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Abstract: Development of biomarkers for neuroinflammation/gliosis is a desirable research goal towards the timely and accurate diagnosis of Alzheimer's disease (AD). Astroglia react to AD pathology with changes in their morphology and increased expression of inflammatory molecules and the enzyme monoamine oxidase B (MAO-B). Recent studies reported that the radiopharmaceutical [18F]SMBT-1 interacts with MAO-B, resulting in higher PET signal in AD subjects compared to cognitively unimpaired (CU) controls. However, the patterns of

[18F]SMBT-1 binding in relation to MAO-B expression in astrocytes and AD pathology remains to be determined. This post-mortem study used in-vitro radiometric binding and autoradiography assays in single blocks of frozen middle temporal gyrus (BA21) to assess the ability of [18]F-SMBT-1 to distinguish AD from related neurodegenerative diseases (PART, FTLD, CBD, PSP) and CU controls. We also examined the relationship between [18]F-SMBT-1 binding and MAO-B protein activity levels as well as MAO-B immunohistochemical localization in the same frozen tissue samples. Compared to CU controls, AD had higher levels of [18F]SMBT-1 which reached statistical significance for the in-vitro binding assay (ANOVA: $F[3, 30] = 5.796$, $P = 0.0030$; Tukey: control < AD, $P = 0.0045$) and a trend for autoradiography. The PART group trended lower than the AD, while the non-AD tauopathy group largely overlapped with the AD group. MAO-B activity was higher in the AD group compared to the other three groups but did not reach statistical significance. Higher [18F]SMBT-1 in-vitro binding levels correlated with higher MAO-B activity in frozen brain tissue homogenates (Spearman $r = 0.6632$, $P = 0.0020$), and [18F]SMBT-1 autoradiography signal corresponded closely to MAO-B immunoreactive astrocytes in sections from the same frozen tissue block. Higher [18F]SMBT-1 autoradiography signal correlated with higher [18F]SMBT-1 binding levels (Spearman $r = 0.7273$, $P = 0.0096$). Our results provide support for the use of [18F]SMBT-1 as a biomarker of reactive astrogliosis associated with high MAO-B expression and AD pathology. Ongoing studies focus on further characterization of the phenotype and proteomic profiles of MAO-B expressing reactive astrocytes in AD brain tissue in larger numbers of cases.

Disclosures: E.E. Abrahamson: None. **L. Shao:** None. **W.R. Paljug:** None. **M.L. Debnath:** None. **V.N. Pivtoraiko:** None. **V.L. Villemagne:** None. **E.J. Mufson:** None. **M.D. Ikonomovic:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.09/B113

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

Support: Warren Alpert Foundation Distinguished Scholars Award
NIH NIA R01AG073918
NIH NIA P30AG066509

Title: Microglial gene expression and morphology are altered in individuals resilient to Alzheimer's disease

Authors: A. N. COCHOIT[1], N. E. KARAGAS[1], C. S. C. JOHNSON[1], S. MAMDE[2], I. C. SMITH[1], A. N. REID[3], K. J. GREEN[1], A. S. PARIHAR[1], C. D. KEENE[4], T. J. GRABOWSKI, Jr.[5], C. S. LATIMER[6], K. Z. LIN[7], S. JAYADEV[1], ***K. E. PRATER**[1];
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Abstract: Alzheimer's disease (AD) is the most common form of dementia, affecting millions of individuals worldwide. Despite the enormous impact on patients and their care partners, limited therapeutic options are available. One research avenue with therapeutic potential is understanding the biology underlying "resilience" to AD. Resilient individuals display amyloid beta plaques and phosphorylated tau tangles neuropathologically, but lack the cognitive decline associated with dementia. Here we report a cohort of 40 human individuals with an average age over 80 years and average post-mortem interval less than 8 hours. The cohort contains 18 resilient individuals (12 female, 6 male, non-cognitively impaired, mini-mental state exam (MMSE) assessment interval less than 12mo from death, average MMSE score=27, average Alzheimer's disease neuropathic change score (avg. ADNC)=2.4), 11 "resistant" aged controls (6 female, 5 male, avg. MMSE=28, avg. ADNC=0.7), and 11 "susceptible" demented individuals (8 female, 3 male, avg. MMSE=19, avg. ADNC=2.8). We performed single-nucleus RNAseq on nuclei enriched for microglia using PU.1 FANS. As expected, we observed multiple microglial phenotypes including multiple forms of homeostatic gene expressing microglia. We demonstrate composition differences in microglia populations displaying classic inflammatory signaling and a shift in inflammatory gene expression in resilient individuals compared to susceptible individuals. We also performed immunohistochemistry on prefrontal cortex. Confocal stitches of the cortex demonstrate an overall shift in microglial morphology between resistant, resilient, and susceptible individuals. Analysis of high magnification images demonstrate that microglial volume, branch length, and process endpoint number differ between groups. These findings suggest that inflammatory microglial morphology is altered in resistant and resilient individuals. Specific targets can now be identified to further new therapeutic development by shifting specific microglial phenotypes in individuals susceptible to AD.

Disclosures: A.N. Cochoit: None. **N.E. Karagas:** None. **C.S.C. Johnson:** None. **S. Mamde:** None. **I.C. Smith:** None. **A.N. Reid:** None. **K.J. Green:** None. **A.S. Parihar:** None. **C.D. Keene:** None. **T.J. Grabowski:** None. **C.S. Latimer:** None. **K.Z. Lin:** None. **S. Jayadev:** None. **K.E. Prater:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.10/B114

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

Support: NIH grant R01AG061708-05
Gift from Acumen Pharmaceuticals

Title: Amyloid beta oligomer-associated glial pathology in Alzheimer's Disease: Identification of an AβO subtype that binds to the earliest reactive astrocytes and is later engulfed by activated microglia

Authors: *D. KRANZ[1], R. B. SILVERMAN[2], W. L. KLEIN[3];
[1]Northwestern Univ., Evanston, IL; [2]Chem., Northwestern Univ., Evanston, IL; [3]Neurobio., Northwestern Univ., Evanston, IL

Abstract: Glial cell activation is considered a primary pathogenic event in Alzheimer's Disease (AD). Molecules that induce the earliest activation of glia in AD, however, have not yet been identified. One possible gliosis-inducing candidate is Amyloid beta oligomers (AβOs), the most toxic form of Aβ. AβOs can activate glia when introduced into culture or rodent brain, but their role in gliosis in the physiological context of AD is not well known. Here, we identify an AβO subtype, probed by clinical trial candidate ACU193, that interacts with the earliest activated glia in 8 week-old 5xFAD brain. While other AβOs accumulate in a halo around Aβ plaques as mice age, ACU193$^+$ AβOs bind in increasing abundance to reactive astrocytes, but then decrease at ~8 months as they become engulfed by activated microglia. To help determine whether ACU193$^+$ AβOs activate glia with which they associate, we orally treated young 5xFAD mice with a novel small-molecule inhibitor of AβO formation (NU-9) for 60 days and analyzed their brains with immunofluorescence microscopy. 5xFAD mice receiving NU-9 had a remarkable 3-fold decrease in reactive astrocyte levels relative to control. Super resolution microscopy on AD human hippocampal tissue shows ACU193$^+$ AβOs colocalize with reactive astrocytes in a similar manner in humans as they do in mice, intimating translational significance in our findings. Taken together, our study further supports AβOs as primary pathological instigators in AD and provides evidence that early induction of reactive astrogliosis is caused by an ACU193$^+$ AβO subtype.

Disclosures: D. Kranz: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Acumen Pharmaceuticals, Inc.. **R.B. Silverman:** None. **W.L. Klein:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Acumen Pharmaceuticals, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acumen Pharmaceuticals, Inc..

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.11/B115

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

Support: NIH/NIA K01AG083128
HMH-BCM grant in ADRD

Title: Restoration of Cognitive Function in Alzheimer's Models by astrocytic NFIA and Sox9

Authors: *D. CHOI, B. DENEEN;
Baylor Col. of Med., Houston, TX

Abstract: Astrocytes are the most abundant and diverse glial cells in the adult brain, comprising 70% of the glial constituency. Astrocytes perform essential tasks for normal brain function and contribute to various neurological disorders, including neurodegenerative diseases such as Alzheimer's disease (AD). However, their role in health and disease remains a mystery. Recently, we found that Sox9 and NFIA contribute to astrocyte-mediated regulation of brain circuits and memory in the brain, and they were highly increased in reactive astrocytes in the AD mouse brain. Although the reactive astrocytes are closely associated with degenerating neurons across multiple brain regions in patients with AD, it is largely unknown how astrocytes contribute to the initiation and progression of AD. To decipher the roles of NFIA and Sox9 in reactive astrocytes associated with AD, we individually knocked them out in astrocytes in the NLGF-APP mouse model of AD and discovered an increase in Aβ production. Next, we used AAV approaches to overexpress NFIA or Sox9 in astrocytes in the NLGF-APP model at time points where mice had existing Aβ plaques. We found a dramatic loss of plaques coupled with the preservation of neurons. Subsequent cognitive behavioral tests demonstrated a concomitant enhancement of memory recall in mice where NFIA or Sox9 were overexpressed. Mechanistically, we found that astrocytes overexpressing NFIA or Sox9 demonstrated increased morphological complexity, coupled with enhanced uptake of Aβ plaques. These observations suggest enhanced astrocytic phagocytosis of Aβ plaques when NFIA or Sox9 is overexpressed, and consistent with this, we identified increased expression of MEGF10, a key phagocytosis gene, in astrocytes overexpressing NFIA or Sox9. Together, these studies reveal that elevated levels of NFIA and Sox9 in astrocytes enhance cognitive function in AD models and likely do so by enhancing the phagocytosis of harmful Aβ plaques.

Disclosures: D. Choi: None. **B. Deneen:** None.

Poster

PSTR154: Glial Contributions to Alzheimer’s Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.12/B116

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

Support: Branch Out Neurological Foundation
Medicine by Design
FDC Foundation

Title: The Impact of Blood-Brain Barrier Modulation by Focused Ultrasound on Oligodendrogenesis for Neurodegenerative Disease

Authors: *K. S. NOSEWORTHY[1], J. SILBURT[2], K. MIKLOSKA[3], K. HYNYNEN[4], I. AUBERT[5];

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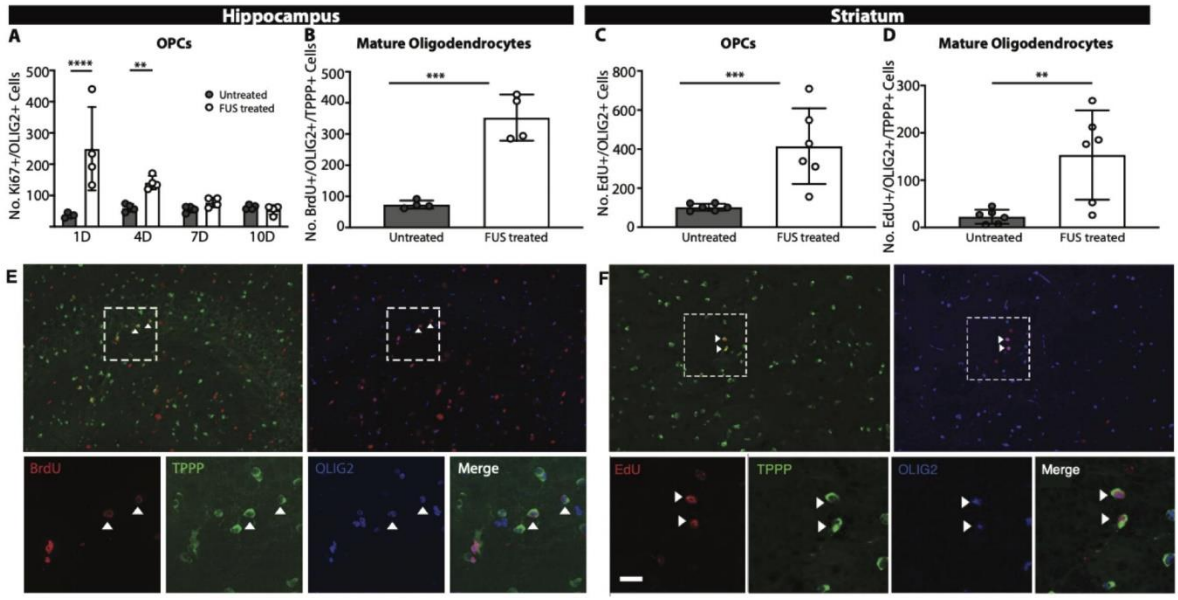
Abstract: Background: Focused ultrasound combined with intravenous microbubbles (FUS) can modulate the blood-brain barrier (BBB) in a controlled, reversible, localized, and non-invasive manner. While initially used to increase the delivery of intravenous therapeutics to the brain, FUS-BBB modulation without intravenous therapeutics can promote elements of brain regeneration, including hippocampal neurogenesis. To further explore the potential regenerative effects of FUS-BBB modulation, we evaluated its impact on the proliferation of oligodendrocyte progenitor cells (OPCs) and oligodendrogenesis in the hippocampus and striatum of adult mice. **Methods:** We use MRI-guided FUS to precisely target the hippocampus or the striatum unilaterally in C57BL/6J mice and induce a local and temporary BBB disruption. Thymidine analogues were administered to label dividing cells post-FUS. Proliferation of OPCs was quantified at 1, 4, 7, and 10 days post-FUS, while mature oligodendrocytes were quantified at 30 days post-FUS. **Results**: FUS-BBB modulation increased the proliferation of hippocampal OPCs by 7-fold ($p<0.0001$) and 2-fold ($p=0.0027$) at 1 and 4 days post-FUS, respectively, and led to a 5-fold ($p=0.0005$) increase in mature oligodendrocytes after 30 days. In the striatum, FUS-BBB modulation significantly increased the generation of OPCs by 4-fold one week post-FUS ($p=0.0002$), which led to a 7-fold ($p=0.0041$) increase in oligodendrogenesis after 30 days. **Conclusions:** This work demonstrates for the first time that FUS-BBB modulation stimulates oligodendrogenesis in the adult hippocampus and striatum, representing neurogenic and non-neurogenic niches, respectively. FUS-mediated oligodendrogenesis could serve as a regenerative treatment in different regions affected by disorders such as in Alzheimer disease and multiple sclerosis. The non-invasive nature of FUS-BBB modulation, in addition to previously reported effects promoting neural plasticity and reducing pathology, positions FUS as a multimodal approach to address diverse aspects of neurological disorders.



Disclosures: K.S. Noseworthy: None. **J. Silburt:** None. **K. Mikloska:** None. **K. Hynynen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); FUS Instruments. **I. Aubert:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.13/B117

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

Support: Grant Agency of the Czech Republic [GACR 309/09/1696 (to JJR)
Spanish Government Plan Nacional de I + D + I 2008-2011, and ISCIII Subdirección General de Evaluación y Fomento de la investigación co-financed by FEDER [PI10/02738 (to JJR)]
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Spanish Ministerio de Economía y Competitividad, RETOS Colaboración [RTC-2013542-1 co-financed by FEDER (to JJR and XB)]
Ministry of Italian University and Research (MIUR) to EG

Title: Alzheimer´S disease and fronto temporal dementia display equivalent astroglial alterations

Authors: *J. RODRIGUEZ;
IKERBASQUE, Bilbao, Spain

Abstract: Alzheimer´s Disease and Fronto Temporal Dementia display equivalent astroglial alterations.

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Pathophysiology of sporadic Alzheimer's disease (SAD) and familial Alzheimer's disease (FAD) remains poorly known, including the exact role of neuroglia and specifically astroglia, in part because studies of astrocytes in human Alzheimer's disease (AD) brain samples are scarce. The same applies to Fronto Temporal Dementia (FTD). We performed an in-depth tri-dimensional (3-D) anatomical and morphometric study of glial fibrillary acidic protein (GFAP)-positive and glutamine synthetase (GS)-positive astrocytes as well as co-localizing populations within the human entorhinal cortex; comparing them with (FTD) and control subjects. We found in SAD and FAD prominent atrophic changes in GFAP and GS astrocytes in the EC of both SAD and FAD characterised by a decrease in area and volume when compared with non-demented control samples (ND). In FTD patients we also found a prominent astrocyte atrophy of GFAP-positive astrocytes and co-expressing GFAP/GS astrocytes, also characterised by a decrease in area and volume similar to either SAD and FAD; whilst minor changes in GS-positive astrocytes in FTD compared to non-dementia controls (ND) samples. This study evidences the importance of astrocyte atrophy and dysfunction in human EC. Furthermore, we did not find neither astrocytic loss nor astrocyte proliferation or hypertrophy (gliosis). In contrast with the astrogliosis classically accepted hypothesis. The studies at this level in the different types of human dementia are scarce. Thus, we could state that AD and FTD are not only a neuropathological diseases but also gliopathological diseases; which in prime affected areas might appear earlier than neuronal alterations. These combined glial and neural alterations are key elements in the disruption of neural networks connectivity, which associated with neurotransmitters imbalance; underlie AD mnesic deficits. Hence, new therapeutic approaches targeting simultaneously glial and neural impairments might be of major relevance for AD and FTD treatment.

Disclosures: J. Rodriguez: None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.14/B118

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

Support: R01NS129788

Title: Testing human astrocyte influence upon synapses within an Alzheimer's disease organoid model

Authors: *S. S. LAVEKAR[1], M. PATEL[2], R. KRENCIK[2];
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Abstract: Astrocytes contribute to the initiation and progression of neurodegeneration in Alzheimer's disease (AD). However, the specific link between astrocyte reactivity within the amyloid microenvironment and the impact on synapses is obscure due to the lack of state-of-the-art tools. Here, we harnessed the combinatorial potential of two emerging technologies that could dissect the relationship of astrocyte reactivity with synapse density and activity. First, we engineered human pluripotent stem cell (hPSC)-derived neurons to express a synaptic reporter. Specifically, the presynaptic protein, synaptophysin, was linked to a genetically encoded calcium sensor (i.e., SYP-jGCaMP8s) to monitor synaptic calcium transients as a readout of activity. We cocultured this reporter with hPSC-derived astrocytes in the form of neural organoids (i.e., Asteroids) and this revealed that astrocytes accelerate the presence of synchronized synaptic calcium transients throughout the entire population. Second, we optimized expansion microscopy techniques to determine if the astrocyte-induced effect on activity is due to increased synapse density. Expansion microscopy decrowds synaptic areas for binding of antibodies before immunolabeling, and increases image resolution by physically expanding the tissue. By applying this technique to Asteroids, we were able to expand neurons and astrocytes by several folds. We are currently combining these approaches to examine the consequence of amyloid oligomers on astrocyte reactivity and synapse activity as an organoid-based AD model system. We expect that optimization and validation of this experimental platform will open avenues to develop biomarkers and test drugs that could help modulate synapse activity, synaptic trafficking and plasticity.

Disclosures: S.S. Lavekar: None. **M. Patel:** None. **R. Krencik:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.15/B119

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

Title: Global deletion of C1q reduces activated astrocytes, rescues synaptic loss independently of amyloid phagocytosis in AD mouse model

Authors: *T. PETRISKO[1], S.-H. CHU[1], A. GOMEZ ARBOLEDAS[2], A. J. TENNER[3];
[2]Mol. Biol. and Biochem., [1]Univ. of California, Irvine, Irvine, CA; [3]Neurobio. and Behavior, The Regents of the Univ. of California, Irvine, Irvine, CA

Abstract: The complement system contributes to enhanced inflammation and cognitive decline in Alzheimer's disease (AD). Previous studies have demonstrated constitutive deletion of the classical initiator protein, C1q, reduces glial activity and attenuates neuronal loss in AD mouse

models. The objective of this study was to determine if global deletion of C1q at two different stages of Alzheimer's disease would reduce neuroinflammation, synaptic loss, and amyloid engulfment. Briefly, $C1qa^{FL/FL}RosaCre^{ERT2}$ mice were crossed to the Arctic AD mouse model to generate WT and Arctic (Arc) $C1qa^{FL/FL}$ mice with and without the $RosaCre^{ERT2}$ transgene. Mice were treated with tamoxifen at either 11-wks or 20-wks of age to induce global C1q deletion. Brains were collected at 10m of age and stained for C3/GFAP and C5aR1/Iba1 to characterize neuroinflammation. Superresolution microscopy was utilized to assess synaptic density of Vglut1-Psd95 synapses while confocal microscopy was used to quantify the phagocytosis of amyloid by microglia. Early deletion of C1q at 11-wks of age failed to reduce microglial reactivity as measured by C5aR1 or Iba1 hippocampal volume while deletion at 20-wks significantly reduced Iba1 volume (29%) alongside a trending reduction in C5aR1 expression (32%). Deletion of C1q at 11-wks of age induced a trending reduction in GFAP volume (25%) while significantly reducing C3 expression (48%). In contrast, deletion at 20-wks failed to reduce GFAP reactivity but a trending reduction of C3 expression was observed (33%). At 10m of age, Arc mice displayed reduced colocalization of Vglut1-Psd95 synapses in the CA3 region of the hippocampus. Deletion of C1q at either 11- or 20- wks of age rescued synaptic loss. Arc mice with C1q deleted at 11-wks displayed increased microglial (Iba1) colocalization with amyloid (6E10) but no changes in lysosome associated phagocytosis of amyloid was observed at either timepoint. The data presented here demonstrates that global deletion of C1q in the Arctic mouse model results in reduced expression of C3 and GFAP in hippocampal astrocytes. Additionally, global C1q deletion rescues synaptic density regardless of the age of deletion independent of amyloid phagocytosis.

Disclosures: T. Petrisko: None. **S. Chu:** None. **A. Gomez Arboledas:** None. **A.J. Tenner:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.16/B120

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

Title: Microglial contribution of pathological Blood-Brain Barrier in a mouse model of Alzheimer's disease

Authors: *L. HOU[1], J. CHENG[1], Z. GUO[1,2], I. TAKEDA[1,2], H. WAKE[1,2];
[1]Nagoya Univ. Grad. Sch. of Med., Nagoya/Aichi, Japan; [2]National Institute for Physiological Sciences, Okazaki, Japan

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by pathological features of accumulation of amyloid plaques and tau tangles. Previous study using Gd-enhanced human magnetic resonance imaging has shown that alteration of the blood-brain barrier (BBB) is detected in the early stages of AD. BBB disruption may adversely affect

neurological function by activating microglia with invading inflammatory mediators in the central nervous system (CNS). In contrast, the infiltration of immune cells into the CNS following BBB disruption may facilitate the removal of Aβ via phagocytosis of microglia and macrophages. However, the time course of BBB disruption in AD pathology as well as the contribution of microglia to this process are not known. In this research, to identify the time course of BBB disruption and their effect on neuron, we used the APP knock-in mouse as AD model (T Saido et al., *Nature Neuroscience*, 2014) to observe the structure of the BBB on different time course using electron microscopy. Our results revealed that BBB leakage start 14 weeks and a tight junction length reduced between12 and16 weeks in AD model mice. We further investigated the dynamics of microglia and BBB permeability over days using in *vivo* two-photon microscopy to quantify the microglial response to BBB disruption. We found that microglia start to accumulate with blood vessels from 13 weeks. These findings suggest that activation of microglia by amyloid-beta may precede and potentially lead to changes in BBB permeability of AD models mice. We are currently using a combination of two-photon microscopy and immuno-electron microscopy to study the role of microglia on BBB disruption and the impact of microglia-directed BBB leakage on AD disease progression.

Disclosures: L. hou: None. **J. cheng:** None. **Z. Guo:** None. **I. Takeda:** None. **H. Wake:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.17/B121

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

Support: NIH Grant 3P01AG073082-02S1

Title: Activation of inhibitory interneurons in Alzheimer's Disease mouse model modifies microglia activation

Authors: *Y. ZHANG[1], J. J. PALOP[2], E. BRADY[3], F. JIANG[3], P. NAMBIAR[4]; [1]Gladstone Inst., Berkeley, CA; [2]Gladstone Inst. & UCSF, South San Francisco, CA; [3]Gladstone Inst., San Francisco, CA; [4]Gladstone Inst. of Neurolog. Dis., San Francisco, CA

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common form of dementia worldwide. Research shows that Aβ accumulation early in AD impairs inhibitory neurotransmission, leading to dysfunction of gamma and theta brain oscillations involved in higher order cognitive processing. Optogenetic activation of parvalbumin (PV)-expressing interneurons at 40-Hz has been shown to induce gamma oscillations, improve Aβ pathology, and alter microglia morphology. Our lab has shown that combined stimulation of PV- and somatostatin (SST)-expressing interneurons (together known as the lhx6-derived interneurons) has a larger, synergistic effect on gamma power compared to just PV-stimulation. Given this interneuron synergy discovery, we investigated how simultaneous PV and SST

interneuron stimulation alters Aβ pathology, microgliosis, transcriptomes, and spontaneous behavior . To do this, we used the PDGF-APPSw,Ind AD model mice with cre-dependent ChR2 expression in Lhx6-derived interneurons (Lhx6-Cre+/- Ai32+/- hAPP-J20+/-). First, mice received optogenetic stimulation of PV- and SST-interneurons in both posterior parietal cortices at theta, gamma, and theta-gamma coupling frequencies respectively. Then, we analyzed functional acute behavior changes using machine learning behavioral phenotyping. We also conducted immunohistochemistry for various AD pathologies and performed single-nuclei RNA sequencing on stimulated and sham-stimulated brains to find activity-dependent differentially expressed genes. As a result of the methods above, we found microglial modification as a result of the interneuron stimulation, revealing a novel mechanism by which neuronal synchronization can alter neuroinflammation in the context of AD pathogenesis.

Disclosures: Y. Zhang: A. Employment/Salary (full or part-time):; Gladstone Institutes. **J.J. Palop:** None. **E. Brady:** None. **F. Jiang:** None. **P. Nambiar:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.18/B122

Topic: B.09. Glial Mechanisms

Support: MODEL AD Grant U54AG054345
TREAT AD Grant U54AG065181, NIH-NIA

Title: Inpp5d haplodeficiency alleviates tau pathology in the PS19 mouse model of Tauopathy.

Authors: *D. SONI[1], P. B.-C. LIN[2], A. LEE-GOSSELIN[3], C. LLOYD[5], E. MASON[4], A. PERKINS[5], M. MOUTINHO[3], B. T. LAMB[6], S. CHU[5], A. OBLAK[6];
[1]indiana university, Indianapolis, IN; [2]Washington Univ., St. Louis, MO; [4]Pharmacol. and Toxicology, [3]Indiana Univ., Indianapolis, IN; [5]Indiana university, Indianapolis, IN; [6]Stark Neurosciences Res. Inst., Indianapolis, IN

Abstract: *Inpp5d* haplodeficiency alleviates tau pathology in the PS19 mouse model of Tauopathy.
Disha M. Soni, Peter Bor-Chian Lin, Audrey Lee-Gosselin, Christopher D. Lloyd, Miguel Moutinho, Bruce T. Lamb, Shaoyou Chu, and Adrian L. Oblak
ABSTRACT Introduction: A noncoding variant (rs35349669) within INPP5D, a lipid and protein phosphatase restricted to microglia in the brain, is linked to increased susceptibility to Alzheimer's disease (AD). While *Inpp5d* is well-studied in amyloid pathology, its role in tau pathology remains unclear.**Methods**: Fluorescence-resonance energy transfer (FRET)-based tau seeding assay was performed on human LOAD brain samples. Furthermore, PS19 Tauopathy mice were crossed with *Inpp5d*-haplodeficient (*Inpp5d*$^{+/-}$) mice to examine the impact of *Inpp5d* in tau pathology.**Results**: A positive correlation was observed between increased tau-seeding and

the expression of *INPP5D* in subjects with late-onset Alzheimer's disease (LOAD). Additionally, Increased INPP5D expression correlated positively with phospho-Tau AT8 in PS19 mice. *Inpp5d* haplodeficiency mitigated hyperphosphorylated tau levels (AT8, AT180, AT100, and PHF1) and motor deficits in PS19 mice. Transcriptomic analysis revealed an up-regulation of genes associated with immune response and cell migration. **Discussion**: Our findings define an association between INPP5D expression and tau pathology in LOAD human brain samples and PS19 mice. Alleviation in hyperphosphorylated tau, motor deficits, and transcriptomics changes in haplodeficient-
Inpp5d PS19 mice indicate that modulation in INPP5D expression may provide therapeutic potential for mitigating tau pathology and improving motor deficits.

Disclosures: D. Soni: None. **P.B. Lin:** None. **A. Lee-Gosselin:** None. **C. Lloyd:** None. **E. Mason:** None. **A. Perkins:** None. **M. Moutinho:** None. **B.T. Lamb:** None. **S. Chu:** None. **A. Oblak:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.19/B123

Topic: B.09. Glial Mechanisms

Title: Elucidating the pathological role of microglial phosphatase, INPP5D, in Alzheimer disease

Authors: *Y.-N. CHU[1], S. TAKATORI[2], T. TOMITA[3];
[1]Univ. of Tokyo, Tokyo, Japan; [2]Univ. Tokyo, Tokyo, Japan; [3]The Univ. of Tokyo, Tokyo, Japan

Abstract: Accumulation of amyloid-β (Aβ) deposition in the brain is a critical factor in the pathogenesis of Alzheimer disease (AD). Genome-wide association studies have identified many genetic variants to be associated with increased AD risk, including those of microglial receptor TREM2. Enhanced Aβ accumulation is observed following TREM2 functional impairment, thus highlighting TREM2's crucial role in Aβ metabolism. Our recent study identified another AD risk gene, INPP5D, as a factor that opposes TREM2 function; however, the precise mechanisms by which INPP5D regulates TREM2 remain unclear. While a previous study suggests a direct competitive relationship between INPP5D and SYK in binding to an essential TREM2 adaptor TYROBP, other works hypothesize INPP5D exerts regulatory effects through its PIP3 phosphatase activity. This research thus aimed to elucidate how INPP5D modulates TREM2 signaling and microglial functions in AD.Using a flow-cytometry-based assay, we quantified the internalization of fluorescent Aβ fibrils (fAβ) by murine primary microglia. Our results demonstrated that while knockdown of *Trem2*, *Tyrobp*, or *Syk* led to decreased fAβ uptake, *Inpp5d* knockdown significantly increased fAβ uptake. We also found that *Inpp5d* reduction did not affect SYK activation levels, as assessed by its phosphorylation, at baseline or upon

stimulation by a TREM2 agonistic antibody. Taken together, our results suggest a regulatory mechanism other than direct competition. Further investigation revealed that *Inpp5d* deficiency in the microglial cell line MG6 increased the phosphorylation of a PIP3 effector AKT, while deficiency of its paralog, *Inppl1*, did not, indicating INPP5D's unique role in TREM2 signaling regulation. Remarkably, INPP5D preferentially downregulated AKT2, while AKT1 played a predominant role in regulating microglial fAβ uptake, highlighting distinct functions of AKT isoforms within the TREM2 pathway. In conclusion, INPP5D opposes TREM2 in regulating microglial fAβ uptake, likely through its unique inhibitory role on specific AKT isoforms rather than direct competition with SYK. These findings provide novel insights into INPP5D's regulatory role in microglial responses in AD, emphasizing the potential for targeted therapeutic interventions aimed at modulating these pathways.

Disclosures: Y. Chu: None. **S. Takatori:** None. **T. Tomita:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.20/B124

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

Support: NIA AG075897
BrightFocus Foundation A2021036S

Title: Investigating the role of neonatal estradiol on microglial-mediated inflammation in AD

Authors: *P. R. KELLER[1], B. T. CASALI[2], E. G. REED[2];
[1]Kent State Univ. Sch. of Biomed. Sciences, Program In Neurosciences, Kent, OH; [2]Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH

Abstract: Microglia, the brain's resident immune cells, play crucial roles across the lifespan, from neurodevelopmental processes such as synaptic pruning and the guidance of axonal growth cones to amyloid plaque remodeling and clearance in Alzheimer's Disease (AD). During development, microglia are intimately involved with the brain's sexual differentiation, themselves exhibiting sex-specific morphology and transcriptomes. Notably, these phenotypes are age-dependent and may translate to increased risk for specific diseases. The current study aims to investigate the role of neonatal hormones on early-life programming of microglia and the subsequent effect on AD onset and progression. We administer estradiol (E2) during the critical period to masculinize female brains and evaluate microglial-mediated responses in the 5xFAD mouse model. Results from the current study will shed light on the effects of early-life programming and early sex hormone pulses on life-long neurodegenerative disease risk/pathogenesis.

Disclosures: P.R. Keller: None. **B.T. Casali:** None. **E.G. Reed:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.21/B125

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

Support: NIH R01AG069447
Craig Fellowship (UICOMP)
Department of Cancer Biology and Pharmacology

Title: Microglia-specific myd88 deficiency ameliorates behavioral deficits in an alzheimer's disease mouse mode

Authors: *A. X. VALLEJOS[1], C. HOLAS[2], K.-I. FUKUCHI[3], J. YANG[4];
[1]Cancer Biol. and Pharmacol., Univ. of Illinois Col. of Med. at Peoria, Peoria, IL; [2]Cancer Biol. and Pharmacol., Univ. of Illinois Col. of Med. Peoria, Peoria, IL; [3]Cancer Biololgy and Pharmacol., Univ. of IL Col. of Med. At Peoria, Peoria, IL; [4]Cancer Biol. & Pharmacol., Univ. of Illinois Col. of Med. At Peoria, Peoria, IL

Abstract: ***Background:*** Alzheimer's disease (AD) is primarily linked to two main features: neurofibrillary tangles (NFT) of hyperphosphorylated tau and amyloid-beta (Aβ) plaques. While genetic factors for some increase the risk by promoting these hallmarks, emerging research also highlights the role of neuroinflammation in AD. This involves the microglial response to Aβ, tau tangles, and inflammatory molecules like IL-1β, which further contributes to the disease's progression.
Objective/Hypothesis: MyD88 is crucial in the MyD88/NF-κB/NLRP3 inflammasome pathway, which promotes inflammation and neurodegeneration. We hypothesize that removing MyD88 in our AD mouse model (TgAPP/PS1) will reduce inflammatory responses to NFTs and Aβ, potentially lessening AD symptoms.
Methods: TgAPP/PS1 mice (AD mouse model) were used in this study. TgAPP/PS1 mice develop Aβ deposits beginning at 4-5 months and increase during aging. We used Cre-LoxP system to achieve microglia-specific knockout of the Myd88 gene in TgAPP/PS1 mice. The mice were divided into 4groups: 1).Cre: MyD88fl/flTgAPP/PS1; 2).MyD88fl/flTgAPP/PS1; 3). Cre: MyD88fl/fl; 4). MyD88fl/fl. At 5months, the mice underwent a battery of behavioral tests, including open field, elevated plus maze and Morris water maze to test anxiety, exploration, as well as spatial learning and memory.
Results: In open field, microglia-specific Myd88 deficiency (KO) decreased traveling activity ($F(1, 37) = 7.34$, $p= 0.01$) and caused an increasing trend in anxiety ($F(1,37) = 7.55$, $p = 0.09$). Consistently, in elevated plus maze, microglia-specific Myd88 KO increased anxiety in TgAPP/PS1 mice by decreasing open duration ($F(1,37) = 10.68$, $p = 0.002$) and increasing enclosed duration ($F(1,37) = 10.28$, $p = 0.003$). In the acquisition phase of Morris water maze, microglia-specific Myd88 KO ameliorated leaning deficits in TgAPP/PS1 mice by decreasing escape latency ($F(4,148) = 3.88$, $p =0.013$) and causing a decreasing trend in distance swum

($F(4,148) = 2.446$, $p = 0.068$). In the probe trial, microglia-specific Myd88 KO rescued memory deficits in TgAPP/PS1 mice by increasing the target quadrant time ($F(1,37) = 4.67$, $p= 0.037$). ***Conclusion/Implications:*** Our findings show that microglia-specific MyD88 KO rescues cognitive deficits in TgAPP/PS1 mice, suggesting microglial MyD88 signaling contributes to AD pathogenesis and could be a therapeutic target.

Disclosures: A.X. vallejos: None. **C. Holas:** A. Employment/Salary (full or part-time):; University of Illinois College of Medicine. **K. Fukuchi:** A. Employment/Salary (full or part-time):; University of Illinois College of Medicine. **J. Yang:** A. Employment/Salary (full or part-time):; University of Illinois College of Medicine.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.22/B126

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

Support: NSERC CGS-D Scholarship
Alzheimer Society London & Middlesex, Dr. Shawn Whitehead
CIHR, Dr. Shawn Whitehead
Alzheimer's Society of Canada, Dr. Shawn Whitehead

Title: Age-dependent changes in circulating microglial extracellular vesicles and cognition in the TgAPP/PS1 Alzheimer's disease rat model

Authors: *S. J. MYERS, B. L. ALLMAN, S. H. PASTERNAK, S. N. WHITEHEAD, A. D. ROSEBOROUGH;
Western Univ., London, ON, Canada

Abstract: Amyloid-β plaques and neurofibrillary tangles are considered the pathological hallmarks of Alzheimer's disease (AD) but individuals with plaques do not always progress to dementia, highlighting the need to identify alternative pathological processes that are more reflective of cognitive function. Human tissue analysis has shown that microglia become activated with advanced age and may be one of the earliest changes to occur in AD. Further, work in our lab has revealed a relationship between increased microglia activation and cognitive impairment. Recent transcriptomic studies have identified a range of microglia activation states that may differ by age, brain region, and disease pathology, however, our current ability to detect microglia activation *in vivo* is limited and lacks specificity. Extracellular vesicles (EVs) serve as a promising measure of brain microglia activation as they can be detected in the peripheral circulation carrying unique surface markers of their cells of origin. This study aimed to identify age- and disease-specific microglial activation markers in plasma-derived EVs as well as impairments in cognition across multiple domains. Male and female wildtype Fischer 344 rats and TgAPP/PS1 double transgenic rats were aged to 3-, 9-, or 15-months-old. Rats underwent

cognitive testing in water-based tasks for executive function and spatial memory, prior to plasma collection and brain tissue extraction at the stated endpoints. To measure circulating microglial EVs, plasma was incubated with fluorescent antibodies against microglial markers TMEM119, TREM2, CD11c, and CD14 and quantified using a nanoscale flow cytometer. Age- and genotype-dependent changes were observed in which 15-month TgAPP/PS1s had significantly increased dual-labeled $TMEM119^{+}/TREM2^{+}$ EVs compared to the 15-month wildtypes. Microglia markers were validated in RNA and protein isolates from rat brain tissue. We also observed age-dependent deficits in both hippocampal-based spatial memory and executive function in the wildtype rats which was exacerbated in the TgAPP/PS1s. Further correlational analyses will explore the relationship between these microglial EVs and various domains of cognition. Overall, detection of activated microglial EVs in the peripheral circulation represents a non-invasive means to measure specific activation phenotypes that can differentiate between age and disease model and expand our understanding of microglia activation and cognition in AD.

Disclosures: S.J. Myers: None. **B.L. Allman:** None. **S.H. Pasternak:** None. **S.N. Whitehead:** None. **A.D. Roseborough:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.23/B127

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

Support: NIH R01AG054672
NIH RF1AG054199
NIH R01AG066429
NIH R01AG072719
NIH R01AG067763 (TI)
NIH R01AG079859 (SI)
In part by Ono Pharmaceutical (TI)

Title: P2rx7 deficiency prevents cognitive deficit, brain atrophy, and tauopathy progression in ps19 mice via suppression of extracellular vesicle secretion and mitochondrial toxicity

Authors: *V. BODART-SANTOS, M. ABDULLAH, J. ELLISON, **S. IKEZU**, T. IKEZU; Dept. of Neurosci., Mayo Clin. Florida, Jacksonville, FL

Abstract: **Introduction:** We previously identified a novel mechanism describing how pathological tau transfers via extracellular vesicles (EVs) in Alzheimer's disease (AD). Targeting EV secretion to mitigate tau transfer is, therefore, a promising therapeutic approach for AD. P2X purinoreceptor 7 (P2RX7), an ATP-gated cationic channel predominantly expressed in microglia, regulates the secretion and biogenesis of EVs. We aim to investigate the effect of *P2rx7*

deficiency in PS19 tauopathy mouse model *in vivo* and by proteomic profiling of brain EVs. **Methods:** PS19:*P2rx7*$^{-/-}$ mice at 9 months of age were tested for fear conditioning (n=25 animals per group) and their brain sections were assessed for cortical and hippocampal atrophy (n=15 animals per group). Tau pathology was evaluated using ELISA and immunofluorescence against phosphorylated (AT8) and misfolded (Alz50) tau (n=10-12 animals per group). *P2rx7*$^{-/-}$ mice were intracranially injected with viral vectors expressing P301L tau in neurons and mEmerald-CD9 in microglia to visualize the secretion of microglial EV *in vivo* (n=4-6 animals per group). Brain EVs samples were subjected to proteomic profiling by mass-spectrometry (n=5 animals per group). **Results:** PS19:*P2rx7*$^{-/-}$ mice showed significant improvement in contextual and cued memory compared to age-matched PS19 mice, which was accompanied by preserved cortical and hippocampal volume, and significant reduction of hippocampal tau pathology and pTau-S396 in sarkosyl-insoluble fraction of brain tissues. *P2rx7* deficiency induce accumulation of TSG101 in microglia in PS19:*P2rx7*$^{-/-}$ mice and reduced microglial mEmerald^{+}-EV secretion in *P2rx7*$^{-/-}$ mice. PS19 mice showed an increased secretion of EVs containing tau into the brain parenchyma, which were strikingly reversed by *P2rx7* ablation. Gene ontology pathway analysis of EV proteome showed significant enrichment of mitochondrial proteins in PS19, which was significantly downregulated in PS19:*P2rx7*$^{-/-}$. **Conclusion:** Our study demonstrated that *P2rx7* deficiency preserve cognitive function and reduces neurodegeneration associated with tau pathology in PS19 mice by dampening EV secretion and EV-mediated tau and mitochondria waste transfer, further indicating the therapeutic potential of targeting *P2rx7* to ameliorate AD progression.

Disclosures: V. Bodart-Santos: None. **M. Abdullah:** None. **J. Ellison:** None. **S. Ikezu:** None. **T. Ikezu:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.24/B128

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

Support: Cure Alzheimer's Fund
NIH Grant 1F31NS130757

Title: Neuronal cytokine IL34 influences plaque pathology, microglia, and behavior in a mouse model of Alzheimer's disease.

Authors: *B. DEVLIN, S. BILBO;
Duke Univ., Durham, NC

Abstract: Microglia-neuron crosstalk is critical for the proper functioning of the central nervous system. As the tissue resident macrophages in the brain, microglia serve essential functions including debris clearance, cytokine production, and the removal of unwanted or damaged

synaptic connections and neurons in development. There is ample evidence that these microglial processes become overactive in diseases such as Alzheimer's, however the signals that properly regulate these processes remain elusive. Recent genome-wide association studies (GWAS), as well as empirical studies, have uncovered a link between the neuron-derived cytokine interleukin-34 (IL34), which signals through the colony stimulating factor 1 receptor (CSF1r) on microglia, and Alzheimer's disease progression. To investigate the mechanisms by which IL34 may influence neuroinflammatory outcomes, we crossed a mouse model of Alzheimer's disease (5XFAD) with IL34 heterozygous mice ($IL34^{LacZ/+}$). We tested WT, 5XFAD, and 5XFAD/IL34 Het mice in open field, elevated plus maze, and Y maze behaviors at both 4 and 6 months. Additionally, we collected brains from these mice and performed histology to quantify amyloid beta plaque pathology along with microglia and synapse endpoints. Our preliminary data demonstrate that while there are no changes at the behavioral level in any of the genotypes tested at 4 months of age, there is a significant reduction in plaque numbers in the motor cortex of 5XFAD/IL34 mice compared to 5XFAD. Further, this change in plaque number coincided with an increase in plaque engulfment by microglia in 5XFAD/IL34 mice, suggesting that plaques are still forming in mice with the additional IL34 mutation, but the microglia are more effective at clearing them. At 6 months of age, we observed a significant increase in percent time spent in the open arms of the elevated plus maze only in 5XFAD/IL34 mice compared to WT mice, suggesting the additional mutation speeds up the progression of the behavioral pathologies seen in 5XFAD mice at older ages. In ongoing experiments, we are testing spatial memory deficits in these mice using the morris water maze and will assess neuroinflammatory endpoints including microglial engulfment of synapses. Furthermore, we will test the effects of virally overexpressing IL34 on older 5XFAD mice (8 months) on the same behavioral and molecular endpoints. In sum, this work points to a mechanistic link between IL34, microglia, and Alzheimer's disease and provides the foundation for investigating the therapeutic potential of targeting the IL34-CSF1r axis in Alzheimer's disease.

Disclosures: B. Devlin: None. **S. Bilbo:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.25/B129

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

Title: Identification and characterisation of CVN293, a brain permeable KCNK13 inhibitor

Authors: *R. WATERS-HALL, B. OSSOLA, C. BENDER, J. R. HARVEY, S. RUSSELL, L. DICKSON, K. MATTHEWS, K. DOYLE, M. LIZIO, X. XU, A. ROWLAND, D. BARKER, K. PAGE, R. BURLI, A. STOTT, N. L. BRICE, L. A. DAWSON, M. CARLTON;
Cerevance Ltd, Cambridge, United Kingdom

Abstract: KCNK13, also known as THIK1, is a 2-pore domain potassium channel and a member of the leak or background potassium (K+) channel family. KCNK13 is preferentially expressed in microglia yet has low expression in peripheral immune cells. KCNK13 is responsible for the K+ efflux in microglia which is required for the activation of the NLRP3 inflammasome. The NLRP3 inflammasome is a multiprotein complex where, once activated, triggers the cascade for the release of proinflammatory cytokines including IL1β. The resultant inflammation is associated with many neurodegenerative diseases including amyotrophic lateral sclerosis, Alzheimer's and Parkinsons's disease. Using Cerevance's proprietary Nuclear Enriched Transcript Sort sequencing (NETSseq) platform, KCNK13 was found to be expressed in microglia isolated from human post-mortem brain tissue. In addition, elevated expression of KCNK13 was observed in cortical samples from patients with Alzheimer's disease. Therefore, our hypothesis was that modulating KCNK13 with a small molecule inhibitor could reduce NLRP3 mediated neuroinflammation, offering a potential treatment for neurodegenerative diseases while sparing peripheral immune suppression. Our candidate compound CVN293 was derived from a high throughput screening campaign and is a potent, selective, brain permeable blocker of KCNK13. CVN293 demonstrated nM potency at human KCNK13, with equipotency observed across species and selectivity observed against family members KCNK6 and KCNK2. Functionally, CVN293 demonstrated a concentration-dependant inhibition of the NLRP3-inflammasome mediated production of IL-1β from LPS-primed murine microglia and good oral bioavailability in pharmacokinetic studies. These findings support the pharmacological mechanism that inhibiting KCNK13 K+ efflux could serve as a therapeutic option for neurodegenerative diseases thus supporting the advancement of CVN293 into clinical trials.

Disclosures: R. Waters-Hall: None. **B. Ossola:** None. **C. Bender:** None. **J.R. Harvey:** None. **S. Russell:** None. **L. Dickson:** None. **K. Matthews:** None. **K. Doyle:** None. **M. Lizio:** None. **X. Xu:** None. **A. Rowland:** None. **D. Barker:** None. **K. Page:** None. **R. Burli:** None. **A. Stott:** None. **N.L. Brice:** None. **L.A. Dawson:** None. **M. Carlton:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.26/B130

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

Title: The Gal-9 expression regulates the microglia activation in a neuroinflammation model induced by the peptide Amyloid-β25-35

Authors: *M. VALENCIA GIL;
Biochem., Natl. AUTONOMUS Univ., CDMX, Mexico

Abstract: The Gal-9expressionregulates the microglia activationinaneuroinflammationmodel induced bythe peptide Amyloid-β_{25-35} Valencia-GilML*[1], Ramírez-Hernández E[1], Sánchez-SalgadoJL[1], Pereyra-Morales MA[1], Segura-Pérez E[1],

Hernández-ZimbrónLF[2]y Zenteno-GalindoE[1] [1]DepartamentodeBioquímica, FacultaddeMedicina, UniversidadNacionalAutónomadeMéxico, CiudaddeMéxico, CDMX, México. [2]EscuelaNacionaldeEstudios Superiores, Universidad NacionalAutónomadeMéxico, León, Guanajuato, México. lucero98valencia15@gmail.comTheneuroinflammationhasbeenrelatedwithneurodegenerativediseases,themostcommonisAlzheimer's disease(AD),whichhasbeenstudiedwithanexperimentalmodeloftheAβ25-35peptide.Thisresponseinvolvestheactivationofmicrogliainthecentralnervoussystem(CNS),withgalectinsaspro-oranti-inflammatoryelements.Gal-9,foundinmicroglia,modulatesthisresponsethroughtheTim-3receptor. Therefore,theactivationofmicrogliaandtheexpressionofGal-9andTim-3wereinvestigatedinamodelofneuroinflammationinducedbyAβ25-35.Thenumberofmicrogliawasquantifiedbyimmunohistochemistryandimmunofluorescenceandtheexpressionof Gal-9andTim-3byWesternBlotinthehippocampus.Themodelshowedanactivationofmicroglia,exacerbatingtheneuroinflammatoryresponseofAβ25-35,alongwithanincreaseintheexpressionofGal-9andTim-3.NeuroinflammationinducedbyAβ25-35impliesaprogressionofAD, whiletheregulationoftheresponsethroughGal-9andTim-3couldbecrucialforfuturetreatments.

Disclosures: M. Valencia Gil: None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.27/B131

Topic: B.09. Glial Mechanisms

Title: Expansion Mass Spectrometry Imaging: High-resolution spatial imaging and quantification of lipids using expansion microscopy

Authors: *B. AKBARI[1], V. WENDT[2], G. CHOPRA[1];
[1]Chem., [2]Purdue Univ., West Lafayette, IN

Abstract: Expansion Microscopy is an innovative imaging technique, offering insights into membrane dynamics, protein-lipid interactions, and spatial organization within cellular compartments as a higher resolution imaging compared with the standard microscopy tools. Mass spectrometry imaging (MSI) excels in the sensitive and simultaneous detection, quantification, and spatial mapping of hundreds of biomolecules such as peptides, proteins, lipids, and various organic compounds within cells and tissues. In this research study, a new technique called expansion mass spectrometry imaging (Ex-MSI) has been developed to improve resolution in lipids within mice tissue samples from perfused Cx3Cr1-GFP mice for method development and age-matched Alzheimer's mice brains (Cx3Cr1-GFP;5xFAD) with green colored microglia for comparison. The Ex-MSI method does not require modifications to

existing mass spectrometers and much easier to adopt by different groups as it involves embedding tissue sections in a crosslinked, water-swellable hydrogel, and indirectly attaching target biomolecules to the gel network. Additionally, we used fluorescence microscopy to identify location of microglia in expanded brain tissue sections of mice, which provided a complimentary label-free and non-destructive insights into the cellular morphology and dynamics in different regions of the brain. By integrating DESI-MSI, expansion microscopy, and fluorescence microscopy, subcellular biomolecules within the brain can be mapped with superior spatial resolution when compared to conventional mass spectrometry. The Ex-MSI method detects subcellular lipid molecules in regions with large lipid droplets or "cellular fat" accumulation in microglial cells. Previously, our group showed that these lipids accumulated microglial cells have phagocytic dysfunction in Alzheimer's disease; the current Ex-MSI method shows differences in these microglial lipids accumulated cell states in a region-specific manner . We believe these comprehensive spatial-molecular maps along with detection of low abundance lipids will provide identification of novel region-specific molecular differences in the brain of Alzheimer's mice.

Disclosures: B. Akbari: None. **V. Wendt:** None. **G. Chopra:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Agilent Instrument In-Kind.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Meditati Inc., BrainGnosis Inc.. Other; NIH National Center for Advancing Translational Sciences U18TR004146 and ASPIRE Challenge and Reduction-to-Practice awards.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.28/B132

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

Support: National Institute of General Medical Sciences - P20AG068053
National Institute on Aging - R01AG062762

Title: Investigating Inflammatory Transcript Levels in a Mouse Model with Altered GABAb Receptors on Glia

Authors: *B. BALSAMO[1], A. A. ORTIZ[2], A. M. OSSE[3], J. W. KINNEY[4];
[1]Univ. of Nevada, Las Vegas, Las Vegas, NV; [2]Brain Hlth., Univ. of Nevada Las Vegas, Las Vegas, NV; [3]Univ. of Nevada Las Vegas, Las Vegas, NV; [4]Dept. of Brain Hlth., Univ. of Nevada Las Vegas, Henderson, NV

Abstract: Alzheimer's Disease (AD) is a prevalent neurodegenerative disease characterized by memory loss, cognitive decline, and neuronal degeneration, affecting nearly 6 million individuals in the United States alone and imposing a significant burden on patients, families, and caregivers. The disease is typified by three primary pathological features: the accumulation of amyloid beta plaques (AB), the formation of hyperphosphorylated tau protein aggregates known as neurofibrillary tangles (NFT), and chronic brain inflammation. Additionally, alterations in numerous brain proteins contribute to the complexity of AD pathology. There have been numerous mechanisms identified associated with inflammatory signaling that have demonstrated impacts on amyloid beta and tau pathology. Our previous work has focused on the role of the metabotropic GABAB receptor on glia that when downregulated results in an exacerbation of amyloid load in the APP/PS1 mouse model as well as results in numerous transcript changes associated with Late Onset Alzheimer's disease (LOAD; Leisgang-Osse 2023). In the present study we investigated numerous mRNA targets associated with transcript changes previously reported using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) to pinpoint alterations at the transcript level. The examination of mRNA level changes that map onto changes previously observed in this model is vital to understand how reduction of GABAB on glia alter amyloid levels. This project is beneficial for advancing our understanding of this novel mechanism in AD pathology.

Disclosures: B. Balsamo: None. **A.A. Ortiz:** None. **A.M. Osse:** None. **J.W. Kinney:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.29/B133

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

Title: Applying Fluorescence Correlation Spectroscopy to Study Amyloid Clearance in Alzheimer's Disease

Authors: *S. BAWARITH[1,2], S. E. FRASER[3], F. SCHNEIDER[1];
[1]USC, Los Angeles, CA; [2]Biomedical Engineering, University of Southern California, Los Angeles, CA; [3]Mol. & Computat. Biol., USC, Los Angeles, CA

Abstract: Alzheimer's Disease (AD) is a progressive neurodegenerative disease characterized by initial memory impairment and cognitive decline that can ultimately affect behavior, speech, and the motor system. The most common characteristics of AD are the abnormal accumulation of amyloid plaques and neurofibrillary tangles. The formation of amyloid plaques is due to the incorrect cleavage of the amyloid precursor protein (APP) by y- secretase. Recently, the FDA approved a drug, Aducanumab, that directly targets amyloid β to drive its clearance. This increased interest in understanding the role of amyloid clearance in AD. We use zebrafish as a model organism to study various scales of amyloid clearance from molecular to organism. We created a transgenic line that expresses the human amyloid β 42 (Aβ42) protein associated with

plaque formation. Aβ42 is fluorescently tagged with mCherry which enables the use of fluorescence correlation spectroscopy (FCS) to quantify Aβ42. This allows us to measure the diffusion dynamics, concentration, and oligomeric state of molecules based on fluorescence fluctuation. Using FCS we can look at fluorescence dynamics of the tagged Aβ42 to quantify the amyloid in fish brains. Confocal microscopy confirms that Aβ42 tagged with mCherry aggregates in clusters in the zebrafish brain and kidney. To study FCS' ability to quantify mCherry we detected a molecular difference between mCherry monomers and dimers in control ex vivo cellular experiments. We found that FCS is able to distinguish mCherry monomers vs dimers. These controls are used to assess the aggregation of Aβ42-mCherry in the cell-types implicated in protein clearance from the brain. After confirming FCS' ability to quantify mCherry in control experiments, we next aim to study mCherry fluorescence in vivo in zebrafish larvae in different cell types. Microglia, the resident macrophages in the central nervous system, are found in the vicinity of amyloid deposits. The roles of microglia in AD development and progression are unclear as they have been reported to be both detrimental and protective to the progression of AD. Our results demonstrate Aβ42 aggregation within the microglia using point FCS. This transgenic model allows for the use of time-lapse microscopy to directly visualize microglia and amyloid plaque interactions. Much is still unknown about the effects of microglial inflammatory response and their effect on amyloid beta clearance; therefore understanding the role of microglia in a disease state allows for the design of relevant therapeutics for AD patients.

Disclosures: S. Bawarith: None. **S.E. Fraser:** None. **F. Schneider:** None.

Poster

PSTR154: Glial Contributions to Alzheimer's Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR154.30/B134

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

Support: NIH U24DK115255
NIH R01DK130913
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Title: Extracellular vesicles modulate neuroglial interaction: possible implication of miR-223-3p between metabolic syndrome and Alzheimer's disease.

Authors: *B. KIM[1], J. M. HAYES[2], C. PACUT[2], E. L. FELDMAN[3];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Neurol., Univ. of Michigan, Ann Arbor, MI; [3]Dept. of Neurol., Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: The metabolic syndrome (MetS) and Alzheimer's disease (AD) share several pathological features, including insulin resistance, abnormal protein processing, mitochondrial dysfunction, and elevated inflammation and oxidative stress. The MetS increases the risk of developing AD, but the precise mechanism remains elusive. Insulin resistance, which develops from a diet rich in sugars and saturated fatty acids, such as palmitate, is shared by the MetS and AD. Extracellular vesicles (EVs) are also a point of convergence, with altered dynamics in both the MetS and AD. The role of palmitate-induced insulin resistance in the brain and its potential link through EVs to AD is unknown. We demonstrate that EVs isolated from palmitate treated oligodendrocytes (ODs) induce insulin resistance in the recipient neurons and prevent neurogenesis. EVs carry cargo ranging from RNAs, protein, and metabolites, and are linked, through intercellular communication, to numerous illnesses. Analysis of miRNA profiles of EVs derived from control (ctlEV) and palmitate (palEV) treated ODs reveal that miR-223-3p is one of the most differentially regulated miRNAs. miR-223-3p levels are lower in type 2 diabetes (T2D) versus control mice and circulating EV-transported miR-223-3p levels predict progression of patients from prediabetes to T2D. In tandem, miR-223-3p levels progressively decrease in serum or circulating EVs from patients with AD and also correlate positively with mini mental state examination scores in AD patients. Our own results indicate that palmitate lowers miR-223-3p levels in OD-derived EVs. Neurons treated with miR-223-3p inhibitor or palEV display mitochondrial dysfunction and increased FoxO3a levels, indicative of insulin resistance. Overall, our findings suggest that in MetS condition, EVs and its cargo miR-223-3p disrupt neuroglial interaction by inducing insulin resistance and mitochondrial dysfunction thus increasing the risk of AD. The authors received funding support from the NIH (U24DK115255, R01DK130913). The authors would like to thank the Sinai Medical Staff Foundation, the Robert E. Nederlander Sr. Program for Alzheimer's Research, the Andrea and Lawrence A. Wolfe Brain Health Initiative Fund, and the NeuroNetwork for Emerging Therapies. The authors appreciate the support from the Michigan Diabetes Research Center for the confocal imaging (NIH P30DK020572, S10OD28612-01-A1).

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Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.01/B135

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

Title: Examining Protein Translation Alterations in Alzheimer's Disease using Drosophila

Authors: *A. DELGADO;
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Abstract: Examining Protein Synthesis Alterations in Alzheimer's disease using a *Drosophila* Tau modelAndy Delgado, Danitra Parker, & Andrew Pickering**Background:** Age-

related neurodegenerative diseases are characterized by excess deposition of misfolded protein aggregates. Dysregulations in protein translation through the mTOR/S6K translation pathway is reported in Alzheimer's disease (AD) and tau interference with ribosomal function is reported in AD and other tauopathies. **Methods:** This study investigates how repressing protein translation at the ribosome through cycloheximide (CHX) feeding modulates longevity in a *Drosophila* tauopathy model as measured by the lifespan assay. **Results:** This study finds that CHX has a protective effect on lifespan in both male and female flies overexpressing human wildtype tau in neurons. **Conclusions:** Further investigation is warranted to determine if this protective effect is due specifically to reduction in global protein translation or interference with tau ribosome interaction. **Future Plans:** Western blotting will be used to determine if the mTOR/S6K pathway is altered in male and female flies overexpressing human wildtype tau in neurons. Polysome profiling and RNA sequencing will also be done to assess the tau flies translatome and puromycin incorporation will be performed to measure their global protein translation.

Disclosures: A. Delgado: None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.02/B136

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

Support: MRC Grant MR/W005506/1

Title: Distance and age-dependency of axonal mitochondrial trafficking deficits and pathology in the PS19 mouse tauopathy model

Authors: *M. H. SABEC[1,2], M. C. ASHBY[2];
[2]Sch. of Physiology, Pharmacology, & Neurosci., [1]Univ. of Bristol, Bristol, United Kingdom

Abstract: Impaired axonal transport is an early pathological hallmark of tauopathy. Consequent disruption to the active trafficking of membrane-bound cargo, such as mitochondria, is hypothesized to contribute to downstream synaptic deficits and axonal dystrophy. However, it is not known if such transport deficits show a selective vulnerability correlated to the distance travelled along the axon. We use PS19 mice, which carry a P301S mutation associated with frontotemporal dementia, to investigate the impact of pathological tau on the trafficking of mitochondria in local versus distal axonal branches across different stages of tauopathy.
We employ a combination of optical *in vivo, in vitro,* and *ex vivo* strategies to quantify putative changes in mitochondrial trafficking dynamics and to examine functional and pathological measures which could be affected by impaired mitochondrial supply within the tau-burdened brain. Mice were injected with viral vectors in the motor cortex to fluorescently dual label axons and mitochondria with distinct constructs within the same neuron. Dynamic mitochondrial trafficking was recorded *in vivo* using two-photon microscopy. Cranial windows were implanted

locally to the injection site or above the ipsilateral somatosensory cortex to grant optical access to local short-range and distal long-range axons, respectively. We show that the P301S tau mutation induced significant decreases in mitochondrial motility and reduced the proportion of motile mitochondria in an age and distance-dependent manner. These recordings were supported by *in vitro* measures of mitochondrial motility recorded by two-photon imaging of cortical slices from PS19 and control littermate mice. In parallel to the dynamic imaging, immunofluorescent staining and quantitative confocal analysis was conducted to evaluated coincident changes in markers of synaptic, axonal, and neuronal health in post-mortem brains.
In this presentation we report a distinct spatial and temporal pattern of mitochondrial trafficking in the physiological and pathological condition. Furthermore, we highlight the potential therapeutic benefit of pharmacological interventions targeted to such early axonal transport deficits as a protection against downstream axonal dysfunction, dysconnectivity, and degeneration.

Disclosures: M.H. Sabec: None. **M.C. Ashby:** None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.03/B137

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

Support: NIH GRANT AG077991

Title: Therapeutic treatment of APPSWE-Tau (TAPP) mice with small molecule OLX-07010 inhibitor of tau self-association

Authors: *D. R. PATEL, P. LOPEZ, D. ROMERO, E. J. DAVIDOWITZ, J. G. MOE; Discovery, Oligomerix, Inc., Bronx, NY

Abstract: Tau aggregation plays a crucial role in the progression of Alzheimer’s disease (AD) and related tauopathies. Interaction between tau aggregation and Aβ pathology accelerates tau pathology, triggering inflammation and exacerbating tau aggregation. This cycle leads to synaptic dysfunction, neuronal death, cognitive impairment, and disease severity. Therefore, targeting tau aggregates has potential to develop effective treatments for AD patients. Our program disrupts early tau aggregation by inhibiting tau self-association. This approach was validated using htau and P301L tau JNPL3 mouse models of tauopathy. These studies showed that OLX-07010, an orally administered molecule, inhibited tau aggregation, and restored impaired motor function (Davidowitz et al., 2020, PMID: 31771053; 2023 PMID:37556474). The current study was conducted using APPSWE-Tau transgenic mice, also known as TAPP mice from Taconic Biosciences, NY, that are more representative of AD to evaluate OLX-07010 treatment on tau aggregation. TAPP mice show tau pathology and gliosis by 3 months of age that accelerates due to Aβ co-expression displaying motor impairment at later age. This study was

designed to evaluate OLX-07010 with treatment from three to ten months of age by administration in diets that were blinded for the Vehicle and treatment groups in TAPP mice. There were six groups in the study, a Control group of C57BL/6 (WT) mice (n=15), and five groups of TAPP mice, a Baseline group (n=15), a Vehicle group (n=20) and three treatment groups of age matched TAPP mice. Treatment groups received 20, 40, or 80 mg doses of OLX-07010 (n=20 per dose). Locomotor behavior was assessed using the Rotarod paradigm and Open Field Test (OFT). Cognitive behavior was evaluated using Barnes Maze (BMT) and Novel Object Recognition Tests (NORT). The in vivo phase is complete, and biochemical studies are in progress. Longitudinal assessments done at 3, 7, and 10 months age revealed no significant differences in the Rotarod analyses for WT control group. However, two TAPP mouse treatment groups (that remain blinded through completion of the biochemistry assays) showed a significant reduction in latency-to-fall at 10 months. Similar trends were observed in the OFT. There was a significant increase in spatial memory at the end of the treatment in the WT group and two blinded TAPP groups. The differences suggest that OLX-07010 may have affected the pathology. Data from ongoing biochemical studies will add on and clarify OLX-07010's effects in a more representative AD mouse model. In summary, this approach will provide a more robust understanding of OLX-07010's potential as a novel treatment strategy against AD.

Disclosures: D.R. Patel: A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **P. Lopez:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **D. Romero:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **E.J. Davidowitz:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **J.G. Moe:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc..

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.04/B138

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

Title: Diabetes mellitus exacerbates behavioral deficit in a mouse model of Alzheimer's disease via a site-specific phosphorylation of tau protein

Authors: *Y. ITO[1,3], T. NAKAJIMA[3,2], S. TESHIROGI[3,2], S. YAMAMOTO[3,2], A. OYAMA[3], R. MORISHITA[1], S. TAKEDA[1,3];

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Abstract: Background: Diabetes mellitus is associated with increased risk of developing Alzheimer's disease (AD). Cerebral accumulation of pathological forms of phosphorylated tau mediates neurodegeneration and cognitive decline in AD. Clinical and experimental studies indicate that diabetes mellitus affects the development of tau pathology; however, the underlying molecular mechanisms remain unknown. Objective: In the present study, we used a unique diabetic AD mouse model to investigate the changes in tau phosphorylation patterns occurring in the diabetic brain. Design: Tau-transgenic mice were fed a high-fat diet (n = 24) to model diabetes mellitus. Metabolic parameters and behavioral phenotypes were assessed at eight months of age. Mice were then sacrificed at nine months of age and brains were analyzed biochemically. A quantitative proteomic analysis of protein phosphorylation was performed using brain extracts of tau-transgenic mice. Results: These mice developed prominent obesity, severe insulin resistance, and mild hyperglycemia, which led to early-onset neurodegeneration and behavioral impairment associated with the accumulation of hyperphosphorylated tau aggregates. Comprehensive phosphoproteomic analysis revealed a unique tau phosphorylation signature in the brains of mice with diabetic AD. Bioinformatic analysis of the phosphoproteomics data revealed putative tau-related kinases and cell signaling pathways involved in the interaction between diabetes mellitus and AD. Conclusion: These results suggest that diabetic condition aggravates behavioral deficit in AD via hyperphosphorylation of tau protein. We identified specific phosphosites of tau involved in pathological relationship between diabetes mellitus and AD. These findings offer potential novel targets that can be used to develop tau-based therapies and biomarkers for use in AD.

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Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.05/B139

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

Support: MR/W019914/1

Title: Age-dependent changes in brain rhythms and visual responses in a humanised mouse model of tauopathy

Authors: *F. RIBEIRO RODRIGUES, J. HOLENIEWSKA, S. DE LEO, A. B. SALEEM, S. G. SOLOMON;
Exptl. Psychology, Univ. Col. London, London, United Kingdom

Abstract: Dementia is commonly associated with Tau protein dysfunction, but how Tau dysfunction has an impact on brain activity is not clear. Here we made measurements from mice expressing human Tau ('hTau') or a mutant form of human Tau associated with fronto-temporal dementia ('Tau+'; including both S305N and Intron 10+3G>A mutations). These Tau+ animals show slow disease progression, which may be more aetiologically relevant than previous transgenic models.
We made longitudinal measurements of local field potential (LFP), from 8-21 months of age, in 11 hTau animals (6 female), and 11 Tau+ animals (6 female). LFP electrodes were chronically implanted into prefrontal cortex (PFC), CA1, primary visual cortex (V1), and ectorhinal cortex of the right hemisphere. Measurements were made in awake, headfixed animals allowed to run on a treadmill and placed in front of visual displays.
We measured 'spontaneous' activity during presentation of a blank grey screen, and performed multitaper spectral analysis on common-average referenced brain activity obtained in epochs of resting or aroused states, as indexed by animal locomotion. At younger ages (<15 months) we saw little difference between hTau and Tau+ animals in power spectrum in any brain region. At older ages (>15 months) we saw a small reduction in broadband power in CA1. In our analyses of resting state activity we found no difference in low frequency power (2-10Hz) in hTau and Tau+ animals in any brain region, at younger or older ages. Gamma frequency power (55-85Hz) was lower in CA1 of older Tau+ animals, at least during periods of arousal (normalised hTau gamma power, -0.33±1.11 dB; normalised Tau+ gamma power, -3.58±1.06 dB, mean ± s.e.m; repeated measures ANOVA, p=0.047).
We measured visually evoked potentials (VEPs) to presentation of static grating patterns, and common average-referenced the activity measured across all electrode signals. We found larger VEPs in V1 of older Tau+ animals (Young hTau, 353.9.6±47.8μV; Young Tau+, 474.6±43.2 μV, p=0.077; Old hTau, 334.4±44.0 μV; Old Tau+, 537.5±39.8 μV, p=0.003). The amplitude of VEPs in Tau+ was also increased in older ages relative to younger ages (p=0.007), while VEPs in hTau were similar across ages (p=0.404). We are currently establishing whether these differences are layer-dependent.
In summary, our initial observations show no clear changes in low frequency power in Tau+ animals, unlike in many other mouse models of tauopathy. Visually evoked responses may be a more sensitive indicator of Tau dysfunction in these animals.

Disclosures: F. Ribeiro Rodrigues: None. **J. Holeniewska:** None. **S. de Leo:** None. **A.B. Saleem:** None. **S.G. Solomon:** None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.06/B140

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

Support: NIH Grant R01NS109640
NIH Grant RF1NS109640

Title: Converging Mechanisms of Polyamine Dysregulation in Distinct Neurological Disorders

Authors: *X. TAO, R. G. ZHAI;
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Abstract: Polyamines, mainly including putrescine, spermidine and spermine, are a group of small positively charged compounds, synthesized in cells or taken from exogenous environment. Polyamines interact with negatively charged cellular components, such as nucleic acids, proteins and lipids, to broadly regulate cellular activities. Dysregulation of polyamine metabolism has been observed in numerous pathological conditions, with the detailed mechanisms unclear. Here we explore these mechanisms by comparing the alteration of polyamine pathway and the consequent global changes in gene expression and metabolism between two distinct neurological conditions: Snyder-Robinson Syndrome (SRS), a rare genetic disorder, and Alzheimer's Disease (AD), a common neurological syndrome. SRS is caused by mutations in spermine synthase (SMS). In loss of SMS cells, spermine is reduced and spermidine is accumulated, accompanied by increased spermidine catabolic byproducts, H_2O_2 and aldehydes, which damage membranous structures, including mitochondria and lysosomes. Inhibiting the rate-limiting spermidine catabolic enzyme, Spermine/spermidine acetyltransferase 1 (SAT1), mitigates oxidative stress and restores mitochondrial and lysosomal function in SRS models. RNA sequencing of brain cortex from an SRS mouse model reveals alteration of neurotransmitter pathways. Metabolism analysis of patient cells indicates shift of energy source utilization in SRS. In AD, SMS and multiple other genes in the polyamine metabolism pathway are upregulated, accompanied with accumulation of spermidine or spermine in different tissues. Contrasting SRS, AD presents distinct as well as shared alterations of global gene expression and metabolism. Our findings illuminate the intricacies of polyamine dysregulation in neurological disorders, offering new avenues for therapeutic intervention.

Disclosures: X. Tao: None. **R.G. Zhai:** None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.07/B141

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

Support: NIH Grant R24AG073190
NIA grant U19AG074866

Title: Substantiation of physiological tau 3R and 4R isoform expression in marmoset brain

Authors: *H. HUHE[1,2], S. SHAPLEY[3], D. DUONG[3], F. WU[3], S. HA[4], S.-H. CHOI[4], Y. MOU[4], T. GUIMARÃES[4], A. THATHIAH[4], L. SCHAEFFER[4], J. K. KOFLER[4], G. W. CARTER[5], N. T. SEYFRIED[3], A. C. SILVA[4], S. J. SUKOFF RIZZO[4];
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Pittsburgh, PA; [3]Emory Univ., Atlanta, GA; [4]Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [5]The Jackson Lab., Bar Harbor, ME

Abstract: Common marmosets (*Callithrix jacchus*) have been shown to spontaneously develop pathological hallmarks of Alzheimer's disease (AD) during advanced age, including amyloid-beta plaques, positioning them as a model system to overcome the rodent-to-human translational gap for AD. However, tau expression in the marmoset brain has been understudied. In present study we comprehensively examined 3R and 4R tau expression in the marmoset brain.
To investigate Tau isoform expression in marmosets, brain tissue from eight unrelated marmosets across various ages were evaluated and compared to human postmortem AD tissue. Microtubule-associated protein tau (MAPT) mRNA expression and splicing were confirmed by RT-PCR and DNA sequencing. Tau protein isoforms expression in the marmoset brains were examined by western blot, mass spectrometry, and immunofluorescence/ immunohistochemistry staining. Synaptic Tau expression was analyzed from crude synaptosome extractions by western blot. Our results indicated that 3R and 4R Tau isoforms are expressed in marmoset brains at both transcript and protein levels across ages. Results from western blot analysis were confirmed by mass spectrometry, which revealed that Tau peptides in marmoset corresponded to the 3R and 4R peptides in the human AD brain. 3R Tau was primarily enriched in neonate brains, and 4R enhanced in adult and aged brains. Tau was widely distributed in neurons with localization in the soma and synaptic regions. Phosphorylation residues were observed on Thr-181, Thr-217, and Thr-231, Ser202/Thr205, Ser396/Ser404. Paired helical filament (PHF)-like aggregates were also detected in aged marmosets by western blot. Our results confirm the expression of both 3Rtau and 4RTau isoforms and important phosphorylation residues in the marmoset brain. These data emphasize the significance of marmosets with natural expression of AD-related hallmarks as important translational models for the study of AD.

Disclosures: H. Huhe: None. **S. Shapley:** None. **D. Duong:** None. **F. Wu:** None. **S. Ha:** None. **S. Choi:** None. **Y. Mou:** None. **T. Guimarães:** None. **A. Thathiah:** None. **L. Schaeffer:** None. **J.K. Kofler:** None. **G.W. Carter:** None. **N.T. Seyfried:** None. **A.C. Silva:** None. **S.J. Sukoff Rizzo:** None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.08/B142

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

Support: NIH AG054345
NINDS RF1AG079125

Title: Deciphering Tau Pathology: A Comparative Study of Humanized Tau Mouse Models in Late Onset Alzheimer's Disease

Authors: *A. L. OBLAK[1], M. SASNER[2], M. D. KOOB[3], B. T. LAMB[1], G. W. CARTER[2];
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Abstract: Tau pathology plays a pivotal role in the progression of neurodegenerative processes observed in Late Onset Alzheimer's Disease. This research provides a thorough examination of mouse models designed to replace the native murine tau gene with its human analogue, specifically the MAPT-GR gene. This substitution aims to enhance our understanding of tau's role in neurodegenerative conditions like Alzheimer's disease. Our study employs a comprehensive analytical approach, incorporating biochemical and histopathological techniques, to assess the phenotypic expressions in three distinct mouse models: the MAPT-GR wild-type, the MAPT-GR*IVS10+16, and the MAPT-GR*N279K, at both 4 and 12 months of age. Biochemical assessments focus on profiling different tau isoforms, analyzing post-translational modifications, and exploring tau's interactions with other cellular constituents. These examinations reveal a complex molecular environment that mimics human tau pathology within a murine model. Meanwhile, our histopathological evaluations concentrate on the spatial and temporal development of tau-related abnormalities, such as the emergence of neurofibrillary tangles and the progression of neuronal degradation. Through integrating these diverse analytical methods, our study provides a detailed understanding of how human tau protein impacts mouse brain physiology. These insights are crucial for clarifying tau's involvement in neurodegenerative diseases and could significantly influence the development of targeted therapies.

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Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.09/C1

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

Support: NIH R01AG081426

Title: Potential of a Viral Vector Delivery for a Resilient APOE Variant to Mitigate Tauopathy

Authors: *J. S. THIESCHAFER[1], K. FREDRIKSEN[2], L. LI[1];
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Abstract: Alzheimer’s disease (AD), categorized as either early-onset AD (EOAD - before age 65) or late-onset AD (LOAD - after age 65), is an incurable form of age-related dementia. Apolipoprotein E (APOE) is the strongest genetic risk factor for LOAD. Compared to the most common APOE3 allele, APOE4 increases the risk while APOE2 decreases the risk of AD. Recently, a rare APOE variant, APOE3-R136S (Christchurch, Ch), was found to be protective

against EOAD in a patient carrying the PSEN1-E280A mutation. Intriguingly, in this unique individual, intracellular neurofibrillary tangles (NFTs) were significantly reduced despite the presence of abundant extracellular Aβ plaques. Although the original Ch mutation was found in an APOE3 carrier, we hypothesized that the Ch mutation could counteract the detrimental effects of APOE4. This hypothesis is supported by a recent report demonstrating a decrease of NFTs in a transgenic mouse model of tauopathy carrying human APOE4 with the Ch mutation. To enhance the translational potential of the Ch variant, this project aims to use an adeno-associated viral vector (AAV) - a common delivery mechanism for gene therapy - and assess the effects of expressing APOE4Ch versus APOE4 on the progression of tauopathy. To this end, 10 male and 14 female six-month-old PS19 mice, a widely used and well-characterized mouse model of tauopathy, were bilaterally injected in the hippocampal and overlaying cortical regions with a human APOE4Ch or APOE4 AAV co-expressing the green fluorescent protein (GFP). Examination of injection monitoring mice showed GFP expression in the brain at 2, 4, and 8 weeks post-AAV injection. Behavioral tests are underway at 9 months of age, alongside uninjected PS19 and non-transgenic age and sex-matched controls, to assess motor and cognitive functions, followed by pathological evaluations for hyperphosphorylation of tau/NFTs and cholesterol/lipid-related changes. Results from this study are expected to show the potential of viral delivery of the Christchurch variant of APOE as a therapeutic approach to protect against tauopathy and APOE4-associated AD pathogenesis.

Disclosures: J.S. Thieschafer: A. Employment/Salary (full or part-time):; University of Minnesota - Twin Cities. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH R01AG081426. **K. Fredriksen:** None. **L. Li:** A. Employment/Salary (full or part-time):; University of Minnesota - Twin Cities.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.10/C2

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

Title: The potentiality of intestinal phosphorylated-tau as a risk factor for Alzheimer's disease

Authors: *J. KIM[1], H. JIN[2], H.-I. IM[3];
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Abstract: Alzheimer’s disease (AD) is the most prevalent type of dementia. Although aggregation of amyloid-beta and hyperphosphorylated tau (p-tau) are commonly observed in the patient’s brain, an obvious etiology has yet to be elucidated. To understanding the cause of the

neurodegenerative disease, various strategies are being investigated and one of those approaches is the gut-brain axis. This bidirectional interaction is being expected to anew non-invasive regulatory strategy for regulating brain homeostasis, because of the homologous features between the central and enteric nervous systems. With the aim to better understanding of etiology of Alzheimer's disease, in the present study we investigated the amount of phosphorylated-tau in the gut and brain, in addition to changes of gut microbiota in accordance with age. APP/PS1 transgenic (TG) mice and their wildtype (WT) littermates were acclimated to normal laboratory conditions. To assess the memory impairment, novel object recognition and Y-maze tests were performed. p-tau was quantified via western blot. To confirm the changes of gut microbiota, metagenomic analysis was performed using Illumina platform. The results of gut microbial diversity and taxonomy association analysis were visualized through R software. 12-month-old TG mice significantly underwent memory impairment compared with WT mice in novel object recognition and Y-maze tests. The amount of p-tau in ventral hippocampus, entorhinal cortex (ENT), and nucleus tractus solitarius (NTS) was significantly increased in TG mice. On the other hand, in 8-month-old mice, there was no differences were exhibited in the behavior tests and the protein level in LC and NTS, the regions known as accumulating p-tau in the early stage of AD onset, was only increased in TG mice. The aggregation of p-tau was earlier onset in the gut than the brain. Although the gut microbial diversities were not significantly difference between the two groups, the list of core microbiome corresponding to each group could be estimated via taxonomy association analysis. Consequently, our study sought to discover the accumulation of intestinal p-tau is earlier onset than in the brain, suggesting that can ultimately affect to Alzheimer's disease. Further study will investigate the effects of the analyzed core microbiome on intestinal tau phosphorylation.

Disclosures: J. Kim: None. **H. Jin:** None. **H. Im:** None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.11/C3

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

Support: NIH R00AG061259

Title: Tau induces depth-dependent alterations in cerebral blood oxygenation of arterioles and capillaries in the rTg4510 mouse model of Alzheimer's disease

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MA; [3]Biomed. Engin., Boston Univ., Boston, MA; [4]Radiology, Massachusetts Gen. Hosp., Charlestown, MA; [5]Neurol., Massachusetts Gen. Hosp., Charlestown, MA

Abstract: In Alzheimer's disease (AD), tau accumulation in the form of neurofibrillary tangles (NFTs) and soluble aggregates in brain tissue has been associated with neuronal death, altered neurovascular coupling, and cerebrovascular dysfunction. Notably, considerable deficits in cerebral blood flow (CBF) have been observed in human patients and mouse models of AD. Since the brain is an organ with high metabolism but virtually no metabolic reserves, it relies on uninterrupted blood flow for maintaining neuronal health. Changes in CBF alter oxygen supply and could have a profound effect on neurodegeneration. In fact, oxygen metabolism has been shown to be altered in AD. Therefore, it is likely that the observed neuronal death is, at least in part, driven by inadequate vascular support. We hypothesized that increased NFT deposition would be associated with increased perturbations in oxygen metabolism.To examine the relationship between oxygen metabolism and tau at the level of individual neurons and blood vessels, we performed two-photon phosphorescence lifetime microscopy (2PLM) in awake, young (6 mo, n = 3) and old (12 mo, n = 3) rTg4510 mice and age-matched WT controls (n = 3). Oxyphor2P was used to measure partial pressure of oxygen (pO2), and the fluorescent dye HS84 was used to visualize tau fibrils *in vivo*. We measured intravascular pO2 in arterioles capillaries, and venules at an average of 30 vascular segments per mice across three cortical depths (z = 0, 100, 200 um). We found, in old rTg4510 mice, a trend towards reduced average intravascular pO2 in arterioles and capillaries. In young rTg4510 mice, a trend towards reduced average pO2 in arterioles was seen, in contrast to capillaries, where no significant difference was observed. We also examined the correlation between pO2 values in vessels and their proximity to tangles by calculating the nearest neighbor distance between vessels and tangles. We found that in old rTg4510 mice, there were positive associations between pO2 and distance to the nearest tangle in penetrating arterioles but not in capillaries, suggesting reduced arteriolar pO2 closer to tangles. We did not find any significant associations between tangle location and vascular pO2 in young mice, suggesting that the appearance of tangles may influence vascular pO2 but only as mice age. In conclusion, lower pO2 values in arterioles and capillaries of old rTg4510 mice, but not in young mice, suggests that aging and the accumulation of tangles plays a significant role in cerebral oxygenation and metabolism. Future work will track individual tangles and neurons longitudinally to determine the impact of altered pO2 on cell loss.

Disclosures: N. Ruiz-Uribe: None. **M. Alfadhel:** None. **N. Wolf:** None. **M. Thunemann:** None. **S. Sakadzic:** None. **R.E. Bennett:** None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.12/C4

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

Support: NIH Grant R33 NS115089
NIH Grant R01 AG075809
NIH Grant P30 DA048742

Title: Mild traumatic brain injury evokes tau pathology in mice with full human MAPT gene replacement

Authors: *L. ZECKER[1], R. CARTER[1], S. P. HALEY[1], A. K. NIETZ[1], B. KOTTKE[1], D. LIAO[1], M. D. KOOB[2], T. J. EBNER[1];
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Abstract: Alzheimer's disease and related dementias (ADRDs) continue to be one of the most pressing public health concerns. Despite the severity and impact of ADRDs, the mechanisms and progression are still poorly understood. Several proteins of interest, one of which being microtubule-associated protein tau (MAPT, tau), have been implicated to play a role in ADRD progression, but understanding their involvement in pathology is lacking. MAPT is normally distributed throughout the neuronal axon, however, recent in vitro evidence suggests that brain injury induces the phosphorylation of tau in humans but not mice, which catalyzes the mislocalization of tau into the dendritic spines and soma, a hallmark of neurodegenerative disease. We investigated the involvement of human-tau (TAU) using an in vivo mouse model of repeated mild traumatic brain injury (mTBI), to better understand the mechanisms of formation, distribution, and clearance of hyperphosphorylated TAU (pTAU). To study this, we have developed a mouse model (MAPT-GR) that fully replaces the mouse Mapt gene with the full-length human MAPT gene. Two variants of the human MAPT were studied, the wild-type H2 haplotype, and the N279K variant, one of three MAPT mutations associated with fronto-temporal dementia. We used an open-skull controlled cortical impact (CCI) to deliver three total impacts over three consecutive days to the exposed motor cortex (0.4 m/s, 1 mm depth). We stained for and quantified pTAU (AT8), neurons (NeuN), and microglia (Iba-1). Examining both for cellular mislocalization and background staining of pTAU, mTBI evoked robust pTAU pathology with clear distributional differences at 1 week vs 1 month post-mTBI. At the 1-week time point, we observed pTAU primarily mislocalized to neuronal soma in H2 mice, but showed mixed somatic and glial mislocalization in N279K animals. At the 1-month time point, pTAU distribution shifts to predominantly glial for both H2 and N279K animals. Interestingly, H2 and N279K animals showed similar levels of background pTAU staining at 1-week, but N279K animals showed significantly more pTAU background staining at 1-month compared to H2 animals. Our results suggest that these two MAPT variants react differently to brain injury, with H2 animals showing a different spatial distribution of pTAU at 1-week post-mTBI, but enhanced pTAU clearance at 1-month compared to N279K animals. Tau is normally distributed throughout the neuronal axon, however, we show that pTAU mislocalizes to the soma following mTBI. These results highlight that focal mTBI can be used to trigger pTAU pathology and investigate the exact timing, progression, and underlying mechanisms in ADRD mouse models.

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Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.13/C5

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

Title: Functional biomarkers of disease progression in the P301S mouse model

Authors: *R. BOND, B. A. JENKINS, N. REISING, J. W. RYDER, T. A. DAY;
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Abstract: Background
Tauopathies, such as frontotemporal dementia and parkinsonism linked to chromosome 17, represent a substantial unmet need for which no approved disease-modifying treatments are available. The B6;CBA-Tg(Thy1-MAPT*P301S)2541Godt (P301S) mouse model, characterized by a robust phenotype and rapid disease progression, offers the potential for rapid screening of therapeutics which address the complex interplay of hyperphosphorylated tau, neurodegeneration, and neuroinflammation in human tauopathies. Motor neuron cell death associated with tau pathology in this model produces a distinct motor phenotype which offers the potential for functional biomarkers to track disease progression. The development of robust measures of functional outcomes in this model is complicated by the complexity of the phenotype. Hyperactivity at younger ages may confound rotarod results, and the use of continuous wheel running measures is contraindicated due to the impact of exercise on pathology progression.
Objective
The goal of this study was to identify a functional readout of disease progression in the P301S mouse model of tauopathy that reliably demonstrates change with therapeutic intervention at earlier disease stages.
Methods
Treatment with PLX3397, a potent inhibitor of CSF1R which has been shown to decrease tau pathology in this model, was utilized to evaluate the sensitivity of functional biomarkers to capture changes in pathology progression. Untreated (n=12) and PLX3397 treated (1000 ppm diet; n=12) female P301S mice were evaluated at two-week intervals from 2.5 to 5 months of age. Untreated female B6129F1 wildtype mice (n=12) were utilized as a control. Behavioral and functional assessments included forelimb and hindlimb grip strength measurement and a modified version of the NeuroScore assessment (Hatzipetros et al. 2015). Tau pathology and neuroinflammatory markers in the forebrain, cerebellum, and brainstem were assessed in wildtype and P301S mice at baseline and at 5 months of age.
Results
Age-dependent changes in forelimb and hindlimb grip strength, NeuroScore, and relative body weight were observed in the P301S mice. PLX3397 treatment in P301S mice was associated with protection of neuromuscular function, reduction in tau pathology, and downregulation of inflammatory genes in specific brain regions. Correlation analysis showed a strong association between tau pathology and functional outcomes.

Conclusions
These results demonstrate that grip strength and NeuroScore are sensitive measures to capture treatment effects and serve as functional biomarkers of pathology progression in this line.

Disclosures: R. Bond: A. Employment/Salary (full or part-time):; Eli Lilly and Company, Indianapolis, USA. **B.A. Jenkins:** A. Employment/Salary (full or part-time):; Eli Lilly and Company, Indianapolis, USA. **N. Reising:** A. Employment/Salary (full or part-time):; Eli Lilly and Company, Indianapolis, USA. **J.W. Ryder:** A. Employment/Salary (full or part-time):; Eli Lilly and Company, Indianapolis, USA. **T.A. Day:** A. Employment/Salary (full or part-time):; Eli Lilly and Company, Indianapolis, USA.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.14/C6

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

Support: NIH: R24AG073138; Takeda Pharmaceutical Company Ltd. ; NIH: P51-OD011107; Alzheimer’s Association, AARF-20-685431

Title: Temporal progression of tau containing filament formation in the hippocampus of a tau-based rhesus monkey model of Alzheimer's pathogenesis: an immunogold ultrastructural study.

Authors: *W. JANSSEN[1], D. BECKMAN[2], A. SOWA[1], S. OTT[2], G. B. DINIZ[2], J. MORRISON[2];
[1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]California Natl. Primate Res. Ctr., Univ. of California, Davis, CA

Abstract: Alzheimer's disease (AD) is a devastating condition with minimal treatment options, and promising findings in rodents failing to translate into successful therapies for patients. We have developed a novel non-human primate (NHP) model of tauopathy in AD by performing unilateral injections of an adeno-associated virus expressing a double tau mutation (AAV-P301L/S320F: AAV-2xTau Group; N=8) or an empty vector (CTRL; N=4) into the left entorhinal cortex (ERC) of adult female rhesus macaques. Animals were euthanized three (3M Group; N=4) or six (6M Group; N=4) months after injection. Using confocal microscopy, we have reported an aggressive progression of tau pathology between 3 and 6 months characterized by the spread of pathology-associated tau epitopes throughout the trans-entorhinal circuitry, extensive microglial and astrocytic neuroinflammation, and neuronal loss. Critically, we also reported evidence of misfolded tau propagation through templating of the endogenous tau, similar to what is hypothesized to occur in humans. To obtain further insight into the ultrastructural properties of tau pathology, we are now pursuing immunoelectron microscopy (IEM) in this NHP model, with an initial focus on the CA3 hippocampal field of 6M animals to be followed by additional hippocampal fields and 3M animals. Immunogold reactivity (IR) using

antibodies for early Tau phosphorylation sites (pT181 and pS199) placed affected neurons into various classifications in the tau model, now interpreted as an accurate timeline of the pathogenesis leading to neuronal cell death seen in AD. We classified neurons into four overlapping stages: 1-no effect/minimally impacted neurons, 2- neurons containing abnormally high levels of active cytoplasmic RER/Golgi assembly for filament translation, along with zero to low expression of IR tau filaments, 3-neurons containing large dense propagations of filament production, and tight mitochondria/phospho-tau (p-tau) contacts, 4-neurons with little to zero cytoplasmic organelles remaining, and emptied/hollowed neuronal cytoplasm. Synaptic p-tau IR adjacent to p-tau IR neurons can reflect a model for the trans-neuronal passage of tau pathology within the circuit. Interestingly, not all of the filamentous structures expressed p-tau IR, and studies using additional neurofilament markers will elucidate tau's role within these tau immuno-negative regions. These results begin to highlight both the ultrastructural stages of tau seeding and propagation in an accelerated model of tau pathology which can be used as a powerful translational approach in development of new therapies for AD over a shortened time frame.

Disclosures: W. Janssen: None. **D. Beckman:** None. **A. Sowa:** None. **S. Ott:** None. **G.B. Diniz:** None. **J. Morrison:** None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.15/C7

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

Title: Elucidating mechanisms underlying tau and amyloid-beta proteinopathies in novel mouse models

Authors: *G. PATERNO, K.-M. GORION, B. BELL, Y. XIA, B. I. GIASSON;
Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive and irreversible cognitive decline, which manifests as impairment in memory and executive function. The major neuropathological hallmarks of AD include neurofibrillary tangles composed of hyperphosphorylated tau protein and extracellular plaques composed of amyloid-beta (Aβ) peptides derived from proteolytically cleaved amyloid precursor protein (APP). The exact mechanisms by which both tau and Aβ promote the pathological process and contribute to the cognitive phenotypes typical of AD remain to be elucidated. Moreover, the current paucity of disease-modifying therapies for AD patients highlights the critical need for further investigation aimed at clarifying the roles both tau and Aβ pathologies have in advancing the development of AD. To this end, we have generated several novel transgenic mouse models termed tau **SPAM** (**S**320F **P**301S **a**ggregating **m**utations). We previously characterized the tau **SPAM TgBy** mouse model of tauopathy which develops neurofibrillary tangle-like inclusion pathology throughout the neuroaxis, and importantly, tau transgene expression is below that of endogenous murine tau

(0.7x). Moreover, the tau SPAM TgBy line accrues phosphorylated tau within enteric neurons, and furthermore, a lethal phenotype ensues due to enteric neuron loss. An additional founder line was generated, tau **SPAM TgDy**, which develops neurofibrillary tangle-like pathology, has tau expression below that of murine tau levels, and notably can live beyond 20 months of age. In this work, we have crossed both tau SPAM TgBy and TgDy with the TgCRND8 mouse model of amyloidosis which has Swedish (K670N/M671L) and Indiana (V717F) mutations within APP, therefore, bigenic mice accumulate both tau and Aβ neuropathologies. In these studies, we investigate several features observed in AD such as post-translational modifications, biochemical insolubility, markers of β-sheet conformation, and neuroinflammation. These novel mouse models will allow for the investigation of proteinopathies characteristic of AD and determination of how co-deposition influences pathological inclusion formation *in vivo*. Collectively, the development of novel models of AD-like neuropathology will contribute to our understanding of how these pathologies may be augmented and how other factors may promote the pathological signatures akin to that of bona fide AD.

Disclosures: G. Paterno: None. **K. Gorion:** None. **B. bell:** None. **Y. Xia:** None. **B.I. Giasson:** None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.16/C8

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

Support: R01 AG068215

Title: Aav injection to overexpress mutant human tau in c57bl/6 mouse brains

Authors: *V. BUZINOVA[1], M. T. MAISEL[2], P. SOMPOL[3], M. P. MURPHY[1];
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Abstract: Tau is one of the two main hallmarks of Alzheimer’s Disease (AD) pathology. In AD, this pathology is present in the form of neurofibrillary tangles (NFTs), that are comprised of hyper-phosphorylated tau. Amyloid Beta (Aβ) is the other hallmark of AD and it has been established that the presence of Aβ is a driver for NFTs. While it is ideal to research AD in mouse models that display both pathologies, there are limited options available. So, we started brainstorming different ways to make that possible. One of which is to overexpress tau pathology in mice that have human amyloid beta pathology. First, to gather some feasibility data, I chose to utilize C57/Bl6 mice. I bred some of these mice and when the pups were born, I performed a bilateral injection in the brains of the pups the day they were born (P0) with an adeno-associated virus (AAV) that will either express GFP in neurons, or overexpress tau-GFP pathology. These mice were then aged for 5-6 months. After aging, some mice underwent live two-photon imaging

while other mice went through some behavioral experiments, open field, rotarod and Barnes maze. The mice that underwent live two-photon imaging also went through whisker stimulation testing. During live two-photon imaging, we found that GFP was expressed in the neurons of control mice injected with AAV-GFP control virus. In the experimental mice that were injected with AAV-tau-GFP, we found that tau pathology had been successfully overexpressed in their brain. After doing whisker stimulation on the mice that were imaged, we found that the mice with tau overexpression experienced a noticeable decrease in percent change in diameter and maximum diameter of blood vessels compared to mice without pathology. For cognitive behavior testing, we found some differences, but nothing significant, considering that the mice were still relatively young. There was no difference in results from rotarod testing, which was expected since none of the mice should have displayed any physical ailments.

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Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.17/C9

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

Support: NIH R00EY024653

Title: Circuit-level investigation of tau-mediated neurodegeneration in Alzheimer’s disease

Authors: *B. AKDOGAN[1,2], L. CARRILLO-REID[3], Z. HOSSAIN[4], F. HUSSAIN[4], F. STEFANINI[5], A. KLEINSTEIN[6], X. QIN[6], B. MILLER[4,2], J.-E. MILLER[4,2];
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Abstract: The molecular and cellular pathology in Alzheimer’s disease (AD) and other tauopathies is well-documented, but the changes in the neural activity underlying their core cognitive symptoms are poorly understood. To start addressing this question, we first investigated the changes in large-scale neural activity in the entorhinal-hippocampal circuit in a mouse tauopathy model (PS19). More specifically, we used one-photon calcium imaging to image individual granule cell activity in the dentate gyrus (DG) of freely moving PS19 and control mice in different environments (open field, home cage, and circular arena). Our preliminary findings revealed a significantly lower number of active neurons in 8-month-old PS19 mice, as compared to the age-matched control mice. To determine if this difference in the number of detected active cells was due to neuronal death, a critical pathological feature of AD progression, we analyzed NeuN-stained brain sections obtained from a separate group of animals. Although our results showed a significant reduction in NeuN-labeled nuclei in the lateral entorhinal cortex, an early site of dysfunction in AD that provides direct input to the

hippocampus, we did not find such a change in the DG granule cell numbers. We then further analyzed the baseline neural activity levels and found neural hyperactivity in PS19 mice, irrespective of their movement speed or the contextual environment that they explored. Finally, we examined the effect of contextual changes on neural activity patterns. The analysis of activity rates in different environments revealed less similar activity levels in control mice, but not in PS19 mice. Despite this overall reduction in context selectivity in PS19 mice, our decoding analysis using support-vector machine classifiers revealed high context decoding accuracy in both groups of mice. Taken together, our analyses of neural activity patterns and immunohistological findings demonstrate a functional activity imbalance within the entorhinal-hippocampal circuit and potentially indicate impaired context selectivity in 8-month-old PS19 mice. We are currently conducting two-photon calcium imaging and retrograde viral tracing studies to further characterize the multifaceted nature of the changes in the entorhinal-hippocampal neural dynamics and cognitive abilities in AD, which would collectively help provide a foundation for future therapeutic interventions.

Disclosures: B. Akdogan: None. **L. Carrillo-Reid:** None. **Z. Hossain:** None. **F. Hussain:** None. **F. Stefanini:** None. **A. Kleinstein:** None. **X. Qin:** None. **B. Miller:** None. **J. Miller:** None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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

Support: NIH R01 (1R01NS135592-01)
NIH R03 (AG062883-02)
NIH COBRE (3P20GM121310-05, -05S2, and -06)

Title: Cre-dependent Expression of P301L Mutant Tau and Chemogenetic Manipulations of a Circuit for Sundowning-related Circadian Disturbances

Authors: *E. D. GWALTNEY[1], P. GUPTA[2], A. E. WARFIELD[3], W. D. TODD, III[3];
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Abstract: A common aspect of circadian dysfunction in Alzheimer’s disease (AD) is a “phase delay” characterized by later acrophases and bathyphases of locomotor activity (LMA) and body temperature (Tb) rhythms. In many AD patients, this phase delay is strongly associated with sundowning syndrome, a poorly understood clinical phenomenon which is characterized by wandering, agitation, and aggression during the late afternoon and early evening. In the TAPP (APPSwe-Tau) mouse model of AD, we recently showed that the development of hyperphosphorylated Tau pathology (pTau) in lateral parabrachial neurons of the brainstem is

strongly correlated with a similar phase delay and increased aggression around the active-to-rest phase transition. We further showed that many of these pTau-expressing LPB neurons express dynorphin and project to the major circadian structures of the hypothalamus, the suprachiasmatic nucleus (SCN, the master circadian pacemaker) and its major axonal target, the subparaventricular zone (SPZ). In our ongoing study, we are testing the hypothesis that pTau in the LPB to SCN/SPZ pathway underlies AD-related circadian dysfunction and sundowning-related behavioral disturbances. We employ pathway-specific and genetically targeted manipulations to test the role of pTau in specific LPB subpopulations that project to the circadian system. In WT mice, we utilized the retrograde delivery of Cre recombinase from the SCN and SPZ (adjacent structures), and a Cre-dependent vector expressing the P301L mutation (and subsequently pTau) in only LPB neurons that target the SCN/SPZ. We also used Cre-mouse lines to similarly express pTau in specific LPB subpopulations that differentially project to the circadian system. In both sets of mice, we performed biotelemetry recordings of LMA and Tb rhythms as well as time-dependent aggression tests before and after the expression of Cre-dependent pTau. Similar strategies are also being used to chemogenetically manipulate this pathway in order to test its function in WT and Cre-mice. Preliminary results indicate that expressing Cre-dependent pTau in the LPB to SCN/SPZ pathway results in a phase delay similar to that seen in TAPP mice with LPB pTau and that seen in AD patients with sundowning syndrome. We are currently quantifying time-dependent levels of aggression is these mice, and expect to see an increase around the active-to-resting phase transition similar to TAPP mice with LPB pTau.

Disclosures: E.D. Gwaltney: None. **P. Gupta:** None. **A.E. Warfield:** None. **W.D. Todd:** None.

Poster

PSTR155: Tau: *In Vivo* Models

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

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Race Against Dementia ARUK-RADF-2019a-001
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Title: Synaptic propagation of tau in Alzheimer's disease and Progressive Supranuclear Palsy

Authors: *T. L. SPIRES-JONES;
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Abstract: In Alzheimer's disease (AD) and Progressive Supranuclear Palsy (PSP), tau pathology spreads through the brain in a stereotypical pattern, and where tau pathology appears in the brain, synapse and neuron loss follows. In cell and animal models of tauopathy, pathological tau spreads via synapses and glia have been shown to play a role in tau propagation, the synaptic spread of pathological tau, and tau-induced neurodegeneration. Here we will present data investigating trans-synaptic tau spread in human brain and living human organotypic brain slices. Immunohistochemistry, array tomography, and confocal imaging were used to study post-mortem brain samples from people with Alzheimer's disease, Progressive Supranuclear Palsy, control subjects, and human organotypic brain slices challenged with proteins extracted from PSP brain samples. Data were analyzed with linear mixed effects models to examine the effects of disease and sex with case used as a random effect to avoid pseudoreplication. We observe that in both AD and PSP, oligomeric tau is found in synaptic pairs even in brain regions that are affected late in the disease process. Further, there is an increased ingestion of synapses by astrocytes in these tauopathies. Exposing living human organotypic brain slices to pathological tau derived from PSP brain tissue causes post-synaptic uptake of oligomeric tau and astrogliosis. Together, these data from human brain support the idea that tau pathology spreads through the brain trans-synaptically and that astrocytes play a role in synapse degeneration. In the future, therapies aimed at preventing synaptic spread of tau may be beneficial in AD and PSP.

Disclosures: T.L. Spires-Jones: F. Consulting Fees (e.g., advisory boards); TS-J has received advisory board stipends, consulting fees, or collaborative research funding from Jay Therapeutics, Cognition Therapeutics, Race Against Dementia, and Scottish Brain Sciences..

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.20/C12

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

Title: Immunotherapy targeting toxic forms of tau modulates AD pathology and inflammaging in very old triple-transgenic AD mice

Authors: *A. SCIORTINO[1], F. ALSHAEBI[2], R. KAYED[1];
[1]Neurol., Univ. of Texas Med. Br., Galveston, TX; [2]UTMB, Galveston, TX.

Abstract: Background Rodent models have proved essential in the understanding of Alzheimer's disease (AD). However, the development of effective AD therapies may be hampered by the use of models that only replicate a single element of AD neuropathology and of early time points that may be omitting crucial aspects such age-dependent inflammation and senescence. Tau misfolding and accumulation has been shown to correlate with the progression of AD pathology and the stage of dementia. Several studies demonstrated that tau removal is able to slow down AD pathology propagation and cognitive deterioration in different animal models. **Methods** To capture age-dependent aspects often overlooked in other studies, we treated very

old triple-transgenic AD mice (3xTg AD) with in-house anti-tau antibodies that target specific toxic tau conformations. Twenty-three months old 3xTg AD mice, that replicate both amyloid and tau pathology, as well as other cellular alterations linked to AD, were injected in the tail vein with 120μg of anti-toxic tau antibody or IgG isotype control. Following immunization, cognitive function and brain neuropathology were examined. We used behavioural tests such as the open field test, and immunohistochemistry, immunofluorescence and western blot techniques. **Results** Preliminary data indicate that treatment with conformational antibodies targeting toxic forms of tau reduces the burden of tau deposition, thus modulating the extent of AD pathology. Furthermore, immunotherapy modulated markers of age-related pathology such as HMGB1, suggesting an interplay of toxic tau with senescence and inflammation. **Conclusions** Our study contributes to the investigation of selective tau immunotherapy approaches for AD and tauopathies. Our results suggest that specifically targeting toxic forms of tau modulates AD pathology beyond pathological protein aggregation and could have beneficial effects even at later disease stages.

Disclosures: A. Sciortino: None. **F. Alshaebi:** None. **R. Kayed:** None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.21/C13

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

Title: Immunotherapy Targeting Toxic Tau mitigates Cognitive Decline and Senescence in Tau Mouse Model

Authors: *F. ALSHAEBI[1], R. KAYED[2];
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Abstract: Immunotherapy Targeting Toxic Tau Ameliorates Cognitive Decline and Senescence in a transgenic Tau Mouse Model
Background: Alzheimer's disease (AD) is characterized by the accumulation of tau protein in the brain, leading to the formation of neurofibrillary tangles and contributing to the gradual deterioration of brain function. Cellular senescence occurs as a consequence, leading to cognitive impairment and hastening the aging process. Immunotherapies targeting Aβ and other protein aggregates are also being developed. This study examines immunotherapy in a Mapt (hTau) mouse model, which could potentially become a useful immunological treatment in the future by lowering inflammation and cellular senescence associated with aging.**Methods:**Mapt (hTau) mice were inoculated in the hippocampus with 1 μg of brain-derived Tau oligomers (BDTOs) from AD patients. After seven months, mice were injected in the tail vein with 120 μg of in-house mouse monoclonal anti-toxic tau antibodies (TTCM1-2 and TOMA1-4) or IgG isotype control. Mice were evaluated for cognitive and motor function before euthanasia, and brain neuropathology was investigated with immunohistochemistry, immunofluorescence, and western

blot techniques.**Results:**Tau pathology was investigated in cortex and hippocampus by immunohistochemistry (IHC) and immunofluorescence (IF). Preliminary data revealed that mice treated with anti-toxic tau antibodies exhibited reduced levels of tau deposition and tau phosphorylation, both known to be associated with AD-pathology development. Additionally, a reduction was observed in senescence and inflammation markers compared to the control group.**Conclusions:**The observed decrease in tau accumulation and phosphorylated tau following immunotherapy indicates that the mechanisms responsible for tau phosphorylation and/or aggregation may have been altered. Additionally, the reduction in senescence and inflammation markers suggests that immunotherapeutic strategies targeting tau are a promising approach in the management of tauopathies.

Disclosures: F. Alshaebi: None. **R. Kayed:** None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.22/C14

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

Support: NIH Grant R01-AG077692
Alzheimer's Association AARF-22-972333

Title: Seizure-activated networks exacerbate tau spread in a novel 5XFAD-TRAP Alzheimer's disease model

Authors: *A. BARBOUR[1], A. CHAVEZ[4], A. CHAVEZ[5], E. J. CORNBLATH[1], K. F. HOAG[1], X. LI[2], C. HASSMAN[6,1], S. ZEBROWITZ[7], K. A. DAVIS[1], V. M. LEE[8], D. M. TALOS[9], F. E. JENSEN[3];
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Abstract: The spread of tau along neuronal connections is central to Alzheimer's disease (AD) progression. Seizures are common in AD and we and others have shown that seizures worsen cognitive and pathological outcomes of AD. However, whether seizures affect the spread of tau throughout the brain is unknown. We hypothesized that seizures facilitate the spread of tau via activation of network circuitry. To examine seizure-AD interactions, we crossed the 5 times familial AD (5XFAD) model with TRAP (targeted recombination in active populations) mice (5X-TRAP) to permanently label seizure-activated neurons with tdTomato (tdT). To model tau spread, we used human AD brain-derived tau lysate (AD-tau) seeding in the right hippocampus

and cortex of 5X-/WT-TRAP mice at 3 months of age followed by pentylenetetrazol (PTZ) kindling to induce seizures or control (saline) protocols. On the final day of kindling, 4-hydroxytamoxifen was injected to induce Fos-driven, Cre-mediated tdT expression in seizure-activated neurons and mice were euthanized at 6 months of age. Brains were serially sectioned and fluorescent slide scanning was performed to image phospho-tau (AT8) and tdT throughout the brain. All AT8+ aggregates and tdT+ neurons were mapped to the Allen Brain Atlas with NeuroInfo software to allow for count comparisons between groups and to examine associations between regional tdT+ counts and tau spread. Confirming prior reports in 5XFAD mice, seeded 5X-TRAP mice had elevated tau spread including in the left hippocampus ($p<0.05$, n=7-11), compared to seeded WT-TRAP. In addition, we found that PTZ kindled 5X-TRAP mice had further increases in AT8 aggregates in the right and left thalamus, isocortex, and white matter tracts ($p<0.05$, n=7-11). PTZ kindling tended to increase tdT+ counts across the brain with PTZ kindled 5X-TRAP mice showing increased tdT+ counts compared to PTZ kindled WT-TRAP in the right and left thalamus and isocortex ($p<0.05$, n=7-11). Notably, PTZ kindled 5X-TRAP mice showed significantly increased AT8 aggregates and tdT+ counts in the intralaminar nuclei of the thalamus and the anterior cingulate and somatosensory cortices ($p<0.05$, n=7-11), which are interconnected regions, suggesting that PTZ seizure activity activates cortico-thalamic circuits to facilitate tau spread in 5X-TRAP mice. Indeed, we found a positive correlation between tdT+ counts in the intralaminar nuclei and tau spread in the anterior cingulate cortex ($p<0.05$, $r=0.84$) of PTZ kindled 5X-TRAP mice. Overall, these data indicate that seizure-activated circuitry facilitates tau spread in 5X-TRAP mice, suggesting seizures and hyperactive circuitry as targets to slow tau spread in AD.

Disclosures: A. Barbour: None. **K.F. Hoag:** None. **X. Li:** None. **V.M. Lee:** None. **D.M. Talos:** None. **F.E. Jensen:** None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.23/C15

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

Support: NIH Grant R01-AG077692
AARF-22-972333

Title: Seizure-activated neurons facilitate increased levels of tau spread in 5xFAD mice

Authors: *A. CHAVEZ[1], A. J. BARBOUR[2], K. F. HOAG[3], V. M. LEE[5], D. M. TALOS[6], F. E. JENSEN[4];
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Abstract: Seizures are highly comorbid with Alzheimer's disease (AD) and worsen the symptoms and pathology of AD patients. One of the main markers of AD progression is the spread of tau along neuronal projections. Mice with seizures and 5XFAD genotype increase tau levels and worsened neuropathology (Gourmaud et al. 2022, Brain). Thus, to further understand how seizures affect tau accumulation and transmission in individual neurons, we crossed the 5XFAD mouse model with Targeted Recombination in Active Populations (TRAP) mice to further understand how seizures affect tau accumulation and transmission. The 5xFAD x TRAP (5X-TRAP) model allowed us to permanently label seizure-activated neurons with tdTomato (tdT). To model tau spread, we injected human AD brain-derived tau lysate (AD-tau) unilaterally into the right hippocampus and cortex. Tau injections were performed in 3-month-old prodromal 5XFAD mice, and seizures were induced 2 weeks after the tau injection using pentylenetetrazol (PTZ) seizure kindling (or saline as a control) protocol. 4-hydroxytamoxifen (4-OHT) was administered on the final day of kindling to induce Cre-dependent, Fos-driven, tdT expression. At 6 months the brains were serially sectioned and stained for phospho-tau (AT8) and NeuN via IHC. The sections were slide-scanned and registered to the Allen Brain Institute Atlas. tdT+ and negative (tdT-/NeuN+) neurons were detected and colocalized with aggregates of AT8. AT8 colocalization with neurons increased in the PTZ-seizure-kindled mice. This is notably seen in the left temporal association cortex, the left cortical subplate, and the right midbrain (PTZ effect: $p<0.05$, n=7-11). A higher proportion of PTZ/tdT+ neurons colocalized with AT8 than PTZ/tdT- neurons in the 5X-TRAP thalamus ($p<0.05$, n=7-11), suggesting seizure-activated neurons contribute to tau spread. There was also an increased colocalization in Sal/tdT+ compared to Sal/tdT- in 5X-TRAP in the left thalamus, right hypothalamus, and the left anterior group of the dorsal thalamus ($p<0.05$, n=7-11), suggesting that basally hyperactive neurons may contribute to increased tau spread in non-kindled 5X-TRAP mice. Overall, these data show that hyperactive neurons aid tau spread robustly in the thalamic areas, possibly contributing to tau spread along thalamo-cortical circuits. It will be beneficial to target these hyperexcitable neurons to elucidate the mechanisms underlying the accumulation and transmission of tau, thus identifying novel therapeutic targets to help slow the progression of AD pathology in patients.

Disclosures: A. Chavez: None. **A.J. Barbour:** None. **K.F. Hoag:** None. **V.M. Lee:** None. **D.M. Talos:** None. **F.E. Jensen:** None.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.24/C16

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

Support: JPB Grant MR-2023-4260
BrightFocus Postdoctoral Fellowship Program in Alzheimer's Disease Research A2023002F
BCM CAND Scholars Program

Title: The big tau splice isoform resists Alzheimer's-related pathological modifications

Authors: *D.-E. C. CHUNG[1], X. DENG[1], H. YALAMANCHILI[2], J.-P. REVELLI[1], A. HAN[3], B. TADROS[1], R. RICHMAN[1], M. DIAS[2], F. ALAVI NAINI[1], S. BOEYNAEMS[1], B. HYMAN[4], H. Y. ZOGHBI[1,5];
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Abstract: As Alzheimer's disease (AD) progresses, tau pathology affects selective brain regions such as the cortex and hippocampus while largely sparing the cerebellum and brainstem except for a few deep nuclei. To explain this phenomenon, we evaluated tau isoforms in various brain regions and discovered that an atypical "big tau" isoform is significantly more abundant in the cerebellum and brainstem compared to the cortex and hippocampus. Big tau results from alternative splicing of the *MAPT* gene to include exons 4a and 6 and was previously known to be predominantly expressed in the peripheral nervous system. However, it has remained understudied for several decades, especially in the central nervous system. Intrigued by big tau's unique protein structure with an extremely longer N-terminal region, we examined if big tau possesses distinct properties compared to regular tau isoforms. Examination of endogenous tau isoforms in the brains of wild-type (WT) mice revealed that big tau is significantly less subject to age-dependent hyperphosphorylation and abnormal conformational changes compared to regular tau. Upon assessing big tau's stability, we found that big tau is more robustly ubiquitinated than regular tau, possibly due to additional lysine residues, and is degraded more efficiently in HEK293T cells. Moreover, using a HEK293T-based cellular assay and a peptide array overlay assay, we discovered that big tau binds much more strongly to microtubules than tau441 via additional microtubule-binding sites in its unique N-terminal region. To measure the aggregation propensity of big tau, we performed detergent fractionation using HEK293T cells that express big tau or tau441 (the longest among six regular tau isoforms) carrying an aggregation-driving mutation. Here, we found that big tau demonstrates a significantly reduced aggregation propensity compared to regular tau. We further investigated this in a mouse model by injecting newborn WT pups with adeno-associated viruses (AAVs) to express mutant big tau or tau441. After aging these mice for six to nine months, we found that big tau is significantly less aggregation-prone in the brain. Importantly, we found that AD patients display an elevated expression level of pathology-resisting big tau in the cerebellum, the brain region that lacks severe tau pathology, compared to cognitively normal control individuals. Taken together, our data indicate that big tau is remarkably less prone to pathological changes compared to regular tau. Alternative splicing that leads to the elevated expression level of big tau in the cerebellum and brainstem may contribute to the sparing of these brain regions from tau pathology.

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Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.25/C17

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

Support: National Health and Medical Research Council grant nos. 1143978 and 1176628
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Dementia Australia Research Foundation
Flinders Foundation
A.I. is a National Health and Medical Research Council Emerging Leadership 2 fellow
K.S. is a Scientia Professor Henry Brodaty Post-doctoral Fellow of the Dementia Australia Research Foundation

Title: Remote memory engrams are controlled by encoding-specific tau phosphorylation

Authors: K. STEFANOSKA[1], R. KOSONEN[2], Y. LIN[1], E. PRIKAS[3], L. M. ITTNER[5], ***A. ITTNER**[4];
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Abstract: Correctly storing and recalling memories is key to survival of all animals. The engram represents the physical trace that encodes a specific memory and enables its recall. Failure of the engram is linked to memory loss in Alzheimer's disease. Here, we demonstrate that microtubule-associated protein tau, a central Alzheimer's factor, has a direct function in the engram. Tau is required, specifically during memory formation, for remote, yet not recent recall in mice. Tau is phosphorylated at specific sites during encoding, and ablating site-specific phosphorylation at threonine-205 (T205) lowers precision of engram cell recruitment and precludes efficient remote recall. Vector-based engineering of engram cells reveals that T205 phosphorylation of tau is required to encode memory for recall at remote time points. Our work delineates a role of encoding-associated tau phosphorylation to support an enduring engram. Thus, tau is directly linked to remote memory, offering a new explanation for amnesia in Alzheimer's disease.

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Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.26/C18

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

Support: NIH 1R61NS125280
NIH 5R33NS125280
Leon Levy Fellowship in Neuroscience
Rainwater Charitable Foundation

Title: Circuit mechanisms of Tau-mediated balance deficits: lessons from a zebrafish model for tauopathy

Authors: *Y. ZHU[1], F. AUER[1], K. HAMLING[1], P. LEARY[2], Q. BAI[3], E. A. BURTON[3,4], D. SCHOPPIK[5,6];
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Abstract: How do behavioral deficits arise from molecular abnormalities in neurological disorders? To understand circuit mechanisms underlying behavioral deficits, we focused on the balance behavior, fundamental to locomotion but frequently impaired in neurological disorders. Using a simple vertebrate model, the larval zebrafish, we identified two brainstem vestibular circuits: a direct circuit to the spinal cord controlling postural stability and an indirect hindbrain-midbrain-spinal-cord circuit underlying navigation stability . Next, we examined dysfunction of these circuits in a novel zebrafish model for tauopathy. Targeted expression of human 4R-Tau in brainstem balance nuclei impaired postural and navigation stability in larvae before neurodegeneration occurred. Intriguingly, differential Tau deposition in the indirect/direct hindbrain vestibular circuits correlated with specific behavioral deficits. By combining the natural heterogeneity of Tau expression and variations of individual behavior, we found a strong positive correlation between Tau levels in specific brainstem balance substrates and the severity of balance phenotypes, suggesting that 4R-Tau accumulation impairs circuit-specific neural function in a dose-dependent manner even in the absence of neuronal loss. Together, our results unveil circuit-specific mechanisms of Tau-mediated balance deficits, shedding light on symptom heterogeneity, circuitry susceptibility, and functional deficits in neurodegenerative diseases.

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Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.27/C19

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

Support: NIA, NIH Grant AG053150

Title: Acute daily treatment with OLX-07010, an oral, small molecule inhibitor of tau self-association, caused dose dependent reduction of tau aggregates in aged JNPL3 mice and improved motor function

Authors: D. R. PATEL[1], P. LOPEZ[1], D. ROMERO[1], *E. DAVIDOWITZ[1,2], **J. G. MOE**[1,2]; [1]Discovery, Oligomerix, Inc., Bronx, NY; [2]Oligomerix, Inc., White Plains, NY

Abstract: OLX-07010, an orally available, small molecule inhibitor of tau self-association, was effective inpreventing the accumulation of aggregated tau in studies of htau and P301L tau mouse modelsof tauopathy using chronic preventive or therapeutic treatment paradigms. Chronic treatmentthat was administered in the diets of young mice from 3 to 7 months prevented the accumulationof tau aggregates and also inhibited the progression of tau aggregation and improved motorcoordination of aged P301L tau JNPL3 mice treated from 7 to 12 months (Davidowitz et al.,2020, PMID: 31771053; 2023, PMID: 37556474). The objective of this study was to evaluate thepotential of an acute effect by single daily dosing of OLX-07010 by oral gavage for four weekson tau aggregation in aged JNPL3 mice.Pharmacokinetic (PK) analysis of OLX-07010 in aged JNPL3 mice was performed to determinethe exposure in the brain and serum following a single oral dose via gavage to select doses forthe treatment study. The study was designed with four groups of mice: Baseline (7 months;n=15), Vehicle (n=20) and two treatment groups (20 and 40 mg/kg; n=20 each). Treatment wasadministered daily by oral gavage from nine to ten months-of-age. Cortical levels of aggregatedtau were determined by two independent methods using either PAGE or high-speedcentrifugation to resolve the aggregates paired with immunoblot or AlphaLisa for detection,respectively. Immunoblots were also performed to determine levels of LC3-II to study the effectof treatment on autophagy. Motor behavior was evaluated from 7-10 months of age using OpenField Test and Rotarod assays.PK analysis showed that OLX-07010 entered the brain, and biochemical analyses showed thattreatment caused dose-dependent, statistically significant, reduction of tau aggregates tobaseline levels. Amelioration of motor impairment and reduced grooming, a measure of anxiety,was associated with the reduction in tau pathology. There was also a dose dependent increasein LC3-II in the insoluble protein fraction derived from cortex.The acute daily treatment strategy had a dose-dependent therapeutic effect on tau pathologywith a functional motor behavior readout. The dose dependent increase in LC3-II suggestsincreased autophagy for clearance of protein aggregates. If the acute treatment strategytranslates to the clinic, then changes in biomarkers of tau aggregation and functional readoutsmay enable shorter timelines for trials.

Disclosures: D.R. Patel: A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **P. Lopez:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **D. Romero:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **E. Davidowitz:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **J.G. Moe:** A. Employment/Salary (full or part-time):; Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc..

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.28/C20

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

Support: 24K02268 from Ministry of Education, Culture, Sports, Science, and Technology, Japan
AMED Project for Regenerative/ Cellular Medicine and Gene therapies, JP23bm1423012
AMED-CREST grant JP21gm1410009

Title: Spatiotemporal progression of tau pathology linked to neurodegeneration and neuroinflammation in a novel tauopathy mouse model

Authors: R. YANAI[1], T. T. MITANI[2], E. A. SUSAKI[3], M. SHIMOJO[4], M. HIGUCHI[5], H. R. UEDA[6], *N. SAHARA[7];
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Abstract: Creating a mouse model that recapitulates human tau pathology is essential for developing strategies to intervene in tau-induced neurodegeneration. To date, attempts to generate such models through the overexpression of non-mutant human tau transgenes have yeiled limited success in producing animals with mature tau pathologies or behavior abnormalities. Various lines of transgenic mice expressing P301L/S mutant tau have developed

neurofibrillary pathology in the central nervous system in a promoter-dependent manner. Here, we developed the a novel tauopathy mouse model, termed rTKhomo mouse, by combining a transgenic CaMKII-tTA system with a P301L mutated 1N4R human tau knock-in at the Rosa26 locus with a C57BL/6J background. This model closely mimics human tau pathology, particularly in the hippocampal CA1 region, showing age-dependent tau accumulation, neuronal loss, and neuroinflammation. Notably, whole-brain 3D staining and light-sheet microscopy revealed a spatial gradient of tau deposition from the entorhinal cortex to the hippocampus, similar to the spatial distribution of Braak neurofibrillary tangle (NFT) staging. Furthermore, [^{18}F]PM-PBB3 positron emission tomography (PET) imaging enabled the quantification and live monitoring of tau deposition. The rTKhomo mouse model will serve as a valuable tool for exploring the mechanisms of tauopathy and for developing interventions targeting the spatial progression of tau pathology.

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Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.29/C21

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

Support: NIH Grant R00AG068602
Harrison Gardner, Jr. Award
Karen Toffler Charitable Trust

Title: Aging and context-dependent influence of tau pathology on neuronal activity in ThyTau22 mice

Authors: *T. ZWANG[1], R. E. BENNETT[2], B. HYMAN[2];
[1]Massachusetts Gen. Hosp., Charlestown, MA; [2]Neurol., Massachusetts Gen. Hosp., Charlestown, MA

Abstract: ThyTau22 (MAPT G272V, MAPT P301S) is a model for tau aggregation, which is a pathological hallmark of Alzheimer’s disease. ThyTau22 express human tau and accumulate a variety of tau-related neuropathological changes with age including tau hyperphosphorylation, neurofibrillary tangles, and ghost tangles. We confirmed tau pathology with AT8 staining cross-sectional cohort at ages 3,6,9,12, and 24 months. Histology shows AT8-positive tau tangles generally appear sparingly at 3 months of age, is moderate at 6 months of age, and extensive at 9, 12, and 24 months of age.
Two flexible electrophysiology probes were implanted into a single hemisphere of ThyTau22 or WT littermate mice. One probe was targeted to the CA1 of the hippocampus, and the other to the medial entorhinal cortex, at positions which showed progressive tangle accumulation in our

cross-sectional histology. Recordings were made from 32 electrodes from each probe once per week over 6 months while mice were behaving in a goal oriented learning task along a linear track in virtual reality or while walking with no visual queues. Recordings were started at 3 months of age and continued until 9 months of age to capture the progressive accumulation of tau tangles.
We see significant differences in the aging associated trajectory of neuronal activity in ThyTau22 compared to WT littermates. The neuron firing rates in the hippocampus and entorhinal cortex are relatively stable over months for WT mice and a majority of the same neurons (>98%) are recorded every session throughout the experiment in each mouse. ThyTau22 mice, however, show distinct differences over time including a decrease in firing rate, progressive increase in silent neurons between recording sessions (starting with a range of values for individual mice of 5-10% and increasing to 15-30% by the final session). Thytau22 mice also show hyper-coherent activity compared to WT in theta and gamma, both within and across the hippocampus and entorhinal cortex, which becomes progressively decoherent between recording sessions and eventually becomes less coherent than WT. When comparing electrophysiology during the goal-oriented learning task in virtual reality to electrophysiology that was recorded while a mouse ran with no visual queues during the same session. Switching between these two contexts show a surprising effect on brain activity: during the goal-oriented learning task we observe hyperactivity in the hippocampus that appears to compensate for dysfunction of the entorhinal cortex. Altogether, these data show that the influence of tau on neuron and network activity changes over time and is context dependent.

Disclosures: T. Zwang: None. **R.E. Bennett:** None. **B. Hyman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Novartis. F. Consulting Fees (e.g., advisory boards); Dewpoint, AbbVie, Avrobio, Axon, Biogen, BMS Cell Signaling.

Poster

PSTR155: Tau: *In Vivo* Models

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR155.30/C22

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: T32 DC010775-14

Title: Time course development of deficits in auditory brainstem responses, sensorimotor and learning behaviors in mouse models of tauopathy

Authors: *A. NGUYEN[1,2], X. XU[3], K. JOHNSTON[2];
[1]Univ. of California, Irvine, Santa Ana, CA; [2]Univ. of California, Irvine, Irvine, CA; [3]Anat. and Neurobio., Univ. California, Irvine, Irvine, CA

Abstract: Cognitive impairments are a hallmark of Alzheimer's Disease and Alzheimer's Disease Related Dementias (AD/ADRD) progression in organisms, manifesting in various ways such as changes in sensorimotor skills, auditory processing, and learning and memory capabilities. The PS19 mouse model (P301S humanized tau mutation) drives a form of tau expression in mice that results in frontotemporal dementia. Combining this model with the humanized ApoE ε4 allele (KI) has been shown to result in increased tau deposition and brain atrophy, beginning at young ages (3 months). The analysis of amyloid disease models (such as APP/PS1) show auditory response alterations months before learning deficits emerge in these animals. However, the impacts of tauopathy on auditory processing is still understudied. In this project, we aim to investigate age-related cognitive impairments associated with tauopathy and the ApoE ε4 allele by examining the development of learning behavioral and auditory deficits in PS19 and ApoE4/PS19 mouse models across different age groups (4 months and 7 months old). In our experiment, we utilize auditory brainstem response (ABR), elevated plus maze (EPM), object recognition memory (ORM), object location memory (OLM) and rotarod tests to measure the extent of memory loss and sensorimotor skill deficits while comparing ABR alterations in terms of auditory processing deficiencies. Our analysis indicates that signs of cognitive impairments and decreased anxiety-behaviors are apparent by 7 months of age. Auditory deficiencies are characterized by delays in ABR latency at lower frequencies (4, 8, 12, and 16 kHz) for PS19 mice and fluctuations in ABR amplitudes at 8kHz for ApoE4/PS19 mice compared to their respective control counterparts, indicative of decreased transmission speed and response intensity of sound processing. ApoE4/PS19 mice also show greater motor coordination and balance than the ApoE4 wild type at this age, but both mouse models *do not yet* show significant impact in learning and memory capabilities. Overall, our study determines the time course of the development of auditory, sensorimotor, and memory deficiencies in the PS19 and ApoE4/PS19 mouse models, thereby contributing to our understanding of the developing impacts of tauopathy and the ApoE ε4 allele on neural circuit dysfunction.

Disclosures: A. Nguyen: None. **X. Xu:** None. **K. Johnston:** None.

Poster

PSTR156: Parkinson’s Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: /

Topic: C.03. Parkinson’s Disease

Support: NIH Grant R56 AG078453
NIH Grant DP2 AG086139
American Parkinson’s Disease Association Research Grant

Title: Single-cell whole genome analysis of somatic mutation in Parkinson's disease

Authors: *M. LODATO;
Molecular, Cell, and Cancer Biol., Univ. of Massachusetts Chan Med. Sch., Worcester, MA

Abstract: Normal 0 false false false EN-US X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin-top:0in; mso-para-margin-right:0in; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0in; line-height:107%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Arial",sans-serif; mso-font-kerning:1.0pt; mso-ligatures:standardcontextual;} DNA damage is a hallmark of human aging and certain neurodegenerative disorders. Our recent work demonstrated that this damage results in the accumulation of permanent somatic mutations in single neurons of the human brain during life and in Alzheimer's disease. Somatic mutations are dangerous because they represent permanent changes to the genetic code. Whether an increased burden of somatic mutations is observed across neurodegenerative diseases is unknown. Like Alzheimer's disease, Parkinson's disease (PD) is an age-associated neurodegenerative disorder that displays increased DNA damage, raising the possibility that neurons in human PD brain donors may also show increased somatic mutations. To test this hypothesis, we applied single-cell whole-genome sequencing (scWGS) to 82 single neurons from 13 sporadic, late-stage, postmortem PD donors and 155 neurons from 29 neurotypical controls. We find that, indeed, specific classes of somatic mutation increase in PD neurons relative to controls. The burden of certain classes of mutation correlates with the level of synuclein pathology observed globally across the PD brain, linking canonical PD pathology to somatic mutation and nominating DNA damage and repair pathways that could be intervened upon in the future to slow PD progression. Thus, degeneration of the genetic code accompanies degeneration of the brain in PD, potentially contributing to the cognitive and motor dysfunction associated with this disease.

Disclosures: M. Lodato: None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.01/C23

Topic: C.03. Parkinson's Disease

Support: NIEHS R00ES029986
NIEHS 1R01ES034846
Parkinson's Fundation Stanley Fahn Award 1064207
DOD TERP TX220241
APDA 1058111

Title: Inhalation of the Parkinson's disease risk factor trichloroethylene induces cell cycle dysregulation as a mechanism of cognitive impairment

Authors: *A. ADAMSON[1], N. ILIEVA[2], W. STONE[3], S. PAIR[2], B. R. DE MIRANDA[4]; [1]Univ. of Alabama at Birmingham, Birmingham, AL; [2]Neurol., Univ. of Alabama, Birmingham,

Birmingham, AL; [3]Neurol., Univ. of Alabama, Birmingham, AL; [4]Neurol., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder in which up to 80% of patients develop cognitive impairments. There is much heterogeneity between cognitive phenotypes in PD, yet there are no mechanisms to explain this, and genetic risk factors cannot account for this difference. Environmental contaminants are epidemiologically linked to PD risk. Trichloroethylene (TCE) is an organic solvent that contaminates much of the water and air in the US. TCE is linked to elevated PD risk (OR 1.70, 95% CI: 1.39-2.07), induces dopaminergic neurodegeneration, and has been correlated to poor cognition (OR 1.51, 95% CI: 1.24-1.84); thus it represents a toxicant that could influence PD dementia. Recently, our lab showed that 12 weeks of TCE exposure via inhalation in mice induces parkinsonian pathologies and cognitive impairment. In addition, we observed an increase in senescence alongside an increase in cell cycle protein CDK5 activation within TCE exposed animals, suggesting that cell cycle dysregulation may be a driver of TCE-induced neurodegeneration and cognitive dysfunction. Populations of neural stem cells (NSCs) in the brain that replicate throughout adulthood reveal a influence of the cell cycle on a neuronal population, and loss of the adult-born neurons from NSCs leads to spatial memory impairments. Also, the overactivation of CDK5 has been shown to phosphorylate Tau at PD relevant residues. Therefore, we hypothesized that TCE exposure induces cell cycle dysregulation as a mechanism that induces senescence in NSCs alongside activating CDK5 thereby influencing a PD phenotype with cognitive impairment. To investigate this relationship, we exposed 12-month-old male and female C57BL/6 mice to 100 ppm TCE inhalation or control over 12 weeks in a whole-body passive exposure inhalation chamber. Mice exposed to TCE showed loss of pyramidal neurons in the CA3 of the hippocampus ($p=0.0002$). Concurrently, we observed an increase in the senescent protein p16 in neurons of the subgranular zone of the dentate gyrus ($p=0.0043$). In line with this, we saw a reduction in the number of immature neurons in this region ($p=0.0282$). Additionally, we observed an increase in the activation of CDK5 within pyramidal neurons of the hippocampus ($p = 0.0208$) alongside increased phosphorylated Tau. We then interrogated CDK5 inhibition as a therapeutic target for preventing pTau accumulation in vitro and found that treating cells exposed to TCE with a CDK5 inhibitor reduced accumulation of pTau ($p = 0.0250$). Together, these data highlight that environmental toxicant-induced senescence and over-activated CDK5 could be fundamental new mechanisms that drive cognitive impairment in PD.

Disclosures: A. Adamson: None. **N. Ilieva:** None. **W. Stone:** None. **S. Pair:** None. **B.R. De Miranda:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.02/C24

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS P50 NS123103
ASAP-020572

Title: Comparative morphological analysis of layer V pyramidal tract (PT) and intratelencephalic (IT) neurons in the supplementary motor area (SMA) of control and parkinsonian monkeys

Authors: *R. M. VILLALBA[1], K. E. JAMES[1], M. DATTATREYA[1], Y. SMITH[1,2];
[1]Emory Natl. Primate Res. Center, Emory Univ., Atlanta, GA; [2]Sch. of Medicine, Dept. Neurology. Emory Univ., Atlanta, GA

Abstract: In Parkinson's disease (PD), dysfunction of the basal ganglia-thalamo-cortical loops induced by striatal dopamine (DA) disrupts processing in the primary motor cortex (M1) and supplementary motor area (SMA). Given their position within the motor system, abnormalities in these motor areas are likely to mediate many of the parkinsonian motor signs, but the pathophysiology of motor cortices in parkinsonism remains poorly understood. Given electrophysiological evidence that pyramidal tract (PT), but not corticostriatal, neurons in M1 undergo changes in firing activity in MPTP-treated parkinsonian monkeys, the main goal of this study is to compare the morphology of SMA corticospinal PT neurons and corticostriatal intratelencephalic (IT) neurons between control and parkinsonian monkeys. Following unilateral injections of AAV-retro in the cervical/thoracic spinal cord or sensorimotor putamen, the SMA in both hemispheres was enriched in Golgi-like retrogradely labeled neurons allowing for detailed morphometric analysis of contralateral PT and IT neurons using Neurolucida and NeurolucidaExplorer (MBF Bioscience, VT-USA). Our preliminary morphological analysis in control animals indicates that layer V cortico-spinal neurons in the SMA have larger cell bodies, but shorter and less complex (Sholl analysis, number of intersections) basilar dendrites than IT neurons. The apical dendrites of PT and IT neurons have shown similar dendritic spine density. Preliminary data obtained so far suggest that PT neurons in parkinsonian monkeys have a smaller cell body, a less complex basal dendritic arbor and a reduced spine density on their apical dendrites compared with controls. Ongoing studies of IT corticostriatal neurons in MPTP-treated monkeys are in progress. A deeper understanding of the circuit pathophysiology of specific populations of cortical neurons in parkinsonism will help with the design of cell type-specific therapeutic approaches to modulate the activity of subsets of cortical pyramidal neurons in PD.

Disclosures: R.M. Villalba: None. **K.E. James:** None. **Y. Smith:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.03/C25

Topic: C.03. Parkinson's Disease

Support: NINDS T32NS131190

Title: Methods to image the dopamine transporter (DAT) in vitro and its applications in Parkinson's disease research

Authors: *L. E. HARMON[1], E. KHEZERLOU[2], J. SAENZ[2], C. BROWN[1], R. W. GUNASEKARA[3], P.-Y. PAN[2], S. S. CHANDRA[1];
[1]Neurosci. and Neurol., Yale Univ., New Haven, CT; [2]Neurosci. and Cell Biol., Rutgers Univ. Robert Wood Johnson Med. Sch., Piscataway, NJ; [3]Neurol., Yale Univ., New Haven, CT

Abstract: Parkinson's Disease (PD) is characterized by the progressive loss of dopaminergic (DA) neurons within the substantia nigra (SN), resulting in debilitating motor impairments. The mechanisms of DA vulnerability in PD are incompletely understood, but likely involve dysregulation of dopamine neurotransmission mediated in part by the dopamine transporter (DAT). DAT surface levels are controlled by the endolysosomal system, which includes several proteins mutated in familial and sporadic PD. For example, loss-of-function mutations in the clathrin uncoating chaperone, auxilin, cause early-onset PD. Recently, our lab observed DAT accumulation at axonal terminals and delayed dopamine reuptake in auxilin knockout (KO) mice prior to DA degeneration and motor symptoms (Vidyadhara et al., 2023). To test the hypothesis that DAT recycling and trafficking defects occur in presymptomatic auxilin KO mice, live imaging of DAT *in vitro* is necessary to track DAT endo- and exocytosis with high temporal resolution. Therefore, in conjunction with the Pan Lab, we have developed a mouse DAT-pHluorin probe (mDAT-phluorin), in which a superecliptic pHluorin is inserted into the extracellular domain of mouse DAT. The mDAT-pHluorin has been validated in N2A and primary DA neurons and demonstrates proper pH sensitivity, expression, trafficking, and ligand binding. Currently, we are comparing primary DA neurons expressing mDAT-pHluorin from the SN of auxilin KO and wildtype mice to assess differences in DAT surface fraction, vesicular pH, and magnitude and rates of endo- and exocytosis following neuronal stimulation. One limitation of pHluorins is their loss of fluorescence once internalized. Therefore, we are also employing PRIME (*probe incorporation mediated by enzymes)* imaging, in which a ligand acceptor peptide is fused to DAT (LAP-DAT), allowing fluorescent labelling of surface DAT in live cells. We have successfully synthesized all components for PRIME and shown that LAP-DAT colocalizes with DAT antibodies in transfected HEK293T cells, validating the specificity of labeling. Using stimulated emission depletion microscopy paired with immunofluorescence staining, we will assess the colocalization of DAT with various endolysosomal markers to understand the role of auxilin in controlling post-endocytic fate of DAT. These DAT imaging techniques can then be used in patient iPSC-derived dopaminergic neurons and neurons from other PD mouse models, allowing us to understand whether DAT recycling and trafficking defects are present in multiple forms of Parkinson's Disease.

Disclosures: L.E. Harmon: None. **E. Khezerlou:** None. **J. Saenz:** None. **C. Brown:** None. **R.W. Gunasekara:** None. **P. Pan:** None. **S.S. Chandra:** None.

Poster

PSTR156: Parkinson’s Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.04/C26

Topic: C.03. Parkinson's Disease

Title: Altered synaptic plasticity in the motor cortex of parkinsonian rats

Authors: *T. KO[1], S. YANG[2,3,4], H. SHIN[5], C. YOU[5];
[1]Incheon Natl. Univ., Incheon, Korea, Republic of; [2]Nano-bioengineering, Incheon Natl. Univ., Incheon, Korea, Republic of; [3]Incheon National University, Center for Brain-Machine Interface, Incheon, 22012, Korea, Republic of; [4]gBrain Inc., Incheon, 21984, Korea, Republic of; [5]Bioengineering and Nano-Bioengineering, Incheon Natl. Univ., Incheon, Korea, Republic of

Abstract: Altered synaptic plasticity in the motor cortex of parkinsonian rats
Taewoo Ko[1*], Hongseong Shin[1, 2*], Chaeyeon You[1], Eunyoung Lee[1], Sunggu Yang[1, 2, 3]
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[3]gBrain Inc., Incheon 21984, Republic of Korea.
Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by the degeneration of dopaminergic neurons and significant disruptions in motor function. This study assessed mGluR-dependent synaptic plasticity on motor cortex of PD rats. To establish a PD model in rats 6-hydroxydopamine (6-OHDA) was utilized. Primary motor cortical slices (400 μm) in PD rats were prepared with a vibratome in chilled iced buffer to preserve tissue integrity. Synaptic plasticity in superficial layer of motor cortex was assessed through extracellular field recordings. Results revealed a significant enhancement of in mGluR-dependent synaptic plasticity in the PD group compared to the control group under high-frequency stimulation (HFS; 130 Hz, 1 sec). Conversely, low-frequency stimulation (LFS; 1 Hz, 15 min) resulted in a substantial reduction in mGluR-dependent synaptic response in the PD group. These findings elucidate the altered cellular properties of the motor cortex in PD.Keywords: Parkinson's disease, Synaptic plasticity, Extracellular field recording, Long-term potentiation, Long-term depression, Metabotropic glutamate receptor

Disclosures: T. Ko: None. **S. Yang:** None. **H. Shin:** None. **C. You:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.05/C27

Topic: C.03. Parkinson's Disease

Support: NIH Grant 1RF1AG083753-01

Title: Low-density lipoprotein receptor-related protein 1 facilitates alpha-synuclein uptake in glia

Authors: *Y. LI, Y. CHEN, J. BALS, K. D. COTTER, J. HAINES, Y. SHIN, A. A. DAVIS; Dept. of Neurol., Washington Univ. Sch. of Med., St. Louis, MO

Abstract: α-Synuclein (αSyn) is a major constituent of Lewy bodies (LBs) and Lewy neurites, and its aggregation plays a significant pathogenic role in Parkinson disease (PD). Blocking the transmission of pathological αSyn within neuronal networks represents a promising therapeutic strategy to slow down or reverse the disease progression cascade. Although the exact mechanism underlying the transmission of pathological αSyn remains to be elucidated, mounting evidence suggests that multiple cell surface molecules facilitate αSyn uptake in a range of brain cells including neurons and glia. Neuronal LRP1 was recently shown to mediate uptake of αSyn in iPSC-derived neurons and facilitate spreading of αSyn in mice. Because LRP1 expression is actually enriched in astrocytes and microglia compared to neurons, we hypothesized that LRP1 facilitates αSyn uptake in glia and that this may regulate αSyn clearance. We used genetic and pharmacologic approaches to investigate the role of LRP1 in αSyn uptake using flow cytometry. We found that αSyn monomer uptake was reduced by approximately 80% (mean 77.68 SEM 0.702) in LRP1 KO mouse embryonic fibroblasts (MEFs) compared to WT MEFs, whereas uptake of αSyn pre-formed fibrils (PFFs) was modestly reduced by approximately 5% (mean 5.340 SEM 2.304). We found that receptor-associated protein (RAP), an LRP1 antagonist, strongly reduced αSyn monomer uptake in a dose-dependent manner in both astrocytes and microglia cultured from C57BL/6 mice. LRP1 deficient MEFs exhibited reduced association with fluorescent αSyn PFFs compared to WT following incubation at 4C, suggesting direct surface binding between LRP1 and αSyn. Collectively, our results support the hypothesis that LRP1 regulates conformation-specific αSyn uptake in glia.

Disclosures: Y. Li: None. **Y. Chen:** None. **J. Bals:** None. **K.D. Cotter:** None. **J. Haines:** None. **Y. Shin:** None. **A.A. Davis:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.06/C28

Topic: C.03. Parkinson's Disease

Support: APVV-20-0331
SASPRO 2_1085/01/02
ICGEBCRP/SVK22-04_EC
APVV-20-0447
APP0398

Title: Extracellular vesicles as mediators of Parkinson´s disease: from cell model to patient

Authors: *D. MJARTINOVÁ[1], M. MOMAND[2], L. FIALOVÁ[2], D. FRICOVA[1];
[1]The unit for translational Res. of neurodegenerative Dis., 2nd Neurol. Dept., Fac. of Med., Comenius Univ., Bratislava, Slovakia; [2]Inst. of Neuroimmunology, Slovak Acad. of Sci., Bratislava, Slovakia

Abstract: Extracellular vesicles (EVs) are membrane-enveloped nanoparticles released by various cell types. They are promised to have a role in Parkinson's disease progression because of their capability of spreading several cargoes, including misfolded proteins (particularly alpha-synuclein) or pathological signals (encoded in miRNA). For this reason, they are promising biomarkers of the disease. Since the large heterogeneity of patients (including differences in age, sex, disease stage, treatment strategy, comorbidities, etc.) has been a limitation in recent studies, we have developed an innovative "from cell model to patient" approach utilizing novel cellular models. We have established neuroblastoma and the human neural progenitor cell lines that stably over-express wild-type or mutant (A53T) GFP-tagged alpha-synuclein at two different levels - low and high. Our current research focuses on characterizing the morphology and protein content of EVs released from these cells with a particular emphasis on alpha-synuclein (Nanoparticle Tracking Analysis, immunoblot). Furthermore, we study the biological effect of EVs through co-cultivation experiments with cells. To identify disease-specific patterns, we analyze the miRNA content of EVs (Next Generation Sequencing) followed by comprehensive bioinformatic analysis to capture upregulated and downregulated miRNAs. This approach helps to guide further clustering (unsupervised machine learning) of miRNAs enveloped in EVs from patients suffering from Parkinson's disease. Through this multidisciplinary approach, we aim to elucidate the role of EVs in Parkinson's disease progression and approximate the discovery of novel biomarkers and therapeutic targets.

Disclosures: D. Mjartinová: None. **M. Momand:** None. **L. Fialová:** None. **D. Fricova:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.07/C29

Topic: C.03. Parkinson's Disease

Support: Hope Center Pilot Grant

Title: The Role of Palmitoyl-protein thioesterase 1 Haploinsufficiency in Parkinson Disease

Authors: *J. PORTILLO[1], J. HAINES[3], J. BALS[2], A. A. DAVIS[4], B. A. BENITEZ[5];
[1]Neurol., Washington Univ. in St. Louis, SAINT LOUIS, MO; [2]Washington Univ. in St. Louis, Saint Louis, MO; [3]Neurol., Washington Univ. St. Louis, Saint Louis, MO; [4]Sch. of Med., Saint Louis, MO; [5]Neurol., Harvard Med. Sch. - BIDMC, Boston, MA

Abstract: Parkinson disease (PD) is characterized by aggregation of misfolded alpha-synuclein (α-syn) protein in Lewy bodies and dopaminergic neuron loss. Growing evidence indicates that

misfolded α-syn may propagate between cells in a prion-like manner. The autophagy / lysosome pathway is an essential cellular system that degrades cellular material, such as α-syn, that would otherwise collect and interfere with normal function. Impairment in lysosomal function is thought to contribute to the pathological accumulation and spreading of α-syn aggregates in PD. Genome-wide association studies, whole exome sequencing, and family history studies have associated variants in multiple lysosomal genes with PD risk. One such gene, *CLN1,* which encodes palmitoyl-protein thioesterase 1 (PPT1), is associated with a form of Batten disease in children who inherit two pathogenic variants. Heterozygous carriers of *CLN1* gene variants do not develop the characteristic lysosomal storage disorder, but are at increased risk of developing PD later in life. Despite this association, the molecular and cell biological mechanism of PPT1 contribution to PD risk is unknown. We found increased spreading of α-syn brain pathology following injection of α-syn preformed fibrils in $Ppt1^{+/-}$ mice compared to $Ppt1^{+/+}$ mice. We also found reduced endolysosomal accumulation of α-syn fibrils in primary microglia cultured from $Ppt1^{+/-}$ mice compared to $Ppt1^{+/+}$ mice. In contrast, there was no difference in the accumulation of α-syn fibrils in primary astrocytes according to *Ppt1* genotype, suggesting a cell-type specific contribution of response to aggregated α-syn fibrils**.** To further investigate this, we measured endogenous α-syn levels in brain lysates from two-month-old $Ppt1^{+/+}$, $Ppt1^{+/-}$, and $Ppt1^{-/-}$ mice using an enzyme-linked immunosorbent assay, and interrogated the overall degradation capacity of lysosomes across $Ppt1^{+/+}$, $Ppt1^{+/-}$, and $Ppt1^{-/-}$ mice using a Dye Quenched-Bovine Serum Albumin assay. Our data suggest that *Ppt1* haploinsufficiency compromises lysosomal function in neurons and/or microglia, impairing degradation of misfolded α-syn aggregates and exacerbating spreading of α-syn brain pathology.

Disclosures: J. Portillo: None. **J. Haines:** None. **J. Bals:** None. **A.A. Davis:** None. **B.A. Benitez:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.08/C30

Topic: C.03. Parkinson's Disease

Support: NIH ENDURE Grant R25NS114309

Title: Astrocytes phenotypes are regulated by Maresin-1 under stress conditions

Authors: *T. L. CHANEY;
Neurosci., LSUHSC-NO, New Orleans, LA

Abstract: "Astrocytes phenotypes are regulated by Maresin-1 under stress conditions."
TONYA CHANEY[1], E. Tendayi Mpofu[1] and Jorgelina M. Calandria[1] [1]LSUHSC-NO Neuroscience Center of ExcellenceParkinson's disease (PD) is a neurodegenerative disorder that affects the dopaminergic neurons in the Substantia Nigra (SN). Astrocytes, the largest and most

abundant type of supporting cells in the central nervous system, participate in the detection and communication of stress signals from neurons to microglial cells. For this purpose, astrocytes change their phenotype, in some cases transforming themselves into reactive types with the subsequent release of cytokines and chemokines with the activation of Nuclear Factor Kappa B (NF-kB) transcription factor. These phenotypes direct the defensive neuroprotective efforts to modulate neuroinflammation. We hypothesize that Maresin1(Mar1) induces the anti-inflammatory conversion of astrocytes that in turn send signals to neurons and microglia to exert toxicity or survival on dopaminergic neurons. This hypothesis was tested in vitro using a primary culture of primary astrocytes and cytokines: tumor necrosis factor alpha (TNFα) and interferon (INF) type I or alpha-synuclein (α-syn) preformed fibrils (PFF) in the presence or absence of Mar1. Mar1 decreased the nuclear translocation of p65, a pro-inflammatory member of NF-kB. In addition, we measured the expression of cytokines like upon the treatments and found that PFF increased the production of three key cytokines and chemokines: TNFα, Interleukin 1 beta (IL1β) and CXCL1 and Mar1 brought them back to control levels. These preliminary results suggest that Mar1 may induce the acquired deactivation of astrocytes, previously called A2 state that promotes the survival of neurons. Mar1 also increased the activation of beta-catenin (β-cat) by inducing its translocation into the nucleus. We concluded that astrocytic activation and acquired deactivation via Mar1 took place probably related to their undergoing morphology change. The knowledge obtained about the pathways modulated by Mar1 open novel avenues for the treatment of PD based on the alteration of intrinsic mechanisms of neuroinflammation that underlie the pathology.

Disclosures: T.L. Chaney: None.

Poster

PSTR156: Parkinson’s Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.09/C31

Topic: C.03. Parkinson’s Disease

Title: Nicotine modulates lipid metabolism and reduces α-Synuclein aggregation in Parkinson's disease cell models

Authors: *X. LI[1,2], J. YANG[3,4], H. WANG[3,4], H. CHEN[3,4], H. HOU[1,2], Q. HU[1,2];
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Abstract: Parkinson’s disease (PD) is a chronic, neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the brain and the pathological aggregation of α-synuclein (α-SYN) protein within surviving neurons. The precise mechanisms of the PD remain unmapped, with lipid metabolism dysfunction emerging as a significant area of interest. This study aimed to explore the potential neuroprotective role of nicotine in PD-related lipid

dysregulation and to clarify the mechanisms involved. We developed two cellular models of PD: one by exposing SH-SY5Y cells to 6-hydroxydopamine (6-OHDA) to induce neurotoxicity, and another by stably overexpressing the α-SYN mutant (A53T-GFP) in SH-SY5Y cells to mimic pathogenic protein aggregation. The neuroprotective effects of nicotine on SH-SY5Y cells were evaluated through cellular viability, apoptosis, lipid droplet formation, lipid peroxidation, brain-derived neurotrophic factor (BDNF) levels and the formation of α-SYN-GFP puncta. To evaluate the contribution of α7 neuronal nicotinic acetylcholine receptor (α7-nAChR) to nicotine's neuroprotective effects, methyllycaconitine citrate (MLA), a selective antagonist for the receptor was used. Our findings demonstrate that nicotine significantly mitigates 6-OHDA-induced neurotoxicity in SH-SY5Y cells, enhancing cell viability, reducing apoptosis, and limiting the formation of lipid droplets and peroxidation. Moreover, nicotine was found to restore BDNF levels and inhibit the formation of α-SYN aggregates, as evidenced by a reduction in the number and size of A53T-GFP puncta when nicotine was introduced. Importantly, these protective effects were negated by the presence of MLA, indicating that they are mediated through α7-nAChRs. These insights not only shed light on the molecular underpinnings of nicotine's positive impact on PD progression but also highlight the potential of targeting α7-nAChRs as a therapeutic strategy.

Disclosures: X. Li: None. **J. Yang:** None. **H. Wang:** None. **H. Chen:** None. **H. Hou:** None. **Q. Hu:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.10/C32

Topic: C.03. Parkinson's Disease

Support: ASAP-000301

Title: Bridging Integrator Protein (BIN3) Regulates Vesicular Trafficking in Dopamine Neurons

Authors: *S. MATHIVANAN[1], E. ABELLA[2], Y. TAO[3], L. KONG[4], M. ROUHIARDESHIRI[4], S.-C. ZHANG[5,6];
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Abstract: Expression Quantitative Trait Locus (eQTL) analysis of hundreds of thousands of single cells from human brains revealed altered expression of BIN3 (Bridging Integrator Protein 3) in Parkinson's disease (PD) patient brains. This raises a possibility of BIN3 involvement in PD pathogenesis. To gain insights into the role of BIN3 in dopamine (DA) neurons, we generated BIN3 Knockout (KO) and Overexpression (OE) human stem cell lines by

CRISPR/Cas9. DA neurons derived from BIN3KO stem cells showed a reduction in endocytosis as indicated by a reduced uptake of fluorescent dye (FM1-43), especially when the neurons were stimulated. In contrast, BIN3OE DA neurons exhibited an elevated endocytosis, more obvious when the neurons were activated. These results suggest the role of BIN3 in membrane trafficking. Intracellularly, both BIN3KO and BIN3OE cells displayed an increased accumulation of autophagic vacuoles and enlarged lysosomes in the cell body but fewer in the neurites, suggesting the requirement of tight regulation of BIN3 in controlling vesicular trafficking. Functionally, BIN3KO DA neurons exhibited a decreased firing upon stimulation. Molecular analysis revealed that LC3B protein levels were decreased in BIN3KO DA neurons and increased in BIN3OE DA neurons. These results suggest that BIN3 regulates vesicular trafficking and hence neuronal function, possibly through modulating the autophagic system.

Disclosures: S. Mathivanan: None. **E. Abella:** None. **Y. Tao:** None. **L. Kong:** None. **M. Rouhiardeshiri:** None. **S. Zhang:** Other; BrainXell.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.11/C33

Topic: C.03. Parkinson's Disease

Support: Stichting Parkinson Fonds NL1901

Title: Bh3-only protein noxa is implicated in dopaminergic neuronal loss in the substantia nigra of parkinson's disease patients

Authors: *A. A. DIJKSTRA[1,2], M. ZUIJDWEG[3], L. P. VAN DER HEIDE[3];
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[2]Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands;
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Abstract: Parkinson's Disease (PD) is a common neurodegenerative disease pathologically hallmarked by the loss of dopaminergic neurons in the substantia nigra (SN) and α-synuclein (αsyn)-containing aggregates. Mitochondria-dependent apoptosis regulated by the Bcl2 protein family has been implicated in regulation of dopaminergic neuronal death in PD. BH3-only pro-apoptotic protein Noxa is a particular protein of interest as a result of previous findings from cellular models, however, the expression of the protein in human tissue has not been explored yet.
Here, we performed immunofluorescence on a cohort of human post-mortem tissue of non-neurological control donors and donors with PD. We examined the expression of Noxa in TH-positive dopaminergic neurons and assessed the local αsyn pathology in the SN and the ventral tegmental area (VTA).
Preliminary results show a decreased dopaminergic neuronal density in PD compared to controls

in the SN, but not in the VTA. Surprisingly, an increased expression of Noxa was found in the dopaminergic neurons and in the SN of PD patients compared to controls, but not in the VTA. Moreover, we did not find a correlation between αsyn pathology and Noxa-expression in the SN or VTA.
Overall, our findings implicate a role for mitochondria-dependent apoptosis in the SN of PD patients but not in the VTA. In addition, no relation between Noxa expression and αsyn could be established. Further research is needed to better understand the involvement and regulation of Noxa in dopaminergic cell death, however, this study is a first promising step in understanding the role of mitochondria-dependent apoptosis in PD pathology.

Disclosures: A.A. Dijkstra: None. **M. Zuijdweg:** None. **L.P. Van Der Heide:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.12/C34

Topic: C.03. Parkinson's Disease

Support: Stichting ParkinsonFonds

Title: Investigating the role of Mcl1 and CRMP4 in apoptosis in dopamine neurons

Authors: *D. HAMBERG, M. P. SMIDT, L. P. VAN DER HEIDE;
Swammerdam Inst. for Life Sci., Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disease marked by the selective degeneration of dopaminergic neurons in the substantia nigra, for which there is currently only symptomatic treatment. Finding ways to prevent progression or even cure PD will require a better understanding of what underlies the vulnerability of these dopaminergic neurons. Previous research has indicated a role of intrinsic apoptosis in PD, which is regulated by the Bcl2 family of proteins. Our lab previously established that Mcl1, an anti-apoptotic Bcl2 protein, is a crucial survival factor in dopaminergic neurons. Further mapping of the regulatory network of Mcl1 could provide information on the necessary factors to survival of dopaminergic neurons. To identify promising novel interactions, we performed co-immunoprecipitation experiments on Mcl1 in MN9D mouse dopaminergic cells followed by mass spectrometry. One of the identified proteins is CRMP4, a phosphoprotein involved in microtubule dynamics. CRMP4 is highly expressed during brain development and regulated during axon de- and regeneration. Interestingly, *Crmp4* shows high expression in the midbrain at E14.5 in mice, and this enrichment overlaps with the expression of *Pitx3*, a transcription factor expressed specifically in dopaminergic midbrain neurons, indicating CRMP4 has a function in dopaminergic neurons during development. Potentially, CRMP4 could also be involved in degenerative responses in these dopamine neurons, making the interaction of CRMP4 and Mcl1 an interesting target to study. In MN9D cells, we show that siRNA-mediated knockdown of CRMP4 increases cleaved

caspase-3 levels, and we are investigating if and how this corresponds to intrinsic apoptosis, or whether this occurs via a different mechanism. We have used proximity ligation assay (PLA) to show that Noxa, an anti-apoptotic Bcl2 protein known to function by inhibiting Mcl1, can bind to Mcl1 when transfected in MN9D cells, and that expression of Noxa leads to activation of Bax, suggesting Mcl1 functions by preventing activation of Bax. Currently we are assessing the consequences of knocking down CRMP4 on the interaction between Noxa and Mcl1, as well as the consequences on Bax activation. Together, these results contribute to our understanding of the role of Mcl1 in dopamine neurons, and further studies on the regulatory network of Mcl1 and CRMP4 could provide interesting possibilities in finding ways to halt PD.

Disclosures: D. Hamberg: None. **M.P. Smidt:** None. **L.P. Van Der Heide:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.13/C35

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS R01NS124037

Title: Elucidating the role of Cd38, a Parkinson's risk gene, in astrocytes

Authors: *S. BASAK[1], R. D. HERNANDEZ[2], X. WEI[3], A. COLAFRANCESCO[4], A. M. PICKRELL[5], R. M. COWELL[6], M. L. OLSEN[3];
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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease characterized by motor and cognitive impairments. Previous PD preclinical studies have focused on monogenic causative mutations, although the majority of PD cases are sporadic and are thought to result from genetic and environmental factors. Genome-wide association studies have identified many PD risk variants, including a SNP (single nucleotide polymorphism) variant of *CD38*, a transmembrane glycoprotein that acts as both a receptor and enzyme with NADase and cADPR activity. Our previous work has revealed that *Cd38*-deficient midbrain astrocytes (*$Cd38^{+/-}$ and $Cd38^{-/-}$)* show profound changes in gene expression, including, genes associated with PD, astrocyte reactivity, reactive oxygen species (ROS) production, and cellular metabolism. Further, genetic ontology analysis in these astrocytes revealed metabolic and bioenergetic pathways were over-represented in *Cd38* deficient mice. Functional mitochondrial stress assays indicated increased proton leak, non-mitochondrial oxygen usage, and glycolytic capacity in *$Cd38^{-/-}$* astrocytes with no changes in ATP linked or maximal respiration, perhaps indicating damage to OXPHOS subunits, increased uncoupling protein activity, or oxidative

stress. To understand these findings, here, we aimed to explore how the bioenergetic changes observed in *Cd38*-deficient astrocytes impact the health of dopaminergic neurons in the substantia nigra pars compacta (SNpC). To measure oxidative stress in the SNpC, we administered dihydroethidium into 3-month and 1-year-old mice. The resulting oxidized hydroethidine (Oxhet) levels served as an indicator of superoxide anion formation, particularly within astrocytes and dopaminergic neurons. Further investigation into oxidative stress and its effects on the morphology and function of astrocytes will enhance our understanding of the role of *Cd38* during aging and its impact on the health of dopaminergic neurons, which are highly vulnerable in Parkinson's disease.

Disclosures: S. basak: None. **R.D. Hernandez:** None. **X. Wei:** None. **A. Colafrancesco:** None. **A.M. Pickrell:** None. **R.M. Cowell:** None. **M.L. Olsen:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.14/C36

Topic: C.03. Parkinson's Disease

Title: Identifying regulators of the dopaminergic survival factor Mcl1

Authors: *R. VAN DE VIS, M. SMIDT, L. P. VAN DER HEIDE;
SILS - Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: The major hallmark of Parkinson's disease (PD) is the progressive loss of dopaminergic neurons in the substantia nigra. Loss of these neurons lead to its characteristic motor symptoms. Of the underlying mechanisms driving neurodegeneration, the intrinsic apoptosis pathway has received much attention. The anti-apoptotic Bcl-2 member myeloid cell leukaemia 1 (Mcl1) has recently been identified as a critical factor in mouse dopaminergic cell survival. Modulating Mcl1 expression to stabilize or increase protein levels may pose a promising therapeutical approach. Therefore, understanding the mechanism underlying Mcl1 regulation is crucial. Proteomics analysis of immunoprecipitated Mcl1 in the mouse dopaminergic MN9D cell line revealed the transcriptional regulator Interferon Regulatory Factor 2 Binding Protein Like (Irf2bpl) as an Mcl1 interactor. Duolink® Proximity Ligation Assay confirmed the complex formation between Mcl1 and Irf2bpl in vitro in MN9D cells. Irf2bpl contains two important domains referring to protein function: a DNA binding domain and a RING finger domain, often involved in ubiquitination signalling. Interestingly, mutations in the human *IRF2BPL* gene have recently been linked to the neurological disorder NEDAMSS with severe neurodevelopmental impact, including Parkinson-like symptoms. The recent characterization of additional E3 ubiquitin ligase function, combined with the neurological significance, make Irf2bpl an intriguing target to study its role in Mcl1 regulation as well as the extensive function in dopaminergic cells. Irf2bpl protein overexpression or short hairpin (shRNA)-mediated knockdown in MN9D cells did not alter Mcl1 expression, suggesting no E3

ligase action under these conditions. Next, whole proteome analysis with tandem mass spectrometry of both stable Irf2bpl overexpression or shRNA knockdown MN9D cell lines was performed to explore the general function of Irf2bpl. GO analysis revelaved major alterations in the biological processes involving protein translation, RNA processing and lipid metabolism. Intriguingly, we also discovered novel functions for Irf2bpl in modulating components of the dopamine synthesis pathway. The link between Irf2bpl and dopaminergic identity is novel and may potentially link the pro-survival protein Mcl1 to maintaining this phenotype, as Mcl1 and and Irf2bpl physically interact. Especially given this complex formation, this could suggest an intrinsic connection between neuronal identity and survival.

Disclosures: R. van de Vis: None. **M. Smidt:** None. **L.P. Van Der Heide:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.15/C37

Topic: C.03. Parkinson's Disease

Title: Design, Optimization, and Comparative Analysis of Mid-Throughput Microplate-Based Lysosomal Assays for Compound Profiling and Prioritization

Authors: *K. GUPTA, M. TOH, M. POLATNICK, E. AMORELLI, E. BRITTON, G. GOKULRANGAN, S. AIVAZIDIS, S. S. PIN;
Cerevel Therapeut., Cambridge, MA

Abstract: Lysosomes are cellular organelles that play a pertinent role in maintaining cellular homeostasis, regulating storage, recycling, and degradation of various biomolecules delivered by autophagy and endocytosis. When the lysosome is unable to function properly, there is an abnormal accumulation of substrate macromolecules that leads to lysosomal impairment and promotes dysregulation of signaling pathways. Lysosomal storage diseases, such as Gaucher's disease (GD), are rare disorders caused by defects in genes of enzymes which are involved in lysosomal function. Recent genetic studies have also linked lysosomal dysfunction to various disorders such as Gaucher's Disease disease (GD) to age-dependent neurodegenerative diseases such as Parkinson's Disease disease (PD). In the past, researchers have developed methods to reduce the biomolecular complexity of whole-cell samples by isolating and purifying individual cellular organelles through techniques such as density gradient centrifugation. These methods are aimed at improving assay sensitivity by enhancing the signal-to-noise ratio. However, these traditional approaches for quantifying lysosomal endpoints are typically labor-intensive and time-consuming, limiting their utility in drug discovery due to their low throughput nature. To this end, we aimed to design and optimize various lysosomal assays amenable for lead optimization and preclinical candidate selection of small molecule lysosomal modulators. By using a combination of density gradient ultracentrifugation, Western Blot, LCMS analysis, fluorometric glucocerebrosidase (GluCer), and cathepsin B assays, we aim to characterize and

measure lysosomal function in a more efficient manner. We observed a noticeable increase in the lysosomal markers LAMP2 and glucosylsphingosine levels, as well as a decrease in the non-lysosomal markers like S6K1 and GAPDH in our isolated and purified lysosomal samples derived from whole-cell HeLa Parental cells. This method demonstrated noticeable enrichment and purification of isolated lysosomal samples through density gradient ultracentrifugation with promising preliminary data validating its application for downstream functional assays. While further investigation is needed to fully understand the implication of our findings, our optimized protocol can be crucial for exploring the role of lysosomes in cellular processes and potential therapeutic interventions for lysosomal-related disorders.

Disclosures: K. Gupta: A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **M. Toh:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **M. Polatnick:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **E. Amorelli:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **E. Britton:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **G. Gokulrangan:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **S. Aivazidis:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **S.S. Pin:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics.

Poster

PSTR156: Parkinson’s Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.16/C38

Topic: C.03. Parkinson’s Disease

Support: NIH Grant 5F31NS129265-02
NIH Grant 1F31NS129265-01A1

Title: Dissecting GWAS identified risk variants in Parkinson's diseases-functional role of GPNMB in the pathogenesis of PD

Authors: *R. LO BU, S. POSER, F. SOLDNER;
Albert Einstein Col. of Med., Bronx, NY

Abstract: ABSTRACT Parkinson’s Disease (PD) is the second most common chronic progressive neurodegenerative disease. Epidemiology and population genetics suggest that sporadic PD (>95% of cases) results from a complex interaction between genetic risk, aging, and environmental factors. A detailed understanding of the genetic risk is the first step to elucidating this complex interaction. Genome wide association studies (GWAS) have identified numerous risk variants (e.g., single nucleotide polymorphisms [SNPs]) present in 78 genomic regions associated with an increased risk of developing PD. However, there is little insight regarding which and how these SNPs mechanistically contribute to the development and progression of PD. Since most of the functional SNPs are highly enriched in non-coding regulatory DNA

elements such as distal enhancers, the prevailing theory is that cis-acting effects of the functional non-coding SNPs on gene expression play a significant role in the development of complex diseases. To compile a list of probable causal SNPs in brain enhancers, we integrated GWAS-identified PD-risk variants with epigenomic data identifying brain-specific enhancers and gene expression datasets in primary brain tissue. This analysis revealed multiple candidate PD-risk variants in a microglia-specific enhancer element in the glycoprotein nonmetastatic melanoma protein B (GPNMB) locus. GPNMB is a type 1 transmembrane protein known to be upregulated in the substantia nigra of PD patients. Very little is known regarding its molecular function and how the dysregulation of GPNMB contributes to PD. To address this knowledge gap, this project aims to: (1) to identify the causal risk variant present in this upstream enhancer of GPNMB and characterize the molecular mechanisms by which the causal risk variant dysregulates GPNMB expression in microglia using CRISPR/Cas9-risk variant edited hPSCs, (2) characterize the functional effects of gain and loss of GPNMB on microglial inflammation associated with microglial activation and neurodegeneration associated-inducers. We have identified two candidate risk variants which slightly but significantly alter allele specific expression of GPNMB. In addition, our analysis of changes in the cellular transcriptome after GPNMB gene deletion in hPSC-derived microglia identified alterations in expression levels of multiple key genes associated with CNS inflammation (i.e. NLRP2, NLRP12, NF-kB). Together this work provides invaluable insight into the novel link between non-coding PD risk variants, GPNMB, and inflammation.

Disclosures: R. Lo Bu: None. **S. Poser:** None. **F. Soldner:** None.

Poster

PSTR156: Parkinson’s Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.17/C39

Topic: C.03. Parkinson’s Disease

Title: Mutant ATP13A2 Causes Neuronal Loss and ER Stress in SH-SY5Y Cell Model of Parkinson's Disease

Authors: *T. CHIU, C.-C. CHIU;
Chang Gung Univ., Taoyuan, Taiwan

Abstract: Parkinson’s disease (PD) is the most common neurodegenerative movement disorder. The primary goal of treatment is to alleviate symptoms but stop the neurodegeneration. PD characterized by the loss of dopaminergic neurons in substantia nigra of midbrain, leading to motor disorder such as bradykinesia, resting tremor, or rigidity. Another hallmark is the formation of Lewy bodies composed of abnormally folded α-synuclein. Many studies have been revealed that mutations in the ATP13A2 gene result in autosomal recessive PD (PARK9). ATP13A2 encodes transporter protein on the lysosomal membrane and serves as lysosomal H^+,K^+-ATPase. However, the mechanism which results in PD is still unclear. In this study,

differentiated SH-SY5Y cell model was used to investigate the mechanism of ATP13A2 mutations-induced dopaminergic neuronal cell death. We identified four ATP13A2 mutations from Taiwanese PD patients. The wild-type (WT) and 4 mutant ATP13A2 were successfully overexpressed in SH-SY5Y dopaminergic cells. Compared to WT ATP13A2, expression of mutant ATP13A induced the neuronal death of differentiated SH-SY5Y dopaminergic cells. The number of lysosomes were decreased in SH-SY5Y cells expressing mutant ATP13A2 compared with WT ATP13A2. Moreover, the protein expression of autophagy related protein, including Atg16, Atg7, p62, was significant increased in SH-SY5Y cells expressing ATP13A2 compared with WT ATP13A2. ATP13A2 mutations caused the activation of ER stress and apoptosis in SH-SY5Y dopaminergic cells. Our results suggest that ATP13A2 mutations lead to autophagy impairment and neuronal apoptosis in PD.

Disclosures: T. Chiu: None. **C. Chiu:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.18/C40

Topic: C.03. Parkinson's Disease

Support: ASAP-000592

Title: Exploring the role of Parkinson's disease-linked LRRK2 and VPS35 in cellular senescence

Authors: *J. ROWLANDS[1], M. ERB[4], E. T. WILLIAMS[2], D. J. MOORE[3];
[1]Neurodegeneration, Van Andel Inst., Grand Rapids, MI; [2]Van Andel Inst., Grand Rapids, MI, ; [3]Dept. of Neurodegenerative Sci., Van Andel Inst., Grand Rapids, MI; [4]Neurodegenerative Sci., Van Andel Res. Inst., Grand Rapids, MI

Abstract: Parkinson's disease (PD) is primarily an idiopathic disease, and while up to 10% of PD cases are familial, age remains the strongest risk factor. Cellular senescence, a major driver of aging, is a stable cell cycle arrest triggered in response to various intrinsic and extrinsic stimuli, as well as developmental signals. Such signals include DNA damage, telomere dysfunction, oncogene activation and organelle stress. Senescent cells are characterized by irreversible cell-cycle arrest, organelle abnormalities, altered metabolism and a senescence-associated secretory phenotype (SASP). Current evidence from human brain tissue and experimental models suggests that cellular senescence may play a role in neurodegenerative diseases, such as Alzheimer's disease and PD. Examining the role of cellular senescence in the context of PD-linked gene products may yield a deeper understanding of disease pathophysiology. We selected two PD genes linked to late-onset, autosomal dominant disease (LRRK2 and VPS35), where cellular senescence may plausibly influence the late onset and/or variable penetrance of disease. Mutations in the *leucine-rich repeat kinase 2* (*LRRK2*) and

vacuolar protein sorting 35 ortholog (*VPS35*) genes drive familial forms of PD that are clinically similar to idiopathic disease, and both proteins play distinct roles in the endolysosomal system. Accordingly, we derived primary mouse embryonic fibroblasts (MEFs) from knockin mice harboring G2019S LRRK2 or D620N VPS35 mutations relative to their wild-type (WT) littermates, and MEFs were cultured and aged in the absence or presence of senescence inducers. We used MEFs as a model to assess their replicative ability, viability, bioenergetics and senescence/SASP markers at each doubling. Our data demonstrate that cells expressing PD-linked LRRK2 or VPS35 mutations develop a senescent phenotype earlier than WT cells, accompanied by reduced mitochondrial oxidative phosphorylation, and alterations in retromer and autophagy-lysosomal pathway components. We are currently investigating the impact on senescence markers, including $p16^{Ink4a}$, $p21^{Cip1}$, SASP factors, DNA damage, lysosomal dysfunction, and whether senolytic compounds can reverse these phenotypes. Taken together, these data suggest that PD-linked LRRK2 and VPS35 cells are prone to accelerated senescence, leading to an extended period of cellular dysfunction that may contribute to disease pathophysiology.

Disclosures: J. Rowlands: None. **M. Erb:** None. **E.T. Williams:** None. **D.J. Moore:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.19/C41

Topic: C.03. Parkinson's Disease

Support: NIH Grant R15NS111413-01A1

Title: The Small Molecule, LM11a-31, Inhibits Proteolytic Processing of the p75 Neurotrophin Receptor and is Neuroprotective in a Cell Culture Model of Parkinson's Disease.

Authors: *A. NEWCHURCH, P. POKHAREL, L. DARZI, S. OVERBY, B. R. KRAEMER; Biol. Sci., Univ. of Alabama in Huntsville, Huntsville, AL

Abstract: The p75 Neurotrophin Receptor ($p75^{NTR}$) is a transmembrane protein located in specific regions of the adult nervous system. The receptor mediates diverse neuronal functions, including regulation of survival, synaptic plasticity, and myelination. In numerous biological contexts, signaling by $p75^{NTR}$ occurs through proteolytic processing, which releases cytosolic $p75^{NTR}$ fragments for downstream signaling. We previously reported that activation of $p75^{NTR}$ in this manner is stimulated by oxidative stress in LUHMES cells, a human neuronal cell line derived from the ventral mesencephalon that expresses markers of dopaminergic neurotransmission. Given the susceptibility of dopaminergic neurons in the ventral mesencephalon to oxidative stress and neurodegeneration associated with Parkinson's disease (PD), we sought to identify the role of this cascade on neurodegeneration, as well as to identify molecular mechanisms governing oxidative stress-induced $p75^{NTR}$ processing. Here, we present

evidence that oxidative stress induces $p75^{NTR}$ processing in mesencephalic cells by promoting internalization of $p75^{NTR}$ from the plasma membrane. Activation of $p75^{NTR}$ in this context occurs independently of TrkB and TrkC signaling, since LUHMES cells lack expression of such receptors. However, we found that LUHMES cells express TrkA receptors, which are downregulated in response to oxidative stress. Furthermore, our preliminary data indicate that inhibition of Trk signaling leads to increased $p75^{NTR}$ cleavage. Thus, our findings support a model in which oxidative stress promotes internalization of cell surface-localized $p75^{NTR}$, leading to cleavage of $p75^{NTR}$ in endosomes, while simultaneously downregulating Trk receptors that inhibit $p75^{NTR}$ cleavage. Importantly, we found that this pathway can be targeted with the small molecule $p75^{NTR}$ modulator, LM11a-31. Treatment of neuronal cultures with LM11a-31 significantly reduced $p75^{NTR}$ cleavage and neuronal death induced by oxidative stress. Altogether, our findings reveal novel mechanisms through which oxidative stress promotes $p75^{NTR}$ signaling and highlight $p75^{NTR}$ as a potential pharmacological target for mitigating neurodegeneration associated with PD.

Disclosures: A. Newchurch: None. **P. Pokharel:** None. **L. Darzi:** None. **S. Overby:** None. **B.R. Kraemer:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.20/C42

Topic: C.03. Parkinson's Disease

Support: Grace Science Foundation

Title: Effects of NGLY1 and NFE2L1 expression on the propagation of PD neuropathology

Authors: *B. D. COLÓN[1,2,3], A. CHANDRAN[4,2], J. HENSEL[4,2], S. BOUDOUKHA[5], J.-C. ROCHET[4,2];
[1]Purdue Univ., West Lafayette, IN; [2]Purdue Institute for Integrative Neuroscience, Purdue University, West Lafayette, IN; [3]Borch Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN; [4]Borch Dept. of Medicinal Chem. and Mol. Pharmacol., Purdue Univ., West Lafayette, IN; [5]Grace Sci. Fndn., Menlo Park, CA

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by a selective loss of midbrain dopaminergic (DA) neurons. There are currently no disease-modifying treatments for PD. Lewy bodies, a pathological hallmark of the disease, are intracellular inclusions composed of aggregated forms of the presynaptic protein alpha-synuclein (aSyn). Mechanisms for maintaining proteostasis and processing misfolded proteins are dysregulated in PD as a result of environmental insults or genetic perturbations. In turn, this dysregulation results in aSyn aggregation, leading to further proteasomal dysfunction and oxidative stress as part of a vicious cycle that results in the selective death of DA neurons.

Consequently, strategies to enhance proteasomal activity and increase the antioxidant response have the potential to reduce aSyn aggregation and slow PD progression. NFE2L1 is a master transcription factor expressed in the endoplasmic reticulum and targeted for proteasomal clearance by the ER-associated protein degradation (ERAD) pathway under basal conditions. Proteasome dysfunction leads to an accumulation of NFE2L1, allowing for the protein's translocation (after several processing steps) to the nucleus, where it can induce the expression of genes involved in proteostasis and antioxidant signaling. One of these processing steps involves NFE2L1 de-glycosylation by N-glycanase 1 (NGLY1), resulting in an unconventional asparagine-to-aspartate post-translational modification. How NGLY1 regulates and alters NFE2L1 function has yet to be fully elucidated.
Evidence of parkinsonian symptoms associated with NGLY1 deficiency and of NFE2L1 down-regulation in post mortem PD brains suggests that disruption of the NGLY1-NFE2L1 axis can cause oxidative stress and protein aggregation in the brain, in turn leading to neurodegeneration. We hypothesize that NGLY1-deficient neurons have a proteostasis defect that should be reflected by increased sensitivity to proteasome inhibitors. Consistent with this hypothesis, we found that NGLY1-deficient, iPSC-derived cortical neurons are unable to upregulate proteasome subunit gene expression when treated with the proteasome inhibitor epoxomicin. Current efforts are focused on examining the efficiency of aSyn seeded aggregation in iPSC-derived neurons from NGLY1-deficient patients. The results of these studies will yield insights into molecular mechanisms underlying neurotoxicity associated with defects along the NGLY1-NFE2L1 axis, setting the stage for developing disease-modifying therapies.

Disclosures: B.D. Colón: None. **A. Chandran:** None. **J. Hensel:** None. **S. Boudoukha:** None. **J. Rochet:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.21/C43

Topic: C.03. Parkinson's Disease

Support: AEI-PID2019-109059RB-I00
AEI-PID2022-137076OB-I00
CIBERNED CB06/05/065
CIBERNED CB06/05/0055
Fundación Ramón Areces (CIVP18A3941)

Title: N370S and L444P GBA1 mutations alter neuronal function and structure in Parkinson´s disease

Authors: E. RODRÍGUEZ TRAVER[1], L. SUÁREZ[1], C. CRESPO[2], I. GONZALEZ-BURGOS[1,3], R. VECINO[1,3], R. MORATALLA[1,3], ***C. VICARIO**[1,3];

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Abstract: Mutations in the *glucocerebrosidase1* (*GBA1*) gene are major risk factors for Parkinson´s disease (PD) and dementia with Lewy bodies. To investigate the impact of *GBA1* mutations on neuronal maturation, function and degeneration, we have obtained dopaminergic neurons (DAn) from our repository of induced pluripotent stem cells (iPSCs) derived from PD patients carrying the N370S/wt or the L444P/wt mutation in *GBA1*. Expression of markers of mesencephalic dopaminergic progenitors and neurons (including LMX1A, LMX1B, FOXA2, NURR1, TH, VMAT2, GIRK2, and DAT) was detected in differentiating cultures. iPSC-derived DAn acquired complex morphologies, released dopamine, fired action potentials and showed spontaneous synaptic activity, indicating that they reached a high degree of functional maturation. Electrophysiological recordings revealed a significant increase in the firing rate of N370S/wt *GBA1*-neurons but not in that of L444P/wt neurons. Remarkably, neurons carrying *GBA1* mutations presented abundant degeneration bodies, multilamellar bodies, autophagosomes, and Golgi apparatus dictyosomes, having partially distinct features in neurons carrying the N370S/wt or the L444P/wt *GBA1* mutation. Furthermore, we detected a significant accumulation of alpha-synuclein aggregates in cell bodies and dendrites of N370S/wt *GBA1*-neurons. Our findings indicate that N370S/wt and L444P/wt *GBA1* mutations produce both similar and distinct molecular, electrical and ultrastructural alterations in dopaminergic neurons.

Disclosures: E. Rodríguez Traver: None. **L. Suárez:** None. **C. Crespo:** None. **I. Gonzalez-Burgos:** None. **R. Vecino:** None. **R. Moratalla:** None. **C. Vicario:** None.

Poster

PSTR156: Parkinson’s Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.22/C44

Topic: C.03. Parkinson’s Disease

Support: Research of Korea Disease Control and Prevention Agency Grant 2023-ER1005-00
NRF grant 2022R1C1C2009107
KHIDI & KDRC Grant HU22C0143000022

Title: Single cell and spatial transcriptomic analysis reveals neuronal heterogeneity and molecular characteristics of disease-associated glias in sporadic Parkinson's disease

Authors: *S. YOO[1], K. LEE[1], J. SEO[2], H. CHOI[2], S.-I. KIM[1], Y.-M. SHIM[1], J. CHANG[2], J. KIM[2], J.-K. WON[1], S.-H. PARK[1];
[1]Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of; [2]Soongsil Univ., Seoul, Korea, Republic of

Abstract: Parkinson's disease (PD) is a common neurodegenerative disorder that results in motor dysfunction due to abnormal protein accumulation and the death of certain neurons.

Research suggests that neurodegenerative diseases share common pathological markers such as reactive neuroinflammation, oxidative stress, and inflammatory responses. Understanding the genes that regulate these cellular responses in Parkinson's disease (PD) is critical for diagnosis, understanding the disease, predicting disease progression, and evaluating treatment efficacy, although it is not yet fully understood. In this study, single-nucleus RNA sequencing was performed on the substantia nigra (SubNa) of four sporadic PD patients and four brains diagnosed with primary age-related tauopathy (PART) to serve as a more appropriate control group from a pathological perspective. A total of 33,293 nuclei were examined and eight major cell types were identified by cluster analysis. Neuronal cells were highly heterogeneous with 11 subtypes, showing a decrease in dopaminergic and glutamatergic neurons and a relative increase in GABAergic neurons. Spatial transcriptomic data revealed two distinct spatial correlation groups in neurons by deconvolution, indicating differences between dopaminergic nuclei and surrounding area. Using Monocle-based pseudo-time analysis, two disease-related trajectories were identified in astrocytes and microglia, along with their distinguishing genes. Specifically, distinct spatial distributions of early, intermediate, and late astrocytes were observed in response to PD, and the inflammatory response was shown to play a critical role in PD progression in microglia. Furthermore, gene regulatory networks (GRNs) based on TENET analysis revealed the specific formation of an unfolded protein response/ER stress-related module in microglia and a protective module that inhibits such stress responses in astrocytes. In conclusion, this approach confirmed the genetic and spatial characteristics of PD substantia nigra neurons and demonstrated the heterogeneity of glial cell fate associated with PD.

Disclosures: S. Yoo: None. **K. Lee:** None. **J. Seo:** None. **H. Choi:** None. **S. Kim:** None. **Y. Shim:** None. **J. Chang:** None. **J. Kim:** None. **J. Won:** None. **S. Park:** None.

Poster

PSTR156: Parkinson’s Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.23/C45

Topic: C.03. Parkinson’s Disease

Support: CAPES - 88887.940799/2024-00
FAPESP - 2019/00065-1; 2021/12538-1
CEUA - 2740200319

Title: Nadph Oxidase, Apocynin, And Its Effects On Apoptotic And Survival Pathways In The 6-Ohda Rat Model Of Parkinson’s Disease

Authors: *L. F. A. T. PEDRAO[1], P. O. S. MEDEIROS[2], B. FALQUETTO[2];
[1]Univ. of Sao Paulo, Sao Paulo, Brazil; [2]Dept. of Pharmacol., Univ. of Sao Paulo, São Paulo, Brazil

Abstract: Introduction: Parkinson's disease (PD) is a neurodegenerative disease characterized by the death of dopaminergic neurons in the Substantia Nigra (SN). It presents motor symptoms, such as dyskinesia, postural instability and respiratory problems. It is known that the 6-OHDA animal rat model presents neurodegeneration in respiratory control regions such as nucleus of solitary tract (NTS), retrotrapezoid nucleus (RTN), preBötzinger complex (preBötC) and rostral ventral respiratory group (rVRG), which causes loss in ventilatory function. Oxidative stress seems to be the main cause of this impairment in breathing and previous work have shown that apocynin (APO) prevented respiratory nuclei degeneration and breathing dysfunction in PD model. **Aim:** Evaluate the effects of the treatment with APO on NOX expression and in the death and survival signaling of respiratory nuclei and respiratory deficits in 6-OHDA animals. **Methods:** 6-OHDA (24µg/µl) or vehicle was injected into male adult rat's striatum. Animals were treated with APO in drinking water for 10 days, starting on the 20th day after PD induction. On the 30th day, the animals were euthanized, their brains were sliced in microtome, and their brainstem was dissected to extract respiratory neurons and perform Western Blot. All animals were submitted to tyrosine hydroxylase (TH)-immunoreactivity (ir) to evaluate SN and confirm PD model. Two-way ANOVA followed by Newman Keuls *post-hoc* was applied with $p<0.05$. **Results:** 6-OHDA reduced TH+ neurons in SN and APO did not reverse it as expected, confirming the PD model (vehicle: 755,49 ± 141,55; 6-OHDA: 207 ± 62,24; vehicle + APO: 711,43 ± 97,52; 6-OHDA + EX: 210,75 ± 80,63 neurons, $F_{1,26} = 213,7$, $p < 0.0001$). Our ELISA protocol showed a reduction NOX2 expression of the rVRG + preBotC nuclei in the 6-OHDA group, and treatment with APO prevented it (Vehicle: 0,2768 ± 0,0711; 6-OHDA: 0,4060 ± 0,0647; Vehicle + APO: 0,2284 ± 0,0686; 6-OHDA + APO: 0,2960 ± 0,0723, $F_{1,13} = 0,8363$, $p = 0,0119$, $F_{1,13} = 5,517$, $p = 0,0353$), but not in the RTN (Vehicle: 0,2027 ± 0,0808; 6-OHDA: 0,1925 ± 0,0757; Vehicle + APO: 0,1876 ± 0,0426; 6-OHDA + APO: 0,2592 ± 0,0582, F1,21 = 1,316, p = 0,2643). Also, in the rVRG + preBotC, we saw alterations in the expression of Akt1, GSK3β-p (Ser9), and GSK3β, which were prevented by the treatment with APO ($p<0,05$). Finally, in the RTN, we saw altered expression of Bcl-2, GSK3β and β-catenin, with prevention after treatment with APO ($p<0,05$) **Conclusion:** Survival signaling is important in the respiratory neurodegeneration in 6-OHDA model, and treatment with APO can prevent it, revealing that NOX2 is valuable in the neurodegeneration of respiratory nuclei in the 6-OHDA model.

Disclosures: L.F.A.T. Pedrao: None. **P.O.S. Medeiros:** None. **B. Falquetto:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.24/C46

Topic: C.03. Parkinson's Disease

Support: NIH/NIEHS, 5 R01 ES024745-07 (MCH)
NIH/NIEHS, 5 R01 ES033462-02 (MCH)
MJFF-020697 (MCH)
NIH/NCI 5P30CA023108-40 (Dartmouth Cancer Center)

Title: BRI3 is a monocyte-restricted immune modulator elevated in Parkinson's disease

Authors: *K. PAUL[1], O. WILKINS[1], K. BIGGS[1], F. ANDERSON[1], S. L. LEE[2], F. W. KOLLING[3], M. C. HAVRDA[1];
[1]Geisel Sch. of Med. at Dartmouth, Lebanon, NH; [2]Neurol., Dartmouth Hitchcock Med. Ctr., Lebanon, NH; [3]Ctr. for Quantitative Biol., Geisel Sch. of Med. at Dartmouth, Lebanon, NH

Abstract: Parkinson's disease (PD) is the fastest growing neurodegenerative disease and is characterized by motor symptoms such as gait instability, tremor, and rigidity. PD is associated with chronic neuroinflammation and there is an increasing appreciation of the role of infiltrating peripheral immune cells in modulating inflammation of tissues in the PD central nervous system (CNS). The molecular mechanisms that may dysregulate the peripheral innate immune system in PD remain unclear. Changes in classical and non-classical monocyte proportions, classical monocyte phenotypes, and monocyte infiltration into the CNS have been described previously, but the non-classical CD16 monocyte phenotype in PD and its contribution to neuroinflammation has not been deeply investigated. We evaluated peripheral blood mononuclear cells from male and female PD patients and healthy controls using single cell RNA-sequencing. We identified hundreds of differentially expressed genes in PD peripheral immune cells, indicative of the existence of a PD-specific transcriptomic states across multiple cell lineages that vary by biological sex. Analysis of differentially expressed genes in the CD16 monocyte subtype identified a significant elevation of *Brain Protein I3* (*BRI3*) in male PD patients compared with healthy controls that is not apparent in females with PD. *BRI3* was highly restricted to the monocyte lineage and significantly enriched in the PD CD16 subset, yet the function of BRI3 in immune cells is not well elucidated. In vitro analyses indicate that *BRI3* expression is elevated in monocyte-like cultures differentiated towards the CD16 subtype. Inactivation of *BRI3* in a monocyte cell line promotes cell survival, but it does not impact cell proliferation. *BRI3* inactivation impaired the secretion of inflammatory cytokines and altered pathways related to arginine metabolism, a metabolic program characteristic of invasive monocyte derived macrophages, a cell type found in the PD brain. *BRI3* inactivated monocytes have increased migration through membranes *in vitro*. At this time, we interpret findings to indicate that PD CD16 monocytes with elevated BRI3 have reduced viability and migratory properties and enhanced inflammatory cytokine profile. Forward looking studies will employ gain-of-function and in vivo strategies to rigorously characterize monocyte *BRI3* in the contexts of inflammation, neuro-invasion, and neurodegeneration.

Disclosures: K. Paul: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 63/318,612. **O. Wilkins:** None. **K. Biggs:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 63/318,612. **F. Anderson:** None. **S.L. Lee:** None. **F.W. Kolling:** None. **M.C. Havrda:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 63/086,765, 63/318,612.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.25/C47

Topic: C.03. Parkinson's Disease

Support: Ben and Catherine Ivy Foundation
Midwestern University

Title: Sexually dimorphic alterations in parkin-null Drosophila head low molecular weight thiols

Authors: A. N. JUBA[1], T. MARGARYAN[5], R. HAMEL[2], B. STWALLEY[2], P. P. KEOSEYAN[2], K. L. HOULIHAN[3], T. B. JONES[4], A. TOVMASYAN[5], ***L. M. BUHLMAN**[1]; [1]Biomed. Sci., [2]Arizona Col. of Osteo. Med., [4]Anat., [3]Midwestern Univ., Glendale, AZ; [5]Translational Neurosci., Ivy Brain Tumor Center, Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Parkinson's disease (PD) is a heterogeneous disorder in regard to onset age, progression, non-motor symptoms, and symptom severity. While men are twice as likely to acquire PD, and women have more rapid disease progression, sexual dimorphism in PD pathophysiology is poorly understood. Identifying consequences of rare disease-causing mutations may reveal potential therapeutic targets for all PD patients. Indeed, elevated reactive oxygen species and decreased glutathione antioxidant capacity are implicated in all forms of PD. We are interested in uncovering mechanisms that may trigger oxidative stress in the absence of parkin. Thus, we have used highly selective redox-sensitive fluorescent proteins to report increased protein oxidation, hydrogen peroxide levels, and glutathione redox disequilibrium in mitochondria of degenerating dopaminergic neurons of parkin-null *Drosophila*. However, using LCMS-MS strategies in young parkin-null *Drosophila* heads, we failed to detect changes in levels of several low molecular weight thiols involved in glutathione synthesis (e.g., methionine, cysteinyl glycine). Here we repeated our studies in control and parkin-null flies separated by sex and have preliminarily detected elevated mitochondrial hydrogen peroxide, brain cystathionine, cysteinyl glycine, and GSH levels in males (oxidized glutathione dimer [GSSG] levels were below detection limits). Increased cystathionine and decreased gamma glutamylcysteine are currently observed in parkin-null flies when including sex as a variable. Our results imply that males may have increased transsulfuration pathway-mediated glutathione synthesis that falls short of antioxidant demand in the absence of parkin. Our data also suggest that parkin-null female glutathione synthesis is not as effectively initiated when hydrogen peroxide levels are elevated. As in rodents, we have provided evidence that glutathione synthesis pathway activity is sexually dimorphic. Similar observations in humans could shed light on sexually dimorphic phenotypes of PD. Better understanding of potential sexually dimorphic PD risk factors could inform symptom management and highlight sex-specific therapeutic targets and strategies.

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Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.26/C48

Topic: C.03. Parkinson's Disease

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R21NS130841
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T32-MH087004
NIH Shared Instrumentation Grant (FAIN: S10OD021838)

Title: Striatal cell-type specific disruption of AMPAR dynamics and subunit composition by LRRK2-G2019S.

Authors: *S. GUPTA[1], G. W. HUNTLEY[2], D. L. BENSON[3];
[1]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Dept. of Neurosci., Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY; [3]Neurosci., Mount Sinai, New York, NY

Abstract: Striatal spiny projection neurons (SPNs) receive glutamatergic synapses principally from cortical cells. Plasticity at such synapses is important for cognitive and other functions. Humans and mice carrying the Parkinson's-linked Lrrk2-G2019S mutation exhibit cognitive impairments associated with corticostriatal dysfunction. The specific mechanisms by which Lrrk2-G2019S regulates AMPA-type glutamate receptor (AMPAR) dynamics and composition, thus shaping neurotransmission and plasticity, at corticostriatal synapses remain unknown. Using slices and cultured neurons from Lrrk2-G2019S knockin mice, in combination with biochemical, STED microscopy and whole-cell recording approaches, we observed a specific increase in surface levels of GluA1-containing AMPARs in SPNs, while GluA2-containing AMPAR levels remained unaffected. Abnormal surface accumulation of GluA1 was independent of PKA activity and was limited to D_1R SPNs. Such GluA1-containing AMPARs resisted internalization, leading to their accumulation in both synaptic and extrasynaptic regions. The trafficking defect in Lrrk2-G2019S SPNs also impaired cLTP-induced insertion of GluA1-containing AMPARs into the synaptic surface, and was selective as transferrin receptors trafficked normally. The aberrant surface buildup of GluA1 had functional ramifications, resulting in significantly increased numbers of calcium-permeable AMPARs but reduced surface mobility within synapses of D_1R SPNs. Together, these data suggest that abnormal AMPAR dynamics at Lrrk2-G2019S D_1R-SPN synapses contribute to corticostriatal dysfunction and underlying cognitive impairments.

Disclosures: S. Gupta: None. **G.W. Huntley:** None. **D.L. Benson:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.28/C49

Topic: C.03. Parkinson's Disease

Support: FAPESP (2022/12788-0)
FAPESP (2020/16320-8)
CNPQ
CAPES

Title: Sex differences in respiratory impairments in a mouse model of Parkinson's disease

Authors: *Y. C. AQUINO[1], G. RODRIGUES[1], T. S. MOREIRA[2], A. T. TAKAKURA[3]; [1]Pharmacol., [2]Univ. of Sao Paulo, Sao Paulo, Brazil; [3]Dept. of Pharmacol., Dept of Pharmacology, Inst. of Biomed. Science, Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: Parkinson's disease (PD) is a neurodegenerative condition characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SN). Individuals with PD exhibit classic motor symptoms such as tremors, and non-motor symptoms including respiratory deficits. Previous studies have shown that the incidence of the disease is two-fold higherin men than in women. Studies have shown that male mice models of PD induced by 6-hydroxydopamine (6-OHDA) exhibit a reduction in basal respiratory frequency associated with degeneration of essential areas in respiratory control such as the pre-Bötzinger complex (preBötC) and the retrotrapezoid nucleus (RTN). However, to date, possible variations in a female mouse model have not been evaluated. Thus, our aim is to investigate the possible differences between males and females and the role of sex hormones in respiratory disorders in the PD model. We used adult female and male C57BL/6 mice (N = 66; CEUA protocol: 3325170822) and performed bilateral ovariectomy (OVX) or orchiectomy (ORX) or sham surgery. Two weeks later, bilateral injection of vehicle or 6-OHDA (10 μg/μl; 0.5 μl) into the striatum was executed to induce the PD model. After 10 days, whole body plethysmography was performed to assess the respiratory parameters of the animals and then they were anesthetized, and perfusion fixed. Our data showed that in PD models there was a 77% reduction in the number of dopaminergic neurons in SN and OVX was able to increase this reduction by 90%. In males, ORX reduced by 12% the number of dopaminergic neurons in control animals but not in PD model. In the preBötC, OVX was also able to increase the reduction in the density of neurons expressing neurokinin 1 receptor (NK1r) in PD models from 25% to 37%. In male mice, 6-OHDA was able to reduce the density of NK1r in preBötC by 24% independently of ORX. Similar results could be observed in the neurodegeneration of RTN phox2b-expressing neurons, as OVX potentiated the reduction from 34.9% to 43.1% in PD models. Respiratory functional parameters showed that PD animals showed a reduction in resting respiratory rate and this response was greater in OVX (Female: Sham+6-OHDA: 160.7 ± 0.7; OVX+6-OHDA: 150.7 ± 2.8 vs. Sham+vehicle: 181.8 ± 0.6; OVX+vehicle: 181. 8 ± 0.6; Male: Sham+6-OHDA: 154.2 ± 2.9; ORX+6-OHDA: 154.4 ± 1.2 vs. Sham+vehicle: 180.8 ± 4.3; ORX+vehicle: 175.3 ± 1.8 bpm). Our results suggest that the OVX was able to potentiate the SN, preBötC and RTN neurodegeneration and the reduction on respiratory frequency induced by 6-OHDA, ORX was

able to induce SN neurodegeneration in control animals. Possibly sex hormones are involved in the effects related to the neuroprotection.

Disclosures: Y.C. Aquino: None. **G. Rodrigues:** None. **T.S. Moreira:** None. **A.T. Takakura:** None.

Poster

PSTR156: Parkinson's Disease: Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR156.29/C50

Topic: C.03. Parkinson's Disease

Support: NIH Grant 1DP2 AG086139
NIH Grant R56 AG078453
AARG-22-973134

Title: Unique neuronal somatic mutation genome signatures and DNA damage mechanisms unearth new targets in late-stage sporadic Parkinson's Disease patients

Authors: *J. ZIEGENFUSS, M. LODATO;
Mol. Cell & Cancer Biol., Univ. of Massachusetts Chan Med. Sch., Worcester, MA

Abstract: Age is the key risk factor for degenerative diseases like Parkinson's Disease (PD), with only a small fraction of cases being inherited. The majority of PD cases are sporadic, underscoring the need to uncover cellular hallmarks and develop research models for this form of the disease. Somatic mutations, which are permanent genomic changes that evade DNA repair mechanisms and accumulate over time, are not limited to dividing cells; they also accumulate in aging human post-mitotic neurons. Understanding the positional context of somatic mutations within the genome represents a valuable indicator of the type of genomic damage within a cell. In fact, these signature analyses are often used to assess tumors and potential therapies to combat cellular dysregulation. Employing single-cell whole-genome sequencing, we aimed to pinpoint unique mutational signatures linked to genomic instability in late-stage sporadic PD patients. This method has the power to reveal new cellular mechanisms and targets relevant to PD-related neuronal changes. Our research shows that PD neurons exhibit a significant number of small unique mutations, indicating an intensified neuron-specific "aging clock" and disrupted DNA break repair processes. Importantly, there is a direct association between the severity of alpha-synuclein aggregate pathology in PD patients and the presence of this particular genomic damage signature. Our identification of PD mutation signatures aligns with other cellular features in patients, such as an oxidative damage repair storm and DNA break repair, suggesting new avenues for targeting pathways critical to PD progression. Through the integration of our genomic, molecular, and cell biology findings, we are developing *in vitro* models that mimic sporadic PD. These models will enable us to target factors that impede the accumulation of PD-related mutations and cellular hallmarks, while promoting cell survival.

Disclosures: J. Ziegenfuss: None. **M. Lodato:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.01/C51

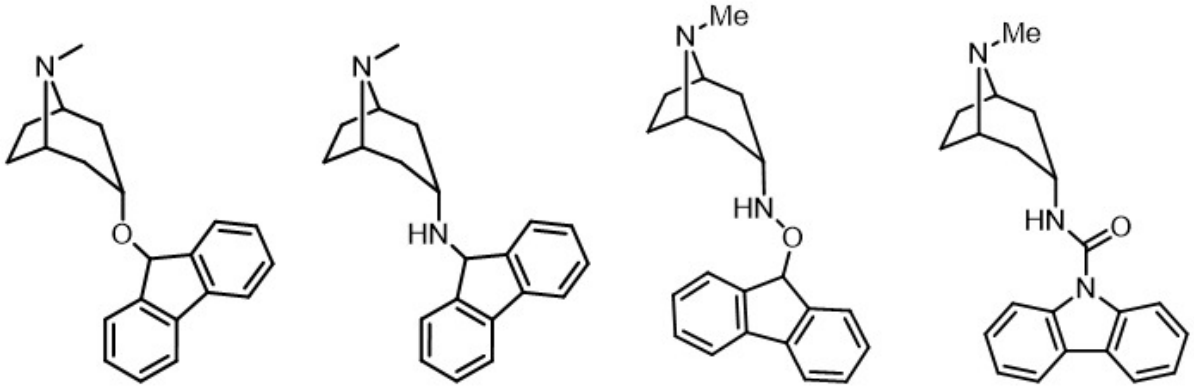
Topic: C.03. Parkinson's Disease

Support: CIIC 056/2024 DAIP UG

Title: Antiparkinsonian alkaloids, effects in a murine model

Authors: *C. ALBA-BETANCOURT[1], E. HERNÁNDEZ VELÁZQUEZ[2], D. GASCA-MARTINEZ[3], A. HERNANDEZ[4], C. SOLORIO ALVARADO[2];
[1]Univ. De Guanajuato, Guanajuato, Mexico; [2]Chem., Univ. de Guanajuato, Guanajuato, Mexico; [3]Behavioral Analysis Unit, Neurobio. Institute, UNAM Campus Juriquilla, Queretaro, Mexico; [4]Inst. de Neurobiologia UNAM, Univ. Nacional Autonoma de Mexico, Querétaro, Mexico

Abstract: Parkinson's disease is a neurodegenerative disorder that is characterized clinically by the presence of the motor triad of akinesia, tremor and stiffness, in addition to non-motor manifestations. One therapeutic strategy is to block the dopamine transporter (DAT) with drugs such as benztropine, which structurally has a benzhydride group that rotates freely. However, due to side effects, involving decreased and unbalanced levels of serotonin and norepinephrine, benztropine has lost popularity. In this project we evaluated benztropine derivatives, previously synthesized by our research group, and which we have named "fluorenatropins", due to the generation of a carbon-carbon bond and thus a fluorene ring, providing rigidity to this group, which we propose they will have a better effect, since it has been shown that the limitation of movement in these groups increases selectivity towards DAT with respect to serotonin transporters (SERT) and norepinephrine (NET), thus limiting unwanted side effects. For this purpose, we used behavioral tests (rotarod, horizontal bars) to evaluate the efficacy of compounds in a mouse model of hemiparkinson, generated by the injection of 6-hydroxydopamine in order to destroy the substantia nigra. From the 8 compounds, the 1A, 1B, 1F and 1G, showed the best improvement in the behavioral test. These compounds contain a bulky ring attached to the benztropine molecule (Figure 1). Mice showed better performance in the rotarod and horizontal bars, compared to the other molecules and to control (L-dopa). With these results we can propose new molecules that have a high potential to reverse the symptoms of Parkinson's disease. Work continues to determine the binding site of these compounds in different brain structures.



1A 1B 1F 1G

Disclosures: C. Alba-Betancourt: None. **E. Hernández Velázquez:** None. **D. Gasca-martinez:** None. **A. Hernandez:** None. **C. Solorio Alvarado:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.02/C52

Topic: C.03. Parkinson's Disease

Support: NSTC-112-2320-B-006-005
NSTC-112-2320-B-006-015-MY3

Title: Dopamine D_3receptor signaling regulates K_{ATP} channel to alleviate the progression of levodopa -induced dyskinesia

Authors: *P.-C. CHEN[1], S.-B. YANG[2];
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Abstract: Long-term treatment with levodopa (L-DOPA) in Parkinson's disease leads to L-DOPA-induced dyskinesia (LID). Dopamine receptors regulate medium-spiny neuronal excitability (MSN) by modulating potassium channel activity. The abnormal activation of MSNs is associated with dyskinesia, but the precise molecular mechanism underlying the dopamine D3 receptor (D3R) and potassium channel interaction in LID remains unclear. Our study aimed to investigate this mechanism by using 6-hydroxydopamine (6-OHDA) to induce hemiparkinsonism in mice and treating them with L-DOPA to induce LID. Our study showed that a D3R antagonist, FAUC365, had a positive impact on alleviating LID progression and

mitigating the degeneration of the nigrostriatal pathway. Interestingly, we found a protein-protein interaction between D3R and ATP-sensitive potassium channels (K_{ATP} channel). Furthermore, we found that the D3R GSK3β/AMPK signaling pathway mediated the increased expression of K_{ATP} channels in the striatum, which suggests the contribution of K_{ATP} channels to LID progression. We also found that using a K_{ATP} channel opener called Diazoxide, co-treated with L-DOPA, attenuated the progression of LID and protected the loss of the nigrostriatal projection. The decreased expression of K_{ATP} channels in the Diazoxide-treated group indicated the vital role of K_{ATP} channels in LID. Overall, our research identifies the critical role of D3R and K_{ATP} channels in LID and provides a novel combination medication strategy.

Disclosures: P. Chen: None. **S. Yang:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.03/C53

Topic: C.03. Parkinson's Disease

Support: CityU 11101922

Title: Dieckol promotes exploratory and motor activity and alters synaptic transmission and plasticity of the longitudinal dentate gyrus network in mice

Authors: *Y. KWON, H. YU, Y. LAU, S. YANG;
Neurosci., City Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Polyphenolic compounds extracted from brown seaweed constitute an Investigational New Drug by the United States Food and Drug Administration (US FDA), and many recent studies have demonstrated their neuroprotective potential against Alzheimer's disease, Parkinson's disease, seizure, and stroke among others. One compound in particular, dieckol (DEK), is known to cross the blood-brain barrier. However, it remains unclear how it affects synaptic transmission or plasticity to influence cognition and behavior. Wild-type C57BL/6J mice were treated with DEK daily for 1 or 2 weeks before behavioural testing and extracellular local field recording of brain slices from the hippocampus and primary motor cortex. DEK-treated mice exhibited greater mobility and exploration in the open-field and Y-maze tests. This was accompanied by altered neuronal excitability (measured by the input-output function) and long-term plasticity in the longitudinal dentate gyrus (DG)-DG synapse, which is a newly identified network in the hippocampus (Choi et al. 2021; Pak et al. 2022). These results suggest that DEK affects motor memory and activity, resulting in increased locomotion and exploration. Therefore, DEK warrants further testing for Parkinson's Disease and in central motor control systems.

Disclosures: Y. Kwon: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Botamedi Brain Health and Medical Care Company Limited. F. Consulting Fees (e.g., advisory boards); Botamedi Brain Health and Medical Care Company Limited. **H. Yu:** None. **Y. Lau:** None. **S. Yang:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.04/C54

Topic: C.03. Parkinson's Disease

Support: MJFF Grant 022480

Title: Discerning the role of myeloid cells in Parkinson's disease

Authors: *A. M. SHAW[1], B. NGUYEN[2], B. A. KILLINGER[2], V. GUPTA[3];
[1]Rush Univ. Med. Ctr., Chicago, IL; [2]Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL; [3]Intrnl. Med., Univ. of Texas Med. Br., Galveston, TX

Abstract: Pathological characterization of Parkinson's disease (PD) includes overexpression and aggregation of the protein alpha-synuclein (α-syn) in neuronal cell bodies known as Lewy bodies, which leads to increased neuroinflammation and subsequent neurodegeneration. These α-syn aggregates over-activate resident microglia via ligation of toll-like receptors (TLRs) and signaling proteins, thereby stimulating pro-inflammatory NFκB and inflammasome pathways. Subsequently, it results in generation of pro-inflammatory cytokines that recruits peripheral monocytes and other immune cells, causing further damage. Thus, targeting peripheral monocyte recruitment and microglial activation could be therapeutic in PD, although agents that can accomplish both are missing. Here we describe a novel approach that targets these pathways using a common molecular target (CD11b) and a novel small molecule therapeutic (LA1). CD11b is an adhesion receptor that is highly and selectively expressed on monocytes and microglia with roles in cell adhesion, migration, tissue recruitment, and phagocytic clearance of pathogens and aggregates. Previously, we discovered that agonism of CD11b via the Conformation-Locking Allosteric agonist (CLOAK), LA1, also acts as an intracellular brake on TLR-stimulated NF-kB and inflammasome pathways, reducing inflammatory activation of myeloid cells. LA1 binds to CD11b on myeloid cells and increases CD11b-dependent adhesion and reduces cell migration. Here, we evaluated the efficacy of LA1 in an a-syn dependent model of PD in mice by stereotaxically injecting an adeno-associated virus (AAV) to overexpress α-syn in the substantia nigra (SN) and subsequently administering either vehicle or LA1 for 4- or 8-weeks. Mice were then anesthetized and euthanized for brain, blood, and spleen collection. We found that agonism of CD11b with LA1, suppressed a-syn mediated pro-inflammatory microglial activation and reduced monocyte infiltration into the brain, thereby reducing neuroinflammation and neurodegeneration.

Disclosures: A.M. Shaw: None. **B. Nguyen:** A. Employment/Salary (full or part-time):; BioSapien. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BioSapien. F. Consulting Fees (e.g., advisory boards); Deep Origin. **B.A. Killinger:** None. **V. Gupta:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 149 Bio, LLc. F. Consulting Fees (e.g., advisory boards); 149 Bio, LLc.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.05/C55

Topic: C.03. Parkinson's Disease

Support: NIH R21 NS128519

Title: Crosstalk between DNA damage and cGAS-STING immune pathway drives neuroinflammation and behavioral dysfunction in mouse model of Parkinson's disease

Authors: *S. KHAN[1], J. XIAO[2], M. KHAN[2];
[1]Neurol., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [2]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Crosstalk between DNA damage and cGAS-STING immune pathway drives neuroinflammation and behavioral dysfunction in mouse model of Parkinson's disease
Sazzad Khan[1], Jianfeng Xiao[1], Mohammad Moshahid Khan[1,2,3]
[1]Departments of Neurology, University of Tennessee Health Science Center, Memphis, USA[2]Neuroscience Institute, University of Tennessee Health Science Center, Memphis, TN, USA[3]Center for Muscle, Metabolism and Neuropathology, Division of Rehabilitation Sciences and Department of Physical Therapy, College of Health Professions, University of Tennessee Health Science Center, Memphis, TN, USA
Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder, characterized by degeneration of dopaminergic neurons in the substantia nigra of the midbrain and loss of both motor and non-motor features. While the molecular mechanisms that regulate the progression of the PD are not fully elucidated, there is evidence to suggest that accumulation of nuclear DNA damage, particularly nuclear DNA double-strand breaks (DDSBs), contribute to the progression of neurodegeneration. In this study we showed that crosstalk between DDSBs and cyclic GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING) immune regulatory pathway drives neuroinflammation which in turn causes dopaminergic neurodegeneration and behavioral deficits in a mouse model of PD. We utilized a mouse model of PD in which an adeno-associated vector 1/2 serotype (AAV1/2) expressing human mutated A53T-α-synuclein (AAV-A53T-syn) was stereotactically injected into the SN of wildtype (WT) mice. WT mice treated with AAV-

A53T-syn display increased expression of γ-H2A.X (Ser139) along with altered expression of DNA repair proteins. Our results showed that DDSBs and reduced expression of DNA repair proteins were further associated with significant increase in cGAS-STING pathway, dopaminergic neuronal loss and behavioral deficits. Interestingly inhibition of DNA damage or deletion of STING significantly prevents loss of dopaminergic neurodegeneration and improve behavioral deficits in PD mice. Our data provide evidence that accumulation of DDSB and/or alteration in DNA DSB repair proteins trigger inflammatory responses in the brain which may further influence degeneration of dopaminergic neurons and behavioral deficits in PD. Thus, targeting the DNA damage response pathway and cGAS-STING together might offer an innovative approach to prevent or slow down PD progression.

Disclosures: S. Khan: None. **J. Xiao:** None. **M. Khan:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.06/C56

Topic: C.03. Parkinson's Disease

Support: MJFF-021161

Title: Impact of the third generation ROCK inhibitor KL-00974 in the rat alpha-synuclein preformed fibril model of Parkinson's Disease

Authors: *M. KUBIK[1], J. P. MACKEIGAN[2], A. STOLL[3], J. PATTERSON[1], C. J. KEMP[1], J. R. HOWE[1], K. MILLER[4], K. C. LUK[5], C. E. SORTWELL[4];
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Abstract: Microglial activation is associated with Lewy Body (LB) deposition which perpetuates a chronic proinflammatory environment believed to exacerbate neurodegeneration in Parkinson's Disease (PD). Rho-associated protein kinase (ROCK1/2) is an intracellular signaling molecule that regulates microglial functions involved in neuroinflammation. The third-generation ROCK1/2 inhibitor, KL-00974, has superior selectivity, potency, and blood-brain barrier penetrance compared to classical ROCK inhibitors (i.e., fasudil). Previously we showed that daily oral KL-00974 administration significantly attenuates microglial activation and prevents nigral neurodegeneration in the alpha-synuclein adeno-associated viral (AAV a-syn) vector model. However, the levels of a-syn in the AAV a-syn overexpression model far exceed those associated with idiopathic PD and the a-syn inclusions that form in the AAV a-syn model lack some key features of LBs. Therefore, in the present study we employed the rat a-syn

preformed fibril (PFF) model, in order to evaluate the impact of KL-00974 during either the LB-like aggregation or the nigrostriatal degeneration stages, in the context of physiological a-syn levels. Rats received intrastriatal injections of a-syn PFFs or monomer and received daily oral administration of varying doses of KL-00974 (0-30 mg/kg/day) for either 60 (aggregation stage) or 120 (degeneration stage) days. In the aggregation stage experiment, KL-00974 did not impact the number of nigral neurons with phosphorylated a-syn (pSyn) inclusions or the number of microglia in the substantia nigra expressing major histocompatibility complex class II immunoreactive (MHC-IIir). Ongoing analyses will assess the impact of KL-00974 on 1) levels pSyn immunoreactivity in the SN and cortex, 2) fibrillar properties of nigral a-syn inclusions, and 3) number and functional phenotype of inclusion-responsive microglia. In the pending degeneration phase experiment, we will assess the impact of KL-00974 on nigral dopamine neuron survival and striatal terminal density. Collectively, these investigations will determine the ability of KL-00974 to provide neuroprotection in the context of a-syn inclusion induced degeneration. Results will provide insights into the disease-modifying potential of KL-00974.

Disclosures: M. Kubik: 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.; MJFF-021161. **J.P. MacKeigan:** None. **A. Stoll:** None. **J. Patterson:** None. **C.J. Kemp:** None. **J.R. Howe:** None. **K. Miller:** None. **K.C. Luk:** None. **C.E. Sortwell:** 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.; MJFF-021161.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.07/C57

Topic: C.03. Parkinson’s Disease

Support: NIH R56 NS109608
R01 NS122805
Arizona Biomedical Research Commission (ABRC) grant ADHS18-198846

Title: Neuroprotective activity of sub-anesthetic ketamine-treatment in the progressive unilateral 6-OHDA-lesion rat Parkinson's disease model is not blocked by antagonizing BDNF signaling

Authors: *S. SINGH[1], C. J. STOPERA[4], M. J. BARTLETT[5], J. STANCATI[6], H. MORRISON[2], K. STEECE-COLLIER[7], T. FALK[3];
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Abstract: Treatment with sub-anesthetic ketamine has been shown to be neuroprotective in rodent models of cerebral ischemia and traumatic brain injury. Our lab has shown that sub-anesthetic ketamine is acutely anti-parkinsonian in a rat 6-hydroxydopamine (6-OHDA) model of Parkinson's disease (PD) and exhibits long-term reduction of L-DOPA-induced dyskinesia via brain-derived neurotrophic factor (BDNF). Here, we evaluated if ketamine also exhibits neuroprotective effects in a progressive 6-OHDA PD model via neurotrophic and/or inflammatory action. Male Sprague-Dawley rats were treated with either ketamine, ketamine + TrkB antagonist ANA-12 (0.5 mg/kg), or vehicle (6-hr treatment; 3 x 20 mg/kg; *i.p.*; 2-hrs apart) beginning 6-hrs prior to aunilateral intrastriatal 6-OHDA lesion (13.75 microg/site), and then treated daily with the 6-hr protocol for 7 days. Using amphetamine-induced rotations (mean ipsilateral rotations ± SEM) to estimate lesion severity, rotational asymmetry was assessed over 90-min. On Day 14 post-lesion, rats treated with ketamine (168 ± 90) showed fewer net ipsilateral rotations compared to vehicle (497 ± 170, and on Day 28, this effect of ketamine (211 ± 116) vs vehicle (666 ± 187; two-tailed t-test; $p<0.05$; n=15) reached significance, suggesting a neuroprotective effect. Rats treated with ketamine and ANA-12 (n = 8) showed a further decrease in net ipsilateral rotations on Day 28 at (-4.5 ± 48). We stained nigral dopaminergic neurons for tyrosine hydroxylase (TH) and unbiased stereology cell count (n=8) is ongoing. Semi-quantitative western analysis showed that relative striatal TH content (Lx/Intact) was increased by 85% in ketamine-treated rats compared to vehicle (n=7). The role of BDNF was evaluated via dual-labeling immunohistochemistry of TH and DAPI with *in situ* hybridization for RNA of BDNF and its receptor, TrkB, in the substantia nigra (SN). The combination of ketamine and ANA-12 was shown to significantly decrease TrkB receptor expression (total Ntrk2) compared to ketamine-only on the ipsilateralhemisphere ($p<0.05$; n=8; ANOVA), but not the contralateral hemisphere. To investigate anti-inflammatory action via activation of microglia, we stained SN and STR with a microglia marker, IBA1, and analyzed branch length/cell and end points/cell. We found that ketaminesignificantly reduced branch length/cell in the intact STR ($p<0.05$). Evaluation of cytokine levels in SN and STR is ongoing. In conclusion, an acute neuroprotective activity of ketamine is further supporting the ongoing clinical evaluation in individuals with PD.

Disclosures: S. Singh: None. **C.J. Stopera:** None. **M.J. Bartlett:** None. **J. Stancati:** None. **H. Morrison:** None. **K. Steece-Collier:** None. **T. Falk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TF has a patent for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease,licensed to PharmaTher Inc.,consulted,travel support from PharmaTher.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.08/C58

Topic: C.03. Parkinson's Disease

Support: CIHR
Montana Molecular

Title: Unraveling Dopamine D1 Receptor Signalling in vivo: Insights from Real-Time Brain Monitoring

Authors: *H. MOHAMMAD;
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Abstract: Despite considerable adverse consequences for patients, L-DOPA has remained the gold standard for the treatment of motor impairment in Parkinson's disease (PD) for the last 50 years. Remarkably, more efficacious drugs have not yet been developed. Dopamine D1 receptors (D1Rs) have long been exploited as a therapeutic target for the motor impairment in PD, but the search for clinically effective D1R agonists was, until recently, limited to drugs built on a catechol scaffold, resulting in poor pharmacokinetics and adverse cardiovascular effects. Recently, the discovery of the first non-catechol D1R agonists has revived clinical interest; these new compounds appear safe, well-tolerated and free of cardiovascular side effects. Non-catechol D1 agonists, as tested in cell culture, showed greater potency in activating G protein-dependent signalling than in promoting β-arrestin recruitment, and were hence initially described as G protein-*biased agonists*. However, it is unknown whether these compounds show similar bias *in vivo*, and what the downstream functional consequences might be. Our approach uses adeno-associated virus (AAV) to express genetically encoded biosensors for cAMP (cADDis) and arrestin (Borealis) in wild-type animals. By using cell-type selective promoters and Cre-recombinase-dependent promoters, we targeted biosensor expression to desired neuronal populations *in vivo*. We apply this approach to a healthy and 6-hydroxydopamine-treated rats, to study how signalling that arises from D1R stimulation is conveyed through G protein-dependent and -independent pathways in a rat model of Parkinson's Disease.

Disclosures: H. Mohammad: None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.09/C59

Topic: C.03. Parkinson's Disease

Support: R01 NIH NINDS (5R01NS122805-03)

Title: In a rodent model of L-DOPA-induced dyskinesia, coupling between primary motor cortex local-field and single-unit activity to movement is suppressed, and this coupling is not restored by low-dose ketamine

Authors: *K. CHINNARAJ[1], A. VISHWANATH[2], M. J. BARTLETT[3], T. FALK[3], S. L. COWEN[2];
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Abstract: Parkinson's Disease (PD) is a neurodegenerative disorder with motor symptoms arising from the loss of dopaminergic neurons. Levodopa (L-DOPA) is the first line treatment of PD, yet prolonged usage leads to L-DOPA induced dyskinesia (LID), characterized by hyperkinetic movements. LID symptoms correlate with finely-tuned gamma (FTG), which is a ~80 Hz oscillation in primary motor cortex (M1) and in basal ganglia subregions. Subanesthetic infusion of ketamine has recently been shown to disrupt FTG and dyskinetic behavior. Ketamine-related gamma has been correlated with increased locomotor activity. However, the relationship between LID-induced oscillations and individual neural responses to movement is less certain. This study examined the relationship between motor cortex local-field potentials and individual neuron activity to movement speed in a preclinical LID model and following ketamine administration. We predicted that dyskinesia would reduce neural correlations to movement speed in M1 and that ketamine would restore these correlations and reduce LID and FTG. Methods: 6-hydroxydopamine hemi-lesioned rat model of PD was used. PD animals were given L-DOPA (12 mg/kg;10 consecutive days) to establish a stable LID model. LID (n = 5) and Sham (n = 5) rats were implanted bilaterally with 16-tetrode hyperdrives (M1: AP 1.5, ML ±2.2, DV 2mm). Local-field potentials and single-unit activity (M1: n=2500 neurons recorded over 10 rats) were recorded following L-DOPA (12 mg/kg, i.p.), ketamine (20 mg/kg, i.p.), or vehicle administration. Movement speed was derived from inertial sensors mounted on the animal's head. Results: In vehicle conditions and in sham-lesioned animals we observed the expected positive correlation between gamma activity (50 - 100 Hz) and movement speed. Unexpectedly, the correlation between gamma, including FTG, and movement was eliminated in the dyskinesia condition, suggesting that dyskinesia suppresses M1 gamma-band activity related to movement. In accord with this observation, individual M1 neurons were less correlated with movement in dyskinesia. Contrary to our hypothesis, ketamine in the dyskinesia condition did not restore the correlation between gamma/FTG or single-unit activity and movement. These results indicate that dyskinesia decouples M1 activity from movement, and that ketamine does not restore this effect. This is surprising as ketamine reduced LID and eliminated LID-associated FTG.

Disclosures: K. Chinnaraj: None. **A. Vishwanath:** None. **M.J. Bartlett:** None. **T. Falk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TF has a patent for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, licensed to PharmaTher Inc., consulted, travel support from PharmaTher. **S.L. Cowen:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.10/C60

Topic: C.03. Parkinson's Disease

Support: Johns Hopkins University
SAMATA THERAPEUTICS

Title: Ketamine-conjugated nanotherapies rescue depressive like-symptoms in a rotenone-induced rat model of Parkinson's disease

Authors: *M. P. AVALOS[1], J. ALLENDE LABASTIDA[1], P. VYAS[1], J. LIU[1], C. FIELDS[2], T. AHMED[2], L. VALLIYAPPAN[2], W. LIYANAGE[3], N. KALE[3], K. M. RANGARAMANUJAM[3], S. KANNAN[1];
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Abstract: Depression is one of the most common psychiatric comorbidities in patients diagnosed with Parkinson's disease (PD). Around 50% of PD patients suffer from depressive disorders in the prodromal phase and most of them develop depressive-like symptoms at some stage of the disease. In addition to the complexity of this dual pathology, the efficacy of current antidepressants (ADs) for PD-related depression is limited since a large number of patients are resistant or partially responders. This poses a challenge for the treatment of this group of patients. In 2019, FDA approved S-ketamine as a nasal spray for adults with treatment-resistant depression and major depressive disorder with acute suicidal ideation or behavior, combined with an oral AD. However, its severe adverse effects, including sedation, dissociation, and abuse liability, require strict control and pose an obstacle in treatment. With the aim of improving the pharmacological intervention, this study explores the efficacy of novel ketamine-conjugated dendrimers in a rotenone (ROT)-induced rat model of PD-related depression. To mimic PD motor and non-motor symptoms, male Sprague Dawley rats were bilaterally infused with ROT (1.5 µg/µl/side), intra-Substantia Nigra pars compacta (SNpc - AP: -5.3 mm, L: ±2.0 mm, DV: -7.8 mm) through stereotaxic surgery. Animals displaying PD phenotype four days after surgical procedure underwent a systemic treatment regime of twice a week i.p. injections of 2.5 mg/Kg (drug-based doses) of ketamine (KET), ketamine-conjugated PAMAM-OH dendrimer (HD-KET), ketamine-conjugated glucose dendrimer (GD-KET) or saline (VEH) until the end of the experimental protocol. Behavioral tests were performed to monitor motor abilities, cognition and depressive-like behaviors. To evaluate the localization of dendrimers in the brain, i.v. injections of GD-Cy3 and HD-Cy5 were performed the day following surgery and, 24 h later, brains were collected for confocal microscopy studies. HD-KET and GD-KET, unlike KET, alleviated depressive-like symptoms by restoring social interaction and sucrose consumption. Interestingly, GD-Cy3 and HD-Cy5 robustly targeted cells into the SNpc of ROT-exposed rats indicating that targeted delivery of these dendrimer drugs to the region of injury was feasible. In vitro studies will be performed to evaluate the intracellular targets of both dendrimers. Our results provide novel promising approaches to implement tailored pharmacological interventions for the treatment of PD-related depression.

Disclosures: M.P. Avalos: None. **J. Allende Labastida:** None. **P. Vyas:** None. **J. Liu:** None. **C. fields:** None. **T. Ahmed:** None. **L. Valliyappan:** None. **W. Liyanage:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SAMATA THERAPEUTICS. **N. Kale:** None. **K.M. Rangaramanujam:** 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.; SAMATA THERAPEUTICS. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SAMATA THERAPEUTICS. **S. Kannan:** 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.; SAMATA THERAPEUTICS. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SAMATA THERAPEUTICS.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.11/C61

Topic: C.03. Parkinson’s Disease

Support: MJFF Andrew West

Title: Novel pharmacodynamic markers and assays in biofluids for LRRK2 kinase activity

Authors: *W. WANG[1], H. LI[1], A. B. WEST[2];
[1]Duke Univ., Durham, NC; [2]Pharmacol., Duke Univ., Durham, NC

Abstract: Parkinson's disease (PD) is an age-related neurodegenerative disorder that profoundly impacts patients' quality of life and imposes a significant burden on healthcare systems. Mutations within the *leucine-rich repeat kinase 2* (*LRRK2*) gene are among the most common genetic risk factors for both familial and sporadic PD. Pathogenic mutations in LRRK2 are known to increase LRRK2 kinase activity. This underscores the therapeutic potential of LRRK2 kinase inhibitors for PD, though pharmacodynamic biomarkers to track LRRK2 kinase activity, for example in response to candidate therapeutics, have not been sufficiently developed for use in routinely collected and banked biofluids. A specific, sensitive, and accessible platform for measuring LRRK2 kinase activity from banked biofluids may facilitate the success of LRRK2-targeting drugs in clinical trials through facilitating measures of target engagement on the patient level for the study duration, as well as assist patient selection for therapeutic intervention based on LRRK2 activity profiles.Herein we introduce an ultra-sensitive single-molecule array assay developed and validated by our laboratory to evaluate extracellular levels of LRRK2, Rab10, and phosphorylated Rab10 (pT73-Rab10) in small volumes of serum and cerebrospinal fluid (CSF).

In mice, strains with PD-linked VPS-35 mutations and over-expression of *SNCA* were treated with different LRRK2 kinase inhibitors to track pharmacodynamic responses in serum related to the ratio of pT73-Rab10 to total Rab10. Rats were also treated with LRRK2 kinase inhibitors, and blood serum samples were collected at different time-points following oral administration of LRRK2-targeting drugs. Finally, pharmacodynamic responses in these markers have been evaluated in non-human primate biofluids treated acutely or chronically with LRRK2 kinase inhibitors. Preliminary results suggest that both serum and CSF concentrations of total LRRK2, and the ratio of pT73-Rab10 to total Rab10, are highly pharmacodynamic and rapidly diminish with LRRK2 inhibition. These studies will reveal the relationship between free drug levels and the fluid biomarkers to help establish a foundation for the successful integration of these markers into ongoing clinical trials for LRRK2-targeting therapeutics.

Disclosures: W. Wang: None. **H. Li:** None. **A.B. West:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.12/C62

Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation Therapeutic Pipeline Program (MJFF-021205)
Cure Parkinson's UK RM2017002125
Sanofi grant

Title: Bruton'S tyrosine kinase (btk) is a druggable therapeutic target for neuroprotection in parkinson's disease

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Abstract: Parkinson's disease (PD) ranks as the second most prevalent neurodegenerative disorder globally. It is characterized by the gradual loss of nigrostriatal dopaminergic neurons and the buildup of α-synuclein aggregates, for which effective treatments to impede disease progression are currently lacking. Unresolving neuroinflammation and NLRP3 activation has been shown to drive PD pathology and progression, making it an attractive therapeutic target for disease modification. Our study aimed to investigate whether Bruton's Tyrosine Kinase (BTK), a pivotal regulatory for NLRP3 inflammasome activation, is activated in experimental PD and if

pharmacological inhibition could be effective to slow or halt disease progression. To confirm activation of BTK, we utilized post-mortem human patient samples. For mechanistic studies on BTK activation and inhibition, we used primary mouse microglia, in addition to two well-established preclinical PD models—the 6-OHDA model and the alpha-synuclein pre-formed fibril (PFF) model. Our observations demonstrate that pathological synuclein activates BTK, subsequently triggering NLRP3 inflammasome activation in microglia. Furthermore, BTK activation parallels NLRP3 activation in the nigrostriatal system of experimental PD models at corresponding time points. Pharmacological inhibition of BTK signaling effectively blocked inflammasome activation in vitro. Moreover, oral administration of the tool compound BTK inhibitor, ibrutinib, reduced NLRP3 inflammasome activation markers and neuropathology in preclinical PD models, while preventing dopaminergic neuron loss and striatal terminal degeneration. In summary, our findings suggest that BTK drives inflammasome activation and neuropathology in PD and that BTK inhibition represents a promising therapeutic strategy for disease modification in PD. Our current studies are evaluating the therapeutic efficacy of second-generation CNS-targeted BTK inhibitor, PRN2675, an analog of the clinical-stage drug Tolebrutinib (Sanofi Genzyme) which displays superior CNS permeability, target coverage and BTK occupancy compared to previous generation BTK inhibitors such as Ibrutinib.

Disclosures: K.H. Bhatt: None. **N. Groves:** None. **N. Jayabalan:** None. **D. Ofengeim:** A. Employment/Salary (full or part-time):; Sanofi. **N.A. Hagan:** A. Employment/Salary (full or part-time):; Sanofi. **R. Gordon:** None. **J. O'Sullivan:** None. **R. Adam:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.13/C63

Topic: C.03. Parkinson’s Disease

Title: Circuit-specific gene therapy reverses core symptoms in a primate Parkinson's disease model

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Abstract: Parkinson’s disease (PD) is a debilitating neurodegenerative disorder. Its symptoms are typically treated with levodopa or dopamine receptor agonists, but its action lacks specificity due to the wide distribution of dopamine receptors in the central nervous system and periphery. Here, we report the development of a gene therapy strategy to selectively manipulate PD-affected circuitry. Targeting striatal D1 medium spiny neurons (MSNs), whose activity is chronically suppressed in PD, we engineered a therapeutic strategy comprised of a highly

efficient retrograde adeno-associated virus (AAV), promoter elements with strong D1-MSN activity, and a chemogenetic effector to enable precise D1-MSN activation after systemic ligand administration. Application of this therapeutic approach rescues locomotion, tremor, and motor skill defects in both mouse and primate models of PD, supporting the feasibility of targeted circuit modulation tools for the treatment of PD in humans.

Disclosures: Y. Chen: None. **Z. Lu:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.14/C64

Topic: C.03. Parkinson's Disease

Support: Insight Centre for Data Analytics, Science Foundation Ireland
Boston Scientific
UCD Ad Astra Support

Title: Estimation of brain tissue electrical conductivity and permittivity in vivo for modelling directional deep brain stimulation leads

Authors: *J. EVERS[1], K. SRIDHAR[2], M. LOWERY[3];
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Abstract: Introduction: Dielectric brain tissue properties are key parameters in computational modelling of bioelectric fields, including those generated by directional leads for deep brain stimulation. However, conductivity and permittivity values are inconsistent across the literature and primarily based on post-mortem recordings, often neglecting the dispersive nature of biological tissue. Here, we recorded rodent dielectric brain properties *in vivo*, fitted the parameters to a Cole-Cole model and performed an *in silico* sensitivity analysis of the effect of conductivity on the electric field distribution and stimulation threshold for neurons. Current steering capabilities of directional electrodes were then investigated *in vivo* and *in silico*. **Methods:** 4-terminal probes for whole brain tissue (interelectrode distance 1.5 mm, electrode Ø 100 µm) and white/grey matter (1 mm, Ø 2 µm) were developed and cell constants for each probe calculated based on complex conductance in 0.1, 0.01 and 0.001 M KCl solution at 25°C with a conductivity of 1.41, 0.141 and 0.0141 S/m. In adult male and female rats (under isoflurane anaesthesia) whole brain post-mortem and *in vivo* and selective grey/white matter *in vivo* dielectric properties (20 Hz - 300 kHz, N = 22) were recorded. Directional and conventional pulse amplitude sets around a segmented DBS electrode were also recorded. A sensitivity analysis on the effect of tissue conductivity and an investigation of current steering was conducted using a DBS finite element model. Analysis by linear mixed models assessing the

effect of tissue property, frequency, group and sex with subject as a random intercept term (R version 4.1.215). Criterion for statistical significance: $p < 0.05$, power: 80%. **Results & conclusions:** Conductivity and permittivity were dispersive across the frequency range examined. *In vivo* conductivity was higher than post-mortem. Conductivity was higher in grey than white matter. The anisotropy ratio between white matter conductivity in corpus callosum (parallel) and internal capsule (perpendicular fibre direction) was 1.56. Permittivity did not differ significantly. *In silico*, lower bulk tissue conductivity led to higher electrical potentials around the lead and a change from 0.2 S/m ± 0.1 S/m results in an up to 53% change in electric potential. Current steering could be observed for directional leads *in vivo* and *in silico*. The results provide estimates of grey and white matter tissue conductivity and permittivity in vivo. The importance of selecting physiologically accurate values for dielectric tissues properties is demonstrated for both conventional symmetrical and directional stimulation leads.

Disclosures: J. Evers: None. **K. Sridhar:** None. **M. Lowery:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.15/C65

Topic: C.03. Parkinson's Disease

Title: Ntx101, an antibody to nitrated alpha synuclein, prevents spread of parkinson's disease pathology in multiple pre-clinical models

Authors: *A. KASHYAP[1], X. XU[1], A. ULUSOY[2], S. WRIGHT[1], K. KLINGER[2], B. LEWIS[1], S. LATHAM[1], D. A. DI MONTE[2], I. GRISWOLD-PRENNER[1];
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Abstract: Parkinson's is a neurodegenerative disease characterized by the pathological accumulation of alpha synuclein (aSyn) within neurons, resulting in motor dysfunction and various non-motor symptoms. aSyn is heavily post-translationally modified by multiple processes, including tyrosine nitration (1). We have developed an antibody, NTX101, that binds a specific nitrated tyrosine on aSyn with high affinity. We hypothesize that blocking the nitrated aSyn (nSyn) nidus of aggregation may prevent the spread of aSyn aggregate pathology in parkinsonian brain. To determine the ability of NTX101 to prevent pathological aSyn brain spreading, efficacy studies were conducted in 2 separate mouse models. As a first model, transgenic mice (M83 mice) overexpressing a human familial parkinsonism mutatant form of aSyn (A53T) had aSyn pathology induced by right striatum injections of human aSyn pre-formed fibrils (PFFs). The second model involved a unilateral injection of adeno-associated viral vectors (AAVs) delivering wild-type human aSyn DNA into the vagus nerve of C57Bl6 mice; at 2 and 3 weeks post AAV administration, mice received intraperitoneal injections of paraquat to promote

aSyn nitration. Antibodies were administered QWX6 in a prevention study, and QWX12 or QWX5 starting 1 week or 5 days after PFF or AAV treatment in intervention studies. Terminal blood and brain samples were collected for pharmacokinetic (PK) and brain immunohistochemistry (IHC); the latter included analyses for phosphoserine-129 aSyn (pS129), an aggregated aSyn marker in PFF-injected mice, and human aSyn in AAV-treated animals. Blinded IHC comparison of various mouse brain regions for NTX101 vs. 9E4, the murine parental antibody of prasinezumab, and/or mouse isotype control was assessed for relative efficacy in preventing pathology spread. NTX101 treatment statistically significantly reduced aSyn aggregates proximal and distal from the site of PFF introduction compared to 9E4 and/or isotype control antibodies. Treatment with NTX101 reduced pS129 aggregates in the substantia nigra and thalamus in the prevention and intervention PFF models. In the AAV-aSyn model, NTX101 treatment strikingly abrogated paraquat-induced aSyn spreading from the dorsal medulla oblongata toward pontine and midbrain regions. These consistent results in different mouse models are strong confirmation of nSyn's role as a pathogenic subspecies driving aggregate spread in the brain; thus, blocking nSyn represents an attractive therapeutic approach against PD. Towards this end, we have recently named a development candidate, NDC-0524, that targets nSyn and are advancing IND-enabling studies.

Disclosures: A. Kashyap: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nitrase Therapeutics. **X. Xu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nitrase Therapeutics. **A. Ulusoy:** None. **S. Wright:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nitrase Therapeutics. **K. Klinger:** None. **B. Lewis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nitrase Therapeutics. **S. Latham:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nitrase Therapeutics. **D.A. Di Monte:** None. **I. Griswold-Prenner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nitrase Therapeutics.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.16/C66

Topic: C.03. Parkinson's Disease

Title: Doxycycline and levodopa improve motor impairment in female and male rats with hemiparkinsonism induced by 6hydroxydopamine

Authors: *E. DEL BEL;
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Abstract: Doxycycline and levodopa improve motor impairment in female and male rats with hemiparkinsonism induced by 6-hydroxydopamine Thaís Antonia Alves-Fernandes [1]; Glauce Crivelaro-do-Nascimento [2]; Maurício Dos- Santos-Pereira [2,5]; Lorena Borges-de Abreu[3]; Airam Nicole Vivanco-Estela [2], Leonardo Calaça Arruda-V Vanderlei [1] R.ta Raisman-Vozari [3]; Patrick P. Michel [3]; E laine Del-Bel [1,2] [1] Physiology and [3]Neurology and Neurosciences, Medical School of Ribeirão Preto - University of Sao Paulo, Ribeirão Preto, Brazil; [2]Dental School of Ribeirao Preto- University of Sao Paulo, Basic and Oral Biology, Ribeirão Preto, Brazil; [4]Sorbonne Université, Paris Brain Institute-ICM, Inserm, CNRS, APHP, Hôpital de la Pitié Salpêtrière, 75013 Paris, France; [5]Stanley Center, Broad Institute, Cambridge, Massachusetts 02142, USA.
We analyze the effect of doxycycline (Doxy) on motor impairment in hemiparkinsonian *Wistar Hannover* rats of both sexes induced with 6-hydroxydopamine (6-OHDA, Ethics Committee 2020.1.473.58.0). Female and male rats underwent stereotaxic surgery for unilateral administration of 6-OHDA (16 μg) in the medial forebrain bundle. Fifteen days after surgery, the impairment of dopaminergic function was assessed using the forepaw adjusting stepping test (forelimb akinesia).. The animals were divided into the following experimental groups: Control (no lesion), 6-OHDA, 6-OHDA+Doxy 40mg/kg (i.p) and 6-OHDA+L-DOPA/benserazide 10mg/kg (s.c.). The treatments continued for 15 days. Tyrosine hydroxylase immunohistochemistry and motor assessments data were analyzed using One-way ANOVA followed by Tukey's multiple comparison tests ($p < 0.05$). The 6-OHDA lesions induced significant and enduring impairments in stepping test movements in both sexes, affecting the contralateral paw. Only male rats with 6-OHDA lesions exhibited a decrease in activity on the actimeter test and shorter latency on the rotarod compared to the control group. Surprisingly, similar to L-DOPA, Doxy reversed the lesion-induced deficits in forelimb akinesia in males and females and improved rotarod motor coordination in male rats. Evaluation of dopaminergic lesions showed similar severity in both sexes, comparable to that of 6-OHDA lesioned rats. In conclusion, Doxy presented an effect on forelimb akinesia equivalent to L-DOPA, in both sexes of animals. This effect persists independently of striatal reinnervation, assuming complete denervation of the striatum induced by 6-OHDA (5 weeks post-lesion).
Keywords: ; neuroprotection; tetracyclines; neurodegenerative disease;
Financial support: CAPES; FAPESP, CNPq

Disclosures: E. Del Bel: None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.17/C67

Topic: C.03. Parkinson's Disease

Title: Nanoparticle-based nose-to-brain delivery of siRNA or miRNA to reduce alpha-synuclein pathology in vitro (SH-SY5Y) and in vivo (Thy1-aSyn line 61)

Authors: *I. DRATH[1], M. FEJA[1], S. WEIß[1], B. GERICKE[1], A. EWE[2], A. AIGNER[3], F. RICHTER[1];
[1]Dept. of Pharmacology, Toxicology and Pharm., Univ. of Vet. Med. Hannover, Hannover, Germany; [2]Dept. of Pharmacology, Pharm. and Toxicology, [3]Univ. of Leipzig, Leipzig, Germany

Abstract: Potential strategies to develop new treatments for Parkinson`s disease (PD) include targeting alpha-synuclein (aSyn) and the use of non-coding RNAs. Thus, downregulation of the major disease-associated protein aSyn by the therapeutic use of small interfering RNA (siRNA) holds great potential. Further, alterations of PD-associated micro RNAs (miRNAs) are found in patients, making the restoration of physiological levels using miRNA mimics an interesting treatment approach. Nevertheless, efficient delivery of small RNA is challenging due to their instability and the need to bypass the blood-brain barrier. Therefore, we developed a non-invasive nanoparticle (NP)-based approach for intranasal delivery of small RNAs, opening this gene therapy strategy to broad clinical application. Uptake of fluorescence-labeled NPs into differentiated SH-SY5Y neuroblastoma cells was confirmed by confocal microscopy. qPCR analysis of aSyn overexpressing SH-SY5Y revealed significant reduction of *SNCA* mRNA levels after treatment with NPs loaded with siRNA targeting human *SNCA* mRNA (siSNCA-NPs). To determine NPs that best distribute the RNAs in the CNS and influence the target protein most efficiently, different AF647-labeled NPs or siSNCA-NPs were administered intranasally to 2-month-old male aSyn overexpressing (Thy1-aSyn, line 61) mice once daily on 4 consecutive days. Labeled NPs distributed extensively across the brain and were detectable in different regions including the olfactory bulb, substantia nigra and prefrontal cortex. Quantitative evaluation by immunohistochemistry revealed that cationic polymers reached the brain in the highest amount. Western blot and qPCR analysis showed, that siSNCA-NPs significantly reduced brain aSyn protein levels as well as *SNCA* mRNA levels. RNA sequencing revealed PD-relevant miRNA alterations in the substantia nigra pars compacta of 6-month-old Thy1-aSyn mice. To find appropriate doses for a longitudinal therapeutic intervention, promising miRNAs and siSNCA were complexed with the most efficient NPs and intranasally applied to 6-month-old transgenic mice for four days. After only four days of intranasal administration, miR-NPs increased miRNA brain levels with a polymer-dependent efficacy. Mice showed no overt adverse behavioral effects nor increased reactive microglia. We are now exploring whether long-term NP-mediated intranasal application of small RNA can improve motor, cognitive and olfactory dysfunctions, reduce aSyn levels and alter miRNA levels in the brain in our PD mouse model, and could thus be a novel therapeutic approach to treat PD.

Disclosures: I. Drath: None. **M. Feja:** None. **S. Weiß:** None. **B. Gericke:** None. **A. Ewe:** None. **A. Aigner:** None. **F. Richter:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.18/C68

Topic: C.03. Parkinson's Disease

Support: NIEHS grant R35-ES030523

Title: Pathologic α-synuclein and Proinflammatory Modulators in Extracellular Vesicles are Reduced in Transgenic SNCA Mice with Dnm1l Heterozygous Knockout

Authors: *H. J. BROWN[1,2,3], R. Z. FAN[4], Y. LAI[4,2], K. TIEU[4,2];
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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder. Disease-modifying therapies for this devastating disease are urgently needed, not only to alleviate symptoms but also to prevent disease progression. Our lab has reported that partial inhibition of dynamin-related protein 1 (Drp1), a well-established regulator of mitochondrial fission, through genetic approaches decreases neurotoxicity and α-synuclein aggregation and transmission between cells *in vitro* whilst improving mitochondrial function and autophagic flux. We further demonstrate the relevance of Drp1 in PD by showing that it is transcriptionally upregulated (p=0.0080) in the ventral midbrain of PD patients compared to healthy age matched controls. Extracellular vesicles (EVs), small lipid nanoscale particles that contain a myriad of different proteins and nucleic acids have been implicated as a pathogenic mechanism of oligomeric α-synuclein transfer between cells in PD aiding in disease progression. More specifically, they serve as a seed for further protein aggregation to occur. Based on prior research, we hypothesized that *in vivo* heterozygous Drp1-KO (*Dnm1l*$^{+/-}$) would reduce the levels of neurotoxic molecules such as α-synuclein and inflammatory modulators in EVs and their subsequent ability to transmit between different cell types *in vitro* and *in vivo.* To test this hypothesis, we crossed our *Dnm1l*$^{+/-}$ mice with transgenic mice overexpressing wild type human α-synuclein under the control of Thy1 promoter (*SNCA*$^{+/-}$). Ultracentrifugation was used to isolate EVs and hereafter were characterized using Nano tracking analysis, transmission electron microscopy and western blot for EV markers CD63, flotillin-1 and, annexin-A2. Our data indicate that *Dnm1l*$^{+/-}$ reduced the total levels of α-synuclein (p=0.0005) and its pathogenic form pS129 (p=0.0185) in the EVs of 12-month-old *SNCA*$^{+/-}$:*Dnm1l*$^{+/-}$ mice compared to *SNCA*$^{+/-}$:*Dnm1l*$^{+/+}$ mice. The levels of inflammatory cytokines such as lipocalin 2 were also significantly reduced (p=0.0028). Furthermore, *Dnm1l*$^{+/-}$ reduced the transmission and subsequent aggregation of α-synuclein in EVs isolated from *SNCA*$^{+/-}$:*Dnm1l*$^{+/+}$ mice administered to primary astrocytes and microglia. Current research focuses on the intracellular protective effects of *Dnm1l*$^{+/-}$ on preventing protein aggregation and inflammation in *SNCA*$^{+/-}$:*Dnm1l*$^{+/+}$ mice when exposed with an inflammatory insult using EVs isolated from C57BL/6 mice treated with lipopolysaccharide. Our data suggests that inhibition of Drp1 may be a promising therapeutic target for PD, by reducing the transmission of α-synuclein and inflammatory mediators between cells.

Disclosures: H.J. Brown: None. **R.Z. Fan:** None. **Y. Lai:** None. **K. Tieu:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.19/C69

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: CONAHCYT Grant N° 1028543
UDG P3E-UDG-2022/2023

Title: Bee venom reduces inflammation and oxidative stress after lipopolysaccharide injection in substantia nigra-striatum axis.

Authors: *A. LOMELI LEPE[1], S. J. LOPEZ-PEREZ[2], J. CASTAÑEDA-CABRAL[3], M. E. URENA-GUERRERO[4], G. GUDIÑO-CABRERA[5];
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Abstract: Neuroinflammation and oxidative stress are important features in the pathogenesis and development of neurodegenerative diseases, the microglial activation and upregulation of pro-inflammatory mediators induce neuronal cell death. Recent studies have shown that bee venom (BV) has beneficial effects on several diseases like synucleinopathies in which the substantia nigra (SN) and striatum (STR) are areas particularly susceptible to neurodegeneration. BV is known to exert anti-inflammatory, anti-oxidative, and anti-immune effects. Here, we investigated the effects of BV over the different inflammatory and oxidative markers, in a lipopolysaccharide (LPS) rat model. We examined whether BV (1.5 mg/kg by acupoint injection ST36 six times every 48 hours) changes the activation of microglia and astrocytes by immunofluorescence in SN and STR brain areas; also quantify the proinflammatory cytokines levels (TNF-α and IL-1β) by ELISA, and estimated the lipid peroxidation and the activity of superoxide dismutase (SOD) and catalase (CAT) by colorimetric kits in LPS-treated rats (2.5 µg by a single dose intranigral injection) in STR. In the LPS-injected rat brain, BV treatment reduced microglia and astrocyte activation in SN and STR. Furthermore, BV decreases IL-1β and lipid peroxidation and increases the CAT activity in the STR. These results indicate that BV can inhibit LPS-induced neuroinflammation and oxidation, also, these results suggest that BV could be a promising treatment option for synucleinopathies and other neurodegenerative diseases.

Disclosures: A. Lomeli Lepe: None. **S.J. Lopez-Perez:** None. **J. Castañeda-Cabral:** None. **M.E. Urena-Guerrero:** None. **G. Gudiño-Cabrera:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.20/

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Dr. Diana and Ziga Elton Laboratory for Molecular Neuroendocrinology
Drs. Ronith and Armand Stemmer, French Friends of Tel Aviv University
Anne and Alex Cohen, Canadian Friends of Tel Aviv University
Exonavis Therapeutics

Title: Adnp/davunetide multi-site protection against tauoapthy

Authors: *I. GOZES;
Sch. of Medicine/Tel Aviv Univ., Tel Aviv, Israel

Abstract: At the turn of the century, we identified activity-dependent neuroprotective protein (ADNP). Unraveling in vivo activities, we developed ADNP deficient mice, discovering that it is essential for embryogenesis/brain formation. Adnp+/- mice survive, but display developmental delays, motor, cognitive and social impairments associated with tauopathy and neurodegeneration. Somatic mutations in ADNP parallel tauopathy in postmortem Alzheimer's disease brains. De novo mutations in ADNP in children cause the ADNP syndrome, and by postmortem analysis, we discovered tauopathy in a brain of a 7-year-old ADNP boy having an ADNP mutation. Modeling this by CRISPR-Cas9 editing in mice (Biol Psychiatry.2022;92:81), we showed protection by the ADNP fragment, investigational drug, davunetide (NAP). The sequence of davunetide (NAPVSIPQ) allows multiple interactions protecting against tauopathy (Eur J Neurosci.2023;58:2641). Specifically, the SIP motif Interacts with microtubule (MT) end binding proteins (EB1 and EB3) enhancing ADNP/SIRT1 (healthy aging)-EB1/EB3 binding, augmenting Tau-MT association. NAPVSIP interacts with proteins containing SH3 domains essential for cytoskeletal function (Mol Psychiatry.2022;27:3316). NAPVSIPQ binds the armadillo domain of beta catenin important for WNT signaling critical for development. ADNP shuttles between the nucleus and the cytoplasm (Mol Psychiatry.2023;28:1946). Importantly, NAPVSIPQ also enters the cell nucleus interacting with an ADNP zinc finger domain (Cells.2023;12:2251), protecting ADNP transcriptional regulation of AKT signaling associated with Tau hyper-phosphorylation. ADNP further interacts with LC3 forming the autophagosome, an interaction that is augmented by davunetide further protective against tauopathy. Conversely, we linked tauopathy with a bi-directional ADNP dysregulation, including ADNP association with Tau mRNA alternative splicing, as well as regulation of steroid hormones toward sexual differences. Thus, ADNP syndrome dysregulation of communicative abilities is accentuated in boys (J Mol Neurosci. 2024;74:15). Furthermore, analyzing clinical trial results, we discovered accelerated disease progression in women suffering from the pure tauopathy progressive supranuclear palsy (PSP), which was partly ameliorated by davunetide treatment (Transl Psychiatry. 2023;13:319) toward new research and development.

Disclosures: I. Gozes: A. Employment/Salary (full or part-time):; Exonavis Therapeutics.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.21/C70

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH Grant DK044442
UH-NEOMED Faculty Scholars

Title: Loss of function of the Takeda G Protein-Coupled Receptor 5 (TGR5) in aged mice is associated with prefrontal cortex and hippocampal pathology and cognitive dysfunction

Authors: S. BOEHME[1], J. LEPP[2], K. CARTER[2], ***S. FLEMING**[3], J. FERRELL[1];
[1]Integrative Med. Sci., Northeast Ohio Med. Univ., Rootstown, OH; [2]Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH; [3]Northeast Ohio Med. Univ., Rootstown, OH

Abstract: The bile acid receptor Takeda G Protein-Coupled Receptor 5 (TGR5) is involved in metabolic regulation and inflammation in the liver and gut. Studies of TGR5 in the brain are limited, but it has been examined as a mediator of gut-brain communication in the context of neuroinflammation, neuropsychiatric function, and neurodegenerative disease. Loss of function of TGR5 in mice is associated with glucose intolerance, bile acid dysfunction, and gut inflammation. However, the long-term effect of TGR5 knockout on the brain and behavior is unknown. In the present study wildtype (WT=13) and TGR5 knockout (TGR5 KO=8) male mice were aged to 22-26 months and tested on a battery of behavioral tests to assess sensorimotor, neuropsychiatric, and cognitive function. In the brain, protein expression was determined for alpha-synuclein, tau, and beta-amyloid within the prefrontal cortex, striatum, hippocampus, and substantia nigra. Expression of genes associated with neuroinflammation, mitochondrial function, and neurotrophic support were measured in the prefrontal cortex and hippocampus. Behaviorally, TGR5 mice showed impairments in cognitive function compared to WT in the Y-maze, object recognition, and Barnes maze tests. In the brain in TGR5 KO mice, phosphorylated alpha-synuclein was increased in the prefrontal cortex and the phosphorylated tau/total tau ratio was increased in the hippocampus compared to WT. Gene expression analysis showed decreased BDNF and increased TNFalpha in the hippocampus and decreased SIRT6 in the prefrontal cortex in TGR5 KO compared to WT mice. These data indicate that loss of TGR5 in aged mice negatively impacts cognitive performance and is associated with pathology in the prefrontal cortex and hippocampus.

Disclosures: S. Boehme: None. **J. Lepp:** None. **K. Carter:** None. **S. Fleming:** None. **J. Ferrell:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.22/C71

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Remodeling of brain energy metabolism in murine models with defect in glycine cleavage

Authors: *A. LOPEZ-RAMIREZ[1], K. HALDAR[2];
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Abstract: Nonketotic hyperglycinemia (NKH) is a neurometabolic disease of varying severity that arises from mutations in the mitochondrial enzyme glycine decarboxylase (GLDC), however, little is known about its consequences on brain energy metabolism. We show that severe active-site mutations in GLDC induced a five-fold increase in brain-glycine levels (to 400 ng/mg tissue) with concomitant depletion of both L and D serine. In their brain proteome, the signature is distinct from wild type and heterozygous mice ($p < 0.01$) and elevation in intermediates of glucose breakdown and mitochondrial oxidative fatty acid metabolism, as well as metabolites that signal energy deprivation and blunt oxidative stress. Comparative analyses showed attenuated mutations induced a two-fold increase in glycine, with no measurable change in levels of L and D serine. Combination of precision genetics, proteome, and metabolite analyses suggests that early changes in the remodeling of brain energy metabolism induced by reduction in GLDC are complex, with implications for treatment of both attenuated and severe NKH disease.

Disclosures: A. Lopez-Ramirez: None. **K. Haldar:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.23/C72

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: Effect of chronic hyperprolactinemia on spike-wave discharges in an animal model of tubulinopathy

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Abstract: The *taiep* rat is the only long-life expectancy model of the human tubulinopathy named hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC), with face validity because it presents a point mutation in the tubulin β 4A gene and with similar findings in MRI features. Furthermore, they present spike-wave discharges (SWDs) on the electroencephalogram (EEG) that resemble absence seizures in humans. Absence seizures occur mainly during childhood, with sexual dimorphism, being girls more affected than boys. Therefore, it is possible that sex hormones such as prolactin may modify SWDs. We previously showed that acute prolactin administration increased the duration of SWDs in adult female *taiep*. The aim of the study was to analyze the effect of chronic hyperprolactinemia on frequency and duration of SWDs. Five-month-old female *taiep* rats were implanted with stainless steel screw electrodes in the cerebral cortex, one week later a 24-h EEG was performed. Subsequently, two adenohypophyses were grafted in the renal capsule to induce hyperprolactinemia, then a 24-h EEG recording was performed one month after the graft. The frequency and duration of the SWDs were quantified and compared with control recordings. Our results shown that hyperprolactinemia significantly increased the mean frequency of SWDs compared to control recordings from 49.67 ± 18.75 to 119.3 ± 25.3 (Mann Whitney U test $P<0.05$), but there were not changes in their mean duration . In conclusion, this neuropeptide modulates SWDs and could be a factor in the regulation of human absence seizures and in their expression during puberty.

Disclosures: E. Hernandez Alvarado: None. **C. Cortes:** None. **J.R. Eguibar:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.24/C73

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Characterization of the transgenic human TDP-43^{Q331K} mouse model of Amyotrophic Lateral Sclerosis (ALS)

Authors: *T. VOGELS;
InnoSer, Bilthoven, Netherlands

Abstract: TAR DNA binding protein 43 (TDP-43) encoded by the TARDBP gene, is a major pathological protein involved in the pathogenesis of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia. TDP-43 pathology is observed in 97% of all cases of ALS, making this protein a major therapeutic target for ALS treatment. Therefore, in this study, we performed an

extensive phenotypic characterisation of a transgenic human TDP-43^{Q331K} (Jackson Laboratories #017933) mouse model of ALS to assess its suitability in preclinical research. Transgenic TDP-43 mice expressing human TDP43 with the Q331K mutation and control mice expressing wild-type (WT) mouse TDP-43 protein were examined at three different ages (4, 6 and 8 months). Motor function was assessed by a battery of tests (spontaneous behaviour in automatic home-cages, inverted grid, weight-lifting test, rotarod, CatWalk test, and grip strength. Nerve conduction was by testing the compound muscle action potential (CMAP). Spinal cord tissue was isolated for histopathology analyses. Blood was collected for neurofilament light chain plasma biomarker analysis. Compared to WT, TDP-43^{QQ331K} mice had significantly lower muscle weight, despite higher body weight. Spontaneous behavior analysis revealed significantly lower activity duration during dark phase, significantly lower on-shelter visit during the dark phase at 6 and 8 months, and significantly longer shelter visits at all timepoints of the TDP-43Q331K mice. Principal component analysis of multiple variables in the automated PhenoTyper home-cages showed consistent phenotypes at different ages, with a progressively larger effect size over time. Muscle strength as measured via inverted grid was significantly lower at 6 and 8 months of age and at all timepoints in the weights-lifting test and rotarod in TDP-43Q331K mice. Reduction of CMAP amplitude was seen in TDP-43 mice, suggesting loss of functional motor axons . The CatWalk test at 7 months and Composite Phenotype Scoring System at 7-9 months also showed deficits in multipe motor function domains. In conclusion, we have shown that the motor deficits in TDP-43^{Q331K} mice mimic the human course of ALS pathophysiology, indicating the suitability of this model in combination with selected behavioral readouts for preclinical studies for new ALS and/ or FTD treatment strategies.

Disclosures: T. Vogels: A. Employment/Salary (full or part-time):; InnoSer.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.25/C74

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: R01AG074552

Title: Behavioral characterization of congenic B6 PS19 transgenic mice

Authors: *K. STANGIS[1], D. A. LAWRENCE[2], G. G. MURPHY[3];
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Abstract: Tauopathies are a family of neurodegenerative diseases characterized by tau deposits within the brain. The two most prevalent types of early-onset dementia, Alzheimer's disease (AD) and Frontotemporal dementia (FTD), fall into this disease class. The PS19 mice carrying

the MAPT P301S mutation, a missense mutation associated with familial FTD, are a widely used tauopathy model. The PS19 transgenic mice express human mutant tau at a level five times greater than endogenous mouse tau, and reportedly display age associated cognitive decline. Cognitive decline is a hallmark symptom of numerous forms of dementia. Animal models which closely emulate progressive memory loss and other cognitive deficits seen in dementia patients serve as valuable research tools in the development of novel therapeutics. Thus, it is essential to define the timeline of cognitive changes as they occur within a specific model. Originally generated within the laboratory of Virginia Lee, the PS19 mice are most well characterized on the mixed B6C3H/F1 and minimally congenic C57BL/6J backgrounds. There is comparatively little literature available describing the behavioral phenotype of commercially available PS19 mice which have been crossed to the C57BL/6NJ background for several generations (Jax No. 024841). When maintained on the mixed or minimally congenic backgrounds, PS19 transgenic mice reportedly display impaired spatial learning and memory in the hippocampal-dependent Morris water maze (MWM) task at 6 months of age, as well as hyperactivity and reduced anxiety-like behavior in the open field. Progressive motor impairment has also been reported between 7 to- 10 months of age. To determine whether PS19 mice maintained on the C57BL/6NJ background (PS19-B6) follow a similar time course of cognitive and motor decline, we conducted a battery of behavioral tests. Behavioral testing conducted at 6 months of age revealed that PS19-B6 transgenic mice display hyperactivity and reduced anxiety-like behavior in the open field. However, we found spatial learning and memory in the MWM to be intact at 6 months of age. Rotarod testing revealed no deficits in motor learning and coordination at 6 months of age, nor at a later timepoint (10 months of age). These findings suggest that hippocampal-dependent deficits of spatial learning and memory emerge at a later timepoint in the PS19 animals maintained on the C57BL/6NJ background than those on the mixed or minimally congenic background. Additional MWM testing will be conducted at 8 months of age, as 10-month rotarod results suggest motor output at this age is sufficiently intact.

Disclosures: K. Stangis: None. **D.A. Lawrence:** None. **G.G. Murphy:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.26/C75

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Capturing Subtle Differences in Mouse Behaviour Induced by Gene Mutations and Pharmacological Intervention using Motion Sequencing

Authors: *G. RIEDEL[1], J. BRAY[2];
[1]Inst. of Med. Sci., Univ. Aberdeen, Aberdeen, United Kingdom; [2]Univ. of Aberdeen, Aberdeen, United Kingdom

Abstract: Measuring behaviour in rodents typically employed point tracking over standard 2-D video to summarise ‘typical’ parameters such as distance, speed, and position. More recently, researchers have utilised depth cameras to track behaviour in 3-D and gain a richer understanding of animal behaviour and behavioural anomalies. Much like with 2-D videos, depth recordings also require identification and labelling of specific movements which leads to results that encompass only behaviours which can be observed by a human. To address these limitations, there has been growing interest in devising unsupervised, data-driven methods that can identify the inherent patterns and delineate how experimental interventions bring about changes in behavioural [Egnor (2016) doi.org/10.1146/annurev-neuro-070815-013845]. A total of 20, 5-month-old, female mice were used for this experiment. Ten NMRI mice were used as controls; ten Line66 mice that express the full-length human Tau protein, carrying a double mutation (P301L & G335D) were used as a model of tauopathy. These mice have abundant tau pathology distributed throughout the brain and present with specific sensorimotor impairments [Melis (2015) doi.org/10.1007/s00018-014-1804-z]. To assess changes in behaviour associated with pharmacological intervention, mice were injected with either Saline (IP) or MK801 (IP: 0.2mg/kg) 30 minutes before the start of the Open Field test. The mice were recorded in the arena (50 x 50 cm) with an overhead Kinect v2 sensor [https://learn.microsoft.com/en-us/windows/apps/design/devices/kinect-for-windows]. The resulting depth videos were fed into a motion sequencing pipeline [Wiltschko (2020) doi.org/10.1038/s41593-020-00706-3]. The unsupervised pipeline was able to identify certain behavioural motifs which significantly differed between MK801 animals and saline animals. Behaviours which increased in usage with MK801 closely matched the known pharmacological effect of MK801 at this dose, namely uncoordinated locomotor activity, bursts of rapid turning behaviour, head weaving and body rolling. MK801 also reduced the usage of certain behaviours compared to saline treated animals, these consisted mainly of vertical movements such as rearing and sniffing. No differences were observed between genotypes. Motion sequencing captured and quantified changes in mouse behaviour induced by MK801. Whilst this experiment could benefit from larger sample sizes, the unsupervised machine learning pipeline reported pharmalogically induced behavoural changes and therefore contributes to the broader validation of such methodologies in behavioural pharmacology research.

Disclosures: G. Riedel: None. **J. Bray:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.27/C76

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH Grant 1RF1 NS117628-01A1

Title: Mri characterization of brain structural changes in a novel rat model of progressive supranuclear palsy

Authors: *S. CLARK[1], K. KALIYAPPAN[2], M. P. LEIGH[2], S. SURESH[2], S. AMEYAA[2], H. NURI[2], J. SPERNYAK[3];
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Abstract: Background: Progressive supranuclear palsy (PSP) is a complex neurodegenerative disorder characterized by motor, cognitive, and emotional impairments, often mimicking Parkinson's disease (PD). A hallmark of PSP is the aberrant aggregation of tau protein into neurofibrillary tangles (NFTs) in brain regions associated with motor control and cognition. Magnetic resonance imaging (MRI) aids in diagnosing PSP by revealing the brain structural changes like midbrain atrophy and ventricular enlargement. Our previous work demonstrated that selective overexpression of wild-type human tau in pedunculopontine tegmentum (PPT) cholinergic neurons induce PSP-like symptoms and pathological tau inclusions. This study aims to evaluate the brain structural changes resulting from tau accumulation in PPT cholinergic neurons, providing insights into the pathomechanisms driving PSP-like symptom development.**Methods:** We utilized a CRE-dependent adeno-associated virus (AAV8) vector to overexpress wild-type human tau in PPT cholinergic neurons of rats. Behavioral assessments were conducted at 5- and 18-months post-infection to evaluate motor, anxiety, and cognitive functions. MRI measures of lateral ventricle and MRS analyses of choline metabolite levels and magnetization transfer ratio (MTR) were performed at 7-, 12-, and 17-months post-infection to assess brain structural changes.**Results:** Our findings revealed significant lateral ventricle enlargement in tau-expressing rats at 12- and 17-months post-infection. Female tau-expressing rats exhibited elevated choline metabolite levels, indicating cellular damage, particularly at 12 and 17 months. Ongoing analyses include evaluating volume changes in the midbrain: pons ratio, volumetric analysis of corpus callosum, inferior colliculus and cerebral aqueduct.**Conclusions:** Our study demonstrates a robust modelling of PSP through selective tau overexpression in PPT cholinergic neurons, mirroring key pathological features observed in PSP patients. The observed lateral ventricle enlargement and alterations in choline metabolites serve as distinguishing markers from PD and highlight the progressive nature of PSP-related brain pathology. Importantly, our findings provide insights into the early onset of PSP pathology relative to symptomatic manifestation, advancing our understanding of disease progression timelines and aiding in the identification of potential PSP-specific biomarkers.

Disclosures: S. Clark: None. **K. Kaliyappan:** None. **M.P. Leigh:** None. **S. Suresh:** None. **S. Ameyaa:** None. **H. Nuri:** None. **J. Spernyak:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.28/C77

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH Grant K00NS108458
BWF 1022360

Title: Tau Pathology Correlates With Motor Deficits in a Mouse Model of Progressive Supranuclear Palsy

Authors: *S. SRIDHAR[1], R. B. CREED[2], A. B. NELSON[3];
[1]Univ. of California, San Francisco, San Francisco, CA; [2]Neurol., Univ. of California, San Fransisco, San Francisco, CA; [3]Neurol., UCSF, San Francisco, CA

Abstract: Progressive Supranuclear Palsy (PSP) is a neurodegenerative movement disorder characterized by impairments in gait, eye movements, and often, cognitive-behavioral function. Gait abnormalities lead to early loss of balance and falls. While the underlying circuitry of this disease is not well understood, post-mortem brain tissue from PSP patients shows pathology including the microtubule binding protein Tau. Early sites of Tau accumulation include the basal ganglia (BG) and midbrain eye movement and locomotor centers. However, little is known about how pathological Tau might lead to the symptoms and signs of PSP. There are no broadly accepted animal models of PSP, which has slowed progress in understanding the pathogenesis and pathophysiology of PSP and the development of therapies. Here we adapted an existing transgenic mouse model expressing mutant human Tau (hTau.P301S), with the goal of examining the relationship of Tau pathology to PSP-like phenotypes. Consistent with prior studies, we find that transgenic Tau mice show progressive gait and balance impairments. At these same later timepoints, we have also newly identified deficits in rapid eye movements. Here, we test whether pathological Tau accumulation in BG and midbrain structures predicts motor deficits. We quantified Tau aggregates using immunohistochemistry and image analysis software (QuPath). We find that Tau transgenic mice show age-dependent Tau accumulation across multiple brain structures, including cortex, basal ganglia, midbrain and brainstem motor areas, and the deep cerebellar nuclei. While Tau aggregation varies between animals, our data suggests that Tau pathology in the mesencephalic locomotor region (including the pedunculopontine and cuneiform nuclei) correlates with gait/balance impairment in Tau transgenic mice. Together, this data connects Tau pathology and PSP-like motor phenotypes in a mouse model.

Disclosures: S. Sridhar: None. **R.B. Creed:** None. **A.B. Nelson:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.29/C78

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: BWF PDEP 1022360
NIH K00NS108458

Title: Impaired Saccadic Eye Movements in Progressive Supranuclear Palsy Mouse Models

Authors: *R. B. CREED[1], S. HARRIS[2], S. SRIDHAR[4], F. DUNN[5], A. B. NELSON[3];
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Abstract: Progressive Supranuclear Palsy (PSP) is a neurodegenerative disease that affects movement, behavior, and cognition. Due to an overlap in symptoms with Parkinson's disease, PSP is considered an atypical parkinsonian disorder, but PSP patients have distinct clinical and pathological features. Clinically, PSP patients have early gait abnormalities, frequent falls, gaze palsy (slowed saccadic eye movements), and tend not to respond to dopamine replacement therapy. Pathologically, aggregated Tau protein (rather than alpha synuclein) accumulates in the brain of PSP patients. As in other neurodegenerative disorders, a combination of cellular dysfunction and cell loss is believed to drive disease symptoms. However, a lack of animal models for PSP has hindered investigation of the causal links between neuropathology, cellular and circuit dysfunction, and symptoms. Here, we have utilized the two different approaches to modeling tauopathy to determine whether Tau pathology is sufficient to recapitulate key PSP phenotypes in mice. We find Tau transgenic mice have impaired motor performance in both the open field and accelerating rotarod test. Additionally, Tau mice also have impaired gait on a linear track. Moreover, we see that overexpression of Tau in both of our models results in eye movement impairments. Overall these findings show that Tau pathology is sufficient to cause locomotor and oculomotor impairments in mice and allows us to model PSP thus providing a platform to investigate the changes in neural structure and function that drive the movement abnormalities seen in disease.

Disclosures: R.B. Creed: None. **S. Harris:** None. **S. Sridhar:** None. **F. Dunn:** None. **A.B. Nelson:** None.

Poster

PSTR157: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR157.30/C79

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: SERB

Title: Prefoldin5 is a Microtubule-associated Protein that Suppresses Tau-aggregation

Authors: *A. BISHT;
Dept. of Biol. Sci., IISER BHOPAL, BHOPAL, India

Abstract: Tauopathy is one of the major neurodegenerative disorders caused by mutations in the microtubule-associated protein Tau. While the current models of Tau function support the idea that decreased Tau-microtubule association leads to microtubule instability, the role of other microtubule-associated proteins in the regulation of Tau-microtubule association remains elusive. Using ommatidial degeneration as a readout, we identified Pfdn5 as a genetic modifier of $hTau^{V337M}$ overexpression defects. We discovered that Pfdn5 colocalizes with the microtubules and loss of Pfdn5 results in disruption of microtubules. *Pfdn5* mutants showed severe reduction in Tubulin levels and enhanced the Tau-induced neurotoxicity and synaptic defects characterized by the accumulation of Tau-aggregates in the motor neuron axons. Tubulin-dependent rescue of Tau-induced synaptic phenotypes critically required the presence of Pfdn5. Remarkably, neuronal expression of Pfdn5 alleviates the age-dependent neurodegeneration and memory deficits induced by $hTau^{V337M}$. Together, our finding demonstrates that (1) Pfdn5 restricts the onset of Tau-induced neurodegeneration and (2) Pfdn5 plays a pivotal role in the amelioration of specific neurodegenerative diseases characterized by the disruption of the microtubule cytoskeleton.

Disclosures: A. Bisht: None.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.01/C80

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

Support: ANR Grant 21-CE37-0033-01

Title: Eeg cross-frequency coupling as putative new neuromarker of cortical dysfunctions in als

Authors: *V. MARCHAND-PAUVERT;
LIB, Sorbonne Univ., Paris, France

Abstract: ALS diagnosis relies on concurrent brain and spinal motor neuron degeneration but the clinical evaluation of cortical dysfunctions is challenged due to muscle atrophy often masking upper motor neuron (UMN) signs. TMS studies have identified imbalanced excitation/inhibition ratio in early stage, indicative of motor cortex hyperexcitability, which is detectable even during presymptomatic stage. However, performing TMS can quickly become difficult as the disease progresses, owing to increased stimulation threshold intensity, limitation of stimulator capacity, and patient discomfort. Consequently, TMS utility is limited for patient follow up, and may be even at early stage of the diagnosis. This underscores the persistent need for neuromarkers of cortical dysfunctions that can be tracked longitudinally over time. EEG can open new avenues and we recently found that cross frequency coupling, precisely the theta-gamma phase-amplitude coupling (PAC) extracted from resting state EEG, can serve as neuromarker of early cortical dysfunctions in ALS (Scekic-Zahirovic et al. Sci Transl Med.

2024). The present study aims to further investigate PAC in patients with ALS including slow signals within the alpha and beta bands, and the link with clinical features and brain structural defect. Scalp EEG (74-channel cap, 10-20 montage, 4-kHz sampling rate, 0.03-1000Hz bandwidth) was collected during resting state, 5 min. with eyes closed and 5 min. with eyes open, in 26 patients with ALS and 26 sex and age matched controls (ID-RCB 2018-A00789-52). Both groups also underwent MRI including structural (3DT1) and diffusion weighted imaging sequences (DWI). Coupling mean modulation index (MI) was evaluated for estimating PAC between slow signals within the theta (4-8 Hz), alpha (8-15 Hz) or beta bands (15-30 Hz), and higher frequency oscillations within the gamma band (30-60 Hz), at the level of five channels covering the sensorimotor cortex of both hemispheres (Fz, Cz, Pz, C3, C4). The integrity of grey and white matter integrity was respectively estimated by calculating the cortical thickness at the level of sensorimotor areas, and the diffusion metrics at different levels along the intracerebral corticospinal tract. Only mean MI for theta-gamma PAC on the dominant hemisphere was decreased in patients, and this result was accompanied and significantly linked to reduced cortical thickness, altered pyramidal tract white matter at the brainstem level, and the mean disease progression rate. This result further supports the link between PAC and UMN degeneration in ALS, and ROC curve indicates that this neuromarker is highly specific and sensitive for patient stratification.

Disclosures: V. Marchand-Pauvert: None.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.02/C81

Topic: C.06. Neuromuscular Diseases

Support: Roche uncodintional grant
MoH RF-2018-12366357

Title: Exploring Therapeutic Mechanisms of Risdiplam Analogues in Spinal Muscular Atrophy Using a Three-Dimensional Stem Cell-Derived Spinal Cord Model

Authors: A. D'ANGELO[1], F. BEATRICE[2], J. ONGARO[3], P. RINCHETTI[4], I. FARAVELLI[5], M. MIOTTO[6], S. LODATO[7], M. NIZZARDO[2], G. P. COMI[5], L. OTTOBONI[2], ***S. CORTI**[2]; [1]Univ. of Milan, Penne, Italy; [2]Univ. of Milan, Milan, Italy; [3]Fndn. IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy; [4]Syst. Biol., Columbia Univ., NEW YORK, NY; [5]Univ. of Milan, Milano, Italy; [6]Humanitas Univ., Milan, Italy; [7]Humanitas Univ., Pieve Emanuele, Italy

Abstract: Objective: To explore the therapeutic mechanisms of a Risdiplam-like compound using a three-dimensional stem cell-derived spinal cord model for Spinal Muscular Atrophy (SMA) and evaluate its ability to reverse the disease's pathological hallmarks.
Background: SMA is a severe neurological disorder characterized by early degeneration of lower

motor neurons, resulting from mutations in the SMN1 gene. Reliable human models are essential for advancing our understanding and treatment of this condition.
Design/Methods: We generated human spinal cord organoids from induced pluripotent stem cells (iPSCs) of SMA type 1 patients (n=3) and healthy controls (n=2). These organoids underwent bi-daily treatment with a Risdiplam-like compound during the first 80 days of development, a period analogous to the first trimester post-conception. Our analyses included bulk transcriptomics, single-cell RNA sequencing, multielectrode array analysis, and immunophenotypic profiling.
Results: In the organoid model, SMA samples exhibited significant cellular and molecular developmental disruptions across multiple cell types, extending beyond motor neurons. The treatment with the Risdiplam-like compound adjusted approximately 15% of the disease-affected genes and demonstrated excellent long-term in vitro tolerance. Critically, it restored the balance between full-length SMN2 transcripts and Δ7 splice variants, and reversed pathological markers in SMA organoids.
Conclusions: Our SMA organoid model proves to be a robust platform for investigating drug dynamics and therapeutic efficacy, highlighting the early and extensive developmental impact of SMA. This study underscores the potential of Risdiplam-like therapies for a comprehensive approach to SMA treatment. Furthermore, our findings contribute significantly to refining Risdiplam therapy and open new pathways for developing adjunct treatment strategies for SMA.

Disclosures: A. D'angelo: None. **F. Beatrice:** None. **J. Ongaro:** None. **P. Rinchetti:** None. **I. Faravelli:** None. **M. Miotto:** None. **S. Lodato:** None. **M. Nizzardo:** None. **G.P. Comi:** F. Consulting Fees (e.g., advisory boards); SAB: Roche, Novartis, Biogen, Sarepta. **L. Ottoboni:** None. **S. Corti:** F. Consulting Fees (e.g., advisory boards); SAB: Roche, Novartis, Biogen.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.03/C82

Topic: C.06. Neuromuscular Diseases

Title: Shifting the Paradigm: PrimeC, an oral candidate for Amyotrophic Lateral Sclerosis, meets primary safety and secondary end points in the phase 2b trial with biomarker-driven focus

Authors: M. CUDKOWICZ[1], V. DRORY[2], A. CHIÒ[3], C. LUNETTA[4], C. SHOESMITH[5], S. ZIMRI[6], ***N. RUSSEK- BLUM**[7], D. SHTOSSEL[8], R. VAN EIJK[9], G. SHAPIRA[10], N. SHOMRON[10], F. TRACIK[11], J. SHEFNER[12];
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Abstract: The objective of this study was to assess the safety, tolerability and preliminary efficacy of PrimeC in patients with amyotrophic lateral sclerosis (ALS) in a randomized, placebo-controlled, double-blind, multi-center Phase 2b trial (PARADIGM; NCT05357950). PrimeC is a unique formulation of ciprofloxacin and celecoxib, targeting key ALS-related pathological mechanisms. This Phase 2b trial aimed to evaluate PrimeC's safety, tolerability and efficacy of PrimeC in patients with ALS. A total of 69 people with ALS were randomized 2:1 to receive PrimeC or placebo for 6 months (one patient was misdiagnosed and was excluded from the pre-defined analysis), followed by a 12-month open-label-extension, during which all participants were administered with PrimeC. Safety, ALSFRS-R, SVC, QoL, ALS associated biomarkers, and survival were collected during the trial. Safety outcomes did not significantly differ between treatment arms. In the Intention-To-Treat (ITT) analysis (n=68), the difference in the adjusted ALSFRS-R score between arms at 6 months was 29.2% (2.232 points/6-month; 95% CI, -0.6 to 5.07; p=0.12). The Per-Protocol (PP) analysis (n=62) showed a 37.4% difference (3.220 points/6-month; 95% CI, 0.337 to 6.1; p=0.03). The difference in the adjusted SVC score between arms at 6 months was 17.2% (PP; p=0.39) and 13.3% (ITT; p=0.5). PrimeC achieved trends toward benefit in the secondary efficacy endpoints of complication-free survival compared to placebo (in several methodologies, including MiToS and King's Advanced Stage-free Survival), reducing the hazard of ALS disease complications or death by up to 53% (King's complication free survival: ITT HR 0.52; 95% CI, 0.24; p=0.1; PP: HR 0.47; 95% CI, 0.21 to 1.06; p=0.07). The PARADIGM trial also evaluated blood-based biomarkers, with TDP43 and PGJ2 serving as primary outcomes. Additional biomarker-driven assays were employed as exploratory endpoints to enhance understanding of ALS drug development, biological activity, and target engagement. Neurofilament light chain (NfL) showed a 4.3% difference between the active and placebo groups (p=0.51; PP). Notably, NfL in patients with a shorter disease duration (i.e., 24, 18, 12, and 9 months) exhibit a more pronounced disparity (up to 29%, p= 0.06) between placebo and active treatments. Given that microRNA regulation is one of the primary proposed mechanisms of action (MoA) of PrimeC, microRNA profiling will potentially delineate differentially expressed microRNAs, thereby reinforcing PrimeC's MoA. In conclusion, these results suggest that PrimeC is safe and may have a positive impact on ALS outcomes, thereby warranting a Phase 3 pivotal trial.

Disclosures: M. Cudkowicz: F. Consulting Fees (e.g., advisory boards); NeuroSense Therapeutics. **V. Drory:** None. **A. Chiò:** None. **C. Lunetta:** None. **C. Shoesmith:** None. **S. Zimri:** A. Employment/Salary (full or part-time):; NeuroSense Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSense Therapeutics. **N. Russek- Blum:** A. Employment/Salary (full or part-time):; NeuroSense Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSense Therapeutics. **D. Shtossel:** A. Employment/Salary (full or part-time):; NeuroSense Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSense Therapeutics. **R. van Eijk:** F. Consulting Fees (e.g., advisory boards); NeuroSense Therapeutics. **G. Shapira:** None. **N. Shomron:** None. **F. Tracik:** A. Employment/Salary (full or part-time):; NeuroSense Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSense Therapeutics. **J. Shefner:** F. Consulting Fees (e.g., advisory boards); NeuroSense Therapeutics.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.04/C83

Topic: C.06. Neuromuscular Diseases

Support: NIH grant P41EB018783
SCIRB C38338GG
Stratton VA Medical Center

Title: Spelling with “yes” and “no”: what to do with fatiguing, error-prone inputs—and how large language models can help

Authors: G. BROWN[1], ***J. HILL**[2];
[1]Neurol., Pennsylvania State Univ., Hershey, PA; [2]Albany Stratton VA Med. Ctr., Albany, NY

Abstract: This study addresses key usability, personalization, and accessibility challenges facing augmentative and alternative communication (AAC) interfaces for people with severe motor impairments. Users limited to "yes" and "no" responses often become fatigued and error-prone, hindering standard keyboard use. Current solutions also tend to offer a restricted set of responses presented visually, which limit personalized expression and are often inaccessible to vision-impaired individuals. Based on these patient-centered design requirements, we developed OnceForYes, a digital assistant facilitating free-text communication through partner-assisted auditory scanning, and tested it by copying text in ideal simulated conditions. First, we demonstrated a 2.8x increase in efficiency (characters typed in a fixed number of yes/no decisions) and >4x increase in robustness (tolerating input error while keeping typos under a fixed target) compared to a traditional fixed letter grid. Second, Context-sensitive word suggestions boosted efficiency up to 2.3x in fixed-grid mode (or 1.2x in Bayesian mode). Additionally, using a large language model, such as GPT2, increased efficiency by up to 79% in fixed-grid mode (or 55% in Bayesian mode) compared to traditional models. These advancements enhance natural language communication, adapting to context and individual patterns. Finally, OnceForYes offers a highly accessible interface that accommodates auditory spelling and a diverse range of input-modalities (e.g., thumb switch, brain-computer interface, etc.). Informed by research with individuals with severe motor impairments, OnceForYes provides fundamental insight into the theory, technology, and systems that are needed to lower the current barriers of communication and restore the power of voice to those most in need.

Disclosures: G. Brown: None. **J. Hill:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US patent application 63/471391.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.05/C84

Topic: C.06. Neuromuscular Diseases

Support: ICMR SRF

Title: Isolation of predominant enterobacteriaceae from amyotrophic lateral sclerosis patients

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Abstract: Abstract:Background ALS, a fatal neurodegenerative disease (NDD), lacks treatment due to its complex pathophysiology. Given this, research targets the gut microbiota, potentially linked to ALS and other NDD. This study aims to explore ALS patients' gut microbiota and compare them with controls, investigating Enterobacteriaceae species via culture-based methods. **Methodology** Between September 2021 and December 2023, 169 stool samples were collected. Among these, 67 were from ALS patients, 60 from their spouses, and 42 from healthy controls (HC). Upon collection, stool samples were immediately cultured followed by biochemical testing of the resulting isolates. To ensure accuracy, isolates were confirmed using conventional biochemical tests alongside validation via an automated VITEK 2 system. For confirmation representative samples underwent 16S rRNA PCR analysis. **Result** We identified six distinct bacterial genera- Escherichia, Citrobacter, Klebsiella, Enterobacter, Proteus, and Serratia - across 169 stool samples collected from 67 ALS patients, 60 spouses, and 42 HC. Demographically, ALS patients comprised 71% male and 19% female, HC group had 80% male and 20% female, and attendees included 70% male and 30% female, with average ages of 51.65 ± 11.4 and 48.95 ± 10.7, respectively. In the ALS group, *Citrobacter freundii* was the most prevalent bacteria, found in 89.55% of cases, followed closely by *Citrobacter diversus* 86.56%, *Escherichia coli* 71.64%, *Klebsiella pneumoniae* 62.68%, *Enterobacter cloacae* 34.32%, *Enterobacter aerogenes* 29.85%, Proteus mirabilis 26.86%, and Serratia marcescens 22.38%. Conversely, *Escherichia coli* dominated HC group 80.95%, followed by *Enterobacter aerogenes* 78.57%, *Serratia marcescens* 61.90%, *Enterobacter cloacae* 59.52%, *Klebsiella pneumoniae* 50.00%, *Citrobacter freundii* 19.04%, *Citrobacter diversus* 14.28%, and *Proteus mirabilis* 14.28%. Within attendees, *Escherichia coli* was most prevalent species 72.13%, followed by *Enterobacter aerogenes* 70.49%, *Enterobacter cloacae* 67.21%, *Klebsiella pneumoniae* 60.65%, *Citrobacter diversus* 29.5%, *Serratia marcescens* 24.59%, *Proteus mirabilis* 24.59%, and *Citrobacter freundii* 22.95%. **Conclusion** Our study delineates distinct gut microbiota profiles in ALS patients compared to healthy controls and spouses. The prevalence of specific Enterobacteriaceae species, particularly *Citrobacter freundii*, suggests potential microbial signatures associated with ALS, warranting further investigation into their role in disease pathogenesis and therapeutic targeting.

Disclosures: P. Gautam: None. **A. Pathak:** A. Employment/Salary (full or part-time):; Associate Professor. **G. Nath:** A. Employment/Salary (full or part-time):; Professor.

Poster

PSTR158: ALS and Motor Neuron Diseases

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Beijing Laboratory of Oral Health (PXM2021_014226_000041）
Beijing Municipal Science & Technology Commission（No.Z231100004823036）
Ractigen Therapeutics (HX-A-2023004).

Title: Rag-17: a promising new gene silencing therapy for sod1-als- early safety and efficacy data from a first-in-human trial

Authors: *J. YE[1], L. JIANG[1,2], L. WANG[1], Y. PAN[1,2], X. WANG[1,2], H. QU[1], X. LIAO[1], X. ZHOU[1], S. ZHANG[3], M. KANG[3], L.-C. LI[3], W. CHEN[1], Y. WANG[1,2,4,5,6,7,8,9];
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Abstract: Abstract for Neuroscience 2024Title: RAG-17: A Promising New Gene Silencing Therapy for SOD1-ALS - Early Safety and Efficacy Data from a First-in-Human Trial **Abstract:** Amyotrophic lateral sclerosis (ALS) is a chronic progressive neurodegenerative disease and the treatment options remain limited. Amyotrophic lateral sclerosis (ALS) patients with mutations in the superoxide dismutase 1(SOD1)gene are a growing target for targeted therapies. While an antisense oligonucleotide (ASO) recently received approval for this specific population, small interfering RNA (siRNA) offers another powerful approach for gene silencing. However, siRNA delivery to the central nervous system (CNS) has hampered its therapeutic potential. RAG-17 is a novel siRNA therapy targeting SOD1, conjugated to the SCAD delivery system for enhanced CNS delivery via intrathecal injection. Preclinical studies demonstrated significant efficacy in delaying disease onset, improving motor function, and extending survival in ALS models.In June 2023, a pioneering open-label, dose-escalation human study (NCT05903690) began to explore RAG-17’s safety, tolerability, pharmacokinetics, and initial efficacy in adults with SOD1

mutation-related ALS. As of December 10, 2023, six participants have been enrolled, receiving between 2 to 6 doses. The doses have been escalated up to 120-150 mg for most, with one participant reaching 180 mg per dose. The study has so far reported no dose-limiting toxicities (DLTs) or serious adverse events (SAEs). The adverse events noted were mild, including muscle tremors and headaches, predominantly after the first dose, resolving on their own without intervention. The plasma concentration of RAG-17 peaked at 12 hours post-administration and gradually declined, clearing within 48 hours. CSF SOD1 protein levels started to decrease immediately after the first dose and continued to drop with additional doses, achieving over a 50% reduction by the 5^{th} dose. Plasma neurofilament light chain (NFL) levels also showed a trend of reduction, exceeding 50% after just five doses.Encouragingly, preliminary efficacy data suggests potential clinical benefit. Among the first three participants receiving at least four doses, two showed stabilization of ALSFRS-R scores, and one even demonstrated improvement in ALSFRS-R and forced vital capacity (FVC) compared to baseline. The final analysis is expected in July 2024.
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Disclosures: J. ye: 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.; Ractigen Therapeutics, Suzhou, China. **L. Jiang:** 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.; Ractigen Therapeutics, Suzhou, China. **L. Wang:** 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.; Ractigen Therapeutics, Suzhou, China. **Y. Pan:** 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.; Ractigen Therapeutics, Suzhou, China. **X. Wang:** 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.; Ractigen Therapeutics, Suzhou, China. **H. Qu:** 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.; Ractigen Therapeutics, Suzhou, China. **X. Liao:** 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.; Ractigen Therapeutics, Suzhou, China. **X. Zhou:** 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.; Ractigen Therapeutics, Suzhou, China. **S. Zhang:** A. Employment/Salary (full or part-time):; Ractigen Therapeutics, Suzhou, China. **M. Kang:** A. Employment/Salary (full or part-time):; Ractigen Therapeutics, Suzhou, China. **L. Li:** A. Employment/Salary (full or part-time):; Ractigen Therapeutics, Suzhou, China. **W. Chen:** 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

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Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.07/C86

Topic: C.06. Neuromuscular Diseases

Support: NIH R21HD102722

Title: Corticomotor excitability & disease progression in ALS: a lower limb focused descriptive study

Authors: *S. DESHMUKH[1,2], M. CUMMINGS[2], A. D. DOSHI[3], S. MADHAVAN[1]; [2]Physical Therapy, [1]Univ. of Illinois at Chicago, Chicago, IL; [3]Neurosci., Univ. of Illinois Chicago, Chicago, IL

Abstract: Introduction: Amyotrophic lateral sclerosis (ALS) is a rapidly advancing neurodegenerative disorder displaying signs of upper motor neuron (UMN) and lower motor neuron (LMN) dysfunction. Transcranial magnetic stimulation (TMS) enables measurement of corticomotor excitability, providing insight into UMN integrity. Some neurophysiological studies using TMS point to corticomotor hyperexcitability as a possible factor leading to neurodegeneration in ALS. Others demonstrated reduced excitability, as shown by motor evoked potential (MEP) amplitude decreases, particularly in the lower limb. The study's objective was to present data for TMS-induced MEPs elicited in the lower limb motor cortex in individuals with different rates of disease progression. Methods: Twelve participants with spinal-onset ALS underwent TMS measurements to measure corticomotor excitability using a double-cone coil oriented in the posterior-anterior direction. Muscle activity was recorded from the tibialis anterior (TA) muscle of both legs with surface electromyography. TMS-induced responses were collected on both sides. MEP amplitude and area were considered primary outcomes of corticomotor excitability. For those with no elicitable MEPs, the highest tolerable intensity was used to confirm the absence of MEPs. We classified participants as MEP+ (present) and MEP- (absent). ALS-specific history, including site and time of symptom onset, was collected. Disease progression, assessed using their progression rate, was calculated based on the ALS functional rating scale - revised (ALSFRS-R) scores and time since disease onset. Using this, participants were classified into slow, medium, and fast progressors. Results: Baseline data (5 males and 7 females with mean age 56±8 years, 26±19 months since symptom onset, 34±7 ALSFRS-R score) were collected as a part of a larger randomized clinical trial. Based on their disease progression rate, there were four slow, five medium and three fast progressors. All twelve participants, irrespective of disease progression, did not demonstrate MEPs and were classified as MEP-.

Discussion: The absence of elicitable MEPs across all participants, regardless of the rate of disease progression, suggests a consistent pattern of severe corticomotor dysfunction in the lower limb motor cortex of individuals with ALS . This observation may reflect a critical stage in corticomotor degeneration that is common across different progression rates. Further research is needed to understand the underlying mechanisms and the role of TMS in assessing disease progression and providing potential prognostic insights for ALS management.

Disclosures: S. Deshmukh: None. **M. Cummings:** None. **A.D. Doshi:** None. **S. Madhavan:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.08/C87

Topic: C.06. Neuromuscular Diseases

Support: VA grant BX005853

Title: Site targeted inhibition of complement prevents neuropathology in the mouse model of amyotrophic lateral sclerosis

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Abstract: Amyotrophic lateral sclerosis (ALS) is a terminal progressive neurodegenerative disorder manifested by muscular weakness and progressive atrophy. Although majority of ALS cases are sporadic (90%), a common genetic mutation in familial ALS is linked to Cu/Zn superoxide dismutase1 (SOD1) gene. Preclinical and clinical studies implicate a role for the complement system in the neuroinflammatory response observed in ALS. In the hSOD1 ^{G93A} mouse model, C3 deposition has been shown to occur in the neuromuscular junction (NMJ) and spinal cord. In this study we evaluated the therapeutic potential of the complement inhibitor, CR2-Crry, which targets deposited C3 and therefore sites of complement activation. Using the hSOD1 mouse model, we initiated intraperitoneal treatment with CR2-Crry at 120 days of age at which time symptoms become apparent. Treatment continued (twice/week) until animals reached the humane endpoint. Treatment of hSOD1 mice with CR2-Crry significantly improved survival, clinical score, and body weight retention compared to vehicle-treated mice. Inhibiting C3 was also found to decrease glial activation in the vicinity of complement activation in both the spinal cord and gastrocnemius muscle. Measured at age 155 days, there was improved motor neuron survival in the spinal cord of CR2Crry-treated mice compared to the vehicle-treated mice. Recently, an anti-C5 monoclonal antibody (Ravulizumab) failed to show efficacy in a clinical trial for ALS. This anti-C5 antibody inhibits complement later in the pathway than CR2-Crry, and unlike CR2-Crry does not affect generation of some earlier bioactive complement activation

products. It will be informative to determine whether anti-C5 treatment is effective in this murine model of ALS.

Disclosures: M. Majumder: None. **D. Borucki:** None. **K. Mallah:** None. **S. Tomlinson:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.09/C88

Topic: C.06. Neuromuscular Diseases

Support: ALS Association Grant 24-PDF-685

Title: Inhibiting dipeptide repeat propagation in C9-ALS

Authors: *S. C. AKERMAN[1], O. SPEAD[2], M. HUANG[2], A. G. THOMAS[3], S. O. VIDENSKY[4], C. M. FARE[1], B. ZAEPFEL[5], C. TALLON[2], B. S. SLUSHER[6], J. D. ROTHSTEIN[7];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease affecting both upper and lower motor neurons. Clinical studies indicate that ALS can start focally and then spread within the spinal cord and in the cortex. Multiple lines of evidence indicate that the disease-causing mutant proteins identified in ALS patients, such as C9orf72-associated dipeptide repeats (DPRs), superoxide dismutase 1, or transactive response DNA-binding protein 43, can form aggregates and these aggregates can spread from cell-to-cell in different ALS models. Understanding how these proteins propagate in ALS models can reveal novel therapeutic targets that can potentially slow down or halt this propagation. One way cell-to-cell communication occurs is through extracellular vesicles (EVs). Exosomes are a major type of EVs that are derived from the endosomal pathway through the formation of intraluminal vesicles that occur with negative curvature of the endosomal membrane. Interestingly, several studies have identified an increase in sphingomyelin (SM) and ceramide in either ALS patients or transgenic mice models. Ceramide is an integral component of exosomal membranes. A major source of ceramide production is through the hydrolysis reaction of SM by the action of neutral sphingomyelinase 2 (nSMase2). Inhibition of exosome biogenesis by nSMase2 both at genetic and pharmacological level, has been shown to halt amyloid-β aggregation and tau propagation in different Alzheimer's Disease models. Interestingly, nSMase2 inhibition has also been shown to play a role in the production of amyloid-beta albeit independent of its involvement in EV production. We hypothesize that exosome-mediated secretion of DPRs is one of the major

contributing factors to the cell-to-cell propagation of these aggregates, although their role in causing cell injury remains unclear in patients as well as endogenous expressing models. Using various model systems, we detected DPR species in isolated EV fractions, in agreement with the current literature. Ongoing experiments suggest that these DPRs can propagate into new recipient cells. Furthermore, using a non-competitive inhibitor against nSMase2 - phenyl(R)-(1-(3-(3,4-dimethoxyphenyl)-2,6-dimethylimidazo[1,2-b]pyridazin-8-yl)pyrrolidin-3-yl)-carbamate (PDDC) - in a C9-AAV mouse model suggests that nSMase2 inhibition has the potential to decrease DPR burden. Mice treated with PDDC compound showed lower p62 inclusions, and lower plasma NfL/GFAP levels. Additionally, PDDC treatment partially restored the lipidomic changes observed in this mouse model.

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Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.10/C89

Topic: C.06. Neuromuscular Diseases

Support: DOD HT94252410122

Title: Protein mimetic antagonists of TDP-43 aggregation mitigate cytotoxic phenotypes in multiple ALS models

Authors: *K. M. REYNOLDS CAICEDO[1], N. STILLMAN[2], A. GROSSBERG[2], D. A. LINSEMAN[2], S. KUMAR[2];
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Abstract: The pathological aggregation of TAR DNA-binding protein-43 (TDP-43) is a hallmark of several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). In this study, we developed several ex vivo and in vitro systems (HEK cells and NSC-34 motor neuronal cells) expressing wild type and mutant TDP43 variants. These cell models were subjected to multiple disease relevant insults including oxidative stress, proteasome stress, preformed TDP-43 fibrils, and neuron-derived exosomes isolated from the plasma of ALS patients, to induce TDP-43 aggregation and cytotoxicity. We then screened a library of synthetic protein mimetics and identified potent antagonists of TDP-43 aggregation that attenuated cytotoxicity in the various ALS model systems. These protein mimetics are structurally and chemically stable in biological milieu and efficiently cross the cell membrane and blood brain barrier. Our results demonstrate the utilization of TDP-43 overexpressing cellular models to identify potent antagonists of intracellular TDP-43

aggregation. This work will aid in expediting the discovery of lead therapeutics for ALS and FTLD.

Disclosures: K.M. Reynolds Caicedo: None. **N. Stillman:** None. **A. Grossberg:** None. **D.A. Linseman:** None. **S. Kumar:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

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Topic: C.06. Neuromuscular Diseases

Support: NIH Grant # UH3NS115608
NIH Grant # R33NS115161

Title: A mechanism based AAV gene therapy approach for Amyotrophic Lateral Sclerosis

Authors: *A. PEETHAMBARAN MALLIKA[1], J. P. LING[2], P. C. WONG[3];
[1]Pathology, Johns Hopkins Med. Inst., Baltimore, MD; [2]Pathology, Johns Hopkins Univ., Baltimore, MD; [3]Dept Pathol, Sch. of Med., Baltimore, MD

Abstract: Emerging evidence supports the notion that loss of splicing repression by TDP-43, an RNA binding protein, initially associated with the pathology in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia, underlies its pathogenesis. We showed previously in motor neurons that splicing repression is a major function of TDP-43, the loss of which occurs during the pre-symptomatic stage of ALS. To complement the loss of TDP-43 splicing repression, we developed an AAV therapeutic approach designed to deliver in motor neurons, a fusion protein, termed CTR, comprised of the N-terminal domain of TDP-43 that recognizes all of its RNA targets and an unrelated but well-characterized RAVER1 splicing repressor. To test this therapeutic approach, we employed a mouse model lacking TDP-43 in spinal motor neurons (*ChAT-IRES-Cre;Tardbpf/f* mice) that exhibits progressive motor neuron loss, axonal degeneration and denervation muscle atrophy. We took advantage of an AAV.PHP.eB vector that enables efficient transduction and accumulation of CTR (AAV.PHP.eB-CTR) in motor neurons by an intravenous (IV) delivery route. Along with control littermates, IV delivery of AAV.PHP.eB-CTR (or AAV.PHP.eB-GFP as control) to early symptomatic (6 week-of-age) *ChAT-IRES-Cre;Tardbpf/f* mice was performed and analyzed for efficacy and toxicity. We show that IV delivery of AAV.PHP.eB-CTR to symptomatic *ChAT-IRES-Cre;Tardbpf/f* mice efficiently transduced spinal motor neurons, attenuated motor neuron loss and consequently extended their lifespan. Corroborating these findings in *ChAT-IRES-Cre;Tardbpf/f* mice, delivery of CTR by AAV prevented inclusion of TDP-43 related cryptic exons in motor neurons and markedly improved muscle mass and strength. Notably, no overt abnormality is observed in aged control mice transduced with AAV.PHP.eB-CTR. These data validate an AAV gene

therapy strategy to complement the loss of splicing repression by TDP-43 and for ALS provide an unprecedented opportunity towards clinical testing of this mechanism-based gene therapy.

Disclosures: A. Peethambaran Mallika: None. **J.P. Ling:** None. **P.C. Wong:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.12/C91

Topic: C.06. Neuromuscular Diseases

Title: Engineering Next-Generation AAV Vectors for Enhanced ALS Therapy

Authors: B. LEE, M. KIM, M. KWON, J. KANG, J. LEE, *J.-W. KIM;
Genixcure, Suwon-si, Korea, Republic of

Abstract: Gene therapy offers immense promise for addressing a wide array of genetic and non-genetic disorders; however, efficient delivery remains a significant obstacle. Recombinant adeno-associated viruses (rAAVs) are commonly employed as vectors for gene therapy, yet their effectiveness is often hampered by their inability to target specific cells or tissues. Consequently, there is a critical need for safer and more efficient rAAV vectors. Recent efforts have concentrated on developing next-generation AAV vectors with improved specificity and delivery efficacy. Computational methods, including machine learning, are increasingly utilized for rAAV capsid engineering. Here we developed InsightMiner^{TM}, a platform for capsid engineering, to enhance the cell- or tissue-specific delivery of rAAV9, a well-established serotype known for its ability to penetrate the blood-brain barrier (BBB). By analyzing various virus species capable of infecting humans and employing machine learning algorithms, specific sequences were identified to improve BBB penetration and central nervous system (CNS) delivery. AAV9 variants were generated by applying specific sequences from InsightMiner^{TM} to enhance BBB penetration and CNS delivery, with XOB-031 demonstrating superior both in vitro and in vivo compared to wild-type AAV9. Subsequently, this platform was applied to target neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). XOB-031 facilitated the efficient delivery of the STMN2 gene into motor neurons of STMN2 knockout mice, thereby enhancing axon regeneration compared to wild-type AAV9. Moreover, XOB-031-STMN2 significantly mitigated behavioral deficits in STMN2 knockout mice. Overall, this study highlights the potential of capsid engineering using human virus data and machine learning to improve therapeutic outcomes in gene therapy, particularly for challenging disorders such as ALS.

Disclosures: B. Lee: None. **M. Kim:** None. **M. Kwon:** None. **J. Kang:** None. **J. Lee:** None. **J. Kim:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.13/C92

Topic: C.06. Neuromuscular Diseases

Title: Modulating TDP-43 aggregation directly and indirectly in C.elegans

Authors: *E. ELSHALIA, N. STILLMAN, S. KUMAR, S. HOROWITZ;
Chem. & Biochem., Univ. of Denver, Denver, CO

Abstract: TDP-43 misfolds and aggregates in multiple diseases, currently with no therapeutic interventions. Here, we tested two different approaches via TDP-43-expressing-*C.elegans*, using either direct and indirect mechanisms to modulate TDP-43 protein aggregation. To directly inhibit TDP-43 aggregation, we tested an Oligopyridylamide (OP) ligand that was shown to directly bind TDP-43 and prevent its aggregation *in vitro*. These experiments showed that the OP prevented TDP-43 aggregation, the development of neurological symptoms, and the accumulation of reactive oxygen species (ROS). In addition to aggregating, TDP-43 is also known to bind a nucleic acid secondary structure known as G-quadruplex (G4) structure, which has been shown previously to be a powerful modulator of protein aggregation. To indirectly modulate TDP-43 aggregation through G4s, we administered 5-aminolevulinic acid (5-ALA), a precursor to protoporphyrins that bind G4s. This G4-targeting treatment ameliorated TDP-43 aggregation and neurodegenerative symptoms, as well as the accumulation of ROS. These results show that both direct and indirect aggregation prevention are possible avenues to pursue for TDP-43 lead therapeutics.

Disclosures: E. Elshalia: None. **N. Stillman:** None. **S. Kumar:** None. **S. Horowitz:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.14/C93

Topic: C.06. Neuromuscular Diseases

Support: CIHR
ALS Canada/Brain Canada
Healey Center

Title: Neuromuscular Junction proteins as potential biomarker in ALS

Authors: *R. ROBITAILLE[1,3], F. PROVOST[4], N. HARDY[5], F. GRÉGOIRE[5], J. MARTINEAU[5], R. NADEAU[7], J. VALLÉE[5], D. ARBOUR[6], M.-S. GAUTHIER[8], M. LAVALLÉE-ADAM[7], B. COULOMBE[9], R. PIOVESANA[2];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the loss of upper and lower motor neurons (MNs). Symptoms include muscle paralysis which rapidly progresses and ultimately results in respiratory failure within 2-5 years after diagnosis. Interestingly, one of the hallmarks of all ALS cases is the destruction of the neuromuscular junction (NMJ). As no cure yet exists, this disease is in desperate need of effective therapeutics and the absence of biomarkers has hampered the development of therapies in ALS. NMJ-related candidate biomarkers were identified from a comparative proteomic study. Importantly, these NMJ-proteins are consistent with the complex and dynamic NMJ denervation process in ALS (axon guidance, extracellular matrix and synaptic properties), whereby cycles of NMJ denervation and reinnervation take place months prior to the complete retraction of the axons within muscles. Our laboratory identified several matrix proteins that are altered during ALS progression. Laminin β2, which is specifically located at the NMJ, is reduced before the appearance of symptoms and remained unchanged during disease progression in $SOD1^{G37R}$ mice. Importantly, its reduction was not related to the innervation status. Using Western blot analyses of symptomatic $SOD1^{G37R}$ mice we found a significant decrease compared to age-matched wild type littermates. Interestingly, consistent with a reduction of laminin β2 at the NMJ, laminins plasma levels were increased in $SOD1^{G37R}$ animals. Our results show that the presence of laminin β2 reflects the state of the disease and support the possibility of using Laminin β2 as a biomarker linked to NMJ in ALS. Understanding the distinctive NMJ protein expression during disease progression will help provide insights into the denervation mechanisms in ALS and help identify potential proteins that could support NMJ repair and integrity. Importantly, NMJ protein detection in blood could be potential candidates for ALS biomarkers for early diagnosis and disease progression assessment.

Disclosures: R. Robitaille: None. **F. Provost:** None. **N. Hardy:** None. **F. Grégoire:** None. **J. martineau:** None. **R. Nadeau:** None. **J. Vallée:** None. **D. Arbour:** None. **M. Gauthier:** None. **M. Lavallée-Adam:** None. **B. Coulombe:** None. **R. Piovesana:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.15/C94

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01NS116262

Title: Intermuscular coherence in the leg as a biomarker for early amyotrophic lateral sclerosis

Authors: *N. P. ISSA[1], S. AYDIN[1], B. SOLIVEN[2], K. REZANIA[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a uniformly fatal disorder that starts with subtle symptoms but progresses to severe disability and death over months and years. On average, diagnosis is delayed by 1-1.5 years from symptom onset, making it difficult to treat and recruit into clinical trials early in the disease process. The ongoing ALS-IMC study (NCT05104710) asks whether Intermuscular Coherence (IMC) can be used as a biomarker for ALS. Initial studies support the diagnostic usefulness of IMC measured in the arm. To determine whether IMC in the leg also carries diagnostic information, we compared IMC between patients with early ALS (uncategorized or possible ALS according to the Awaji Criteria) and neurotypical control subjects studied at the University of Chicago. IMC was calculated from surface electromyography measurements from the tibialis anterior-extensor digitorum brevis muscle pair (TA-EDB) during three 30-second epochs of ankle dorsiflexion and toe extension/spreading. Subjects ranged in age from 50 to 80, and there was no statistical difference in age between the ALS group (N=17, mean±st.dev 64.7±8.0 years) and the Neurotypical group (N=24, 61.8±8.6, p=0.19 Mann-Whitney). IMC in the βγ frequency range (20-40 Hz) was significantly lower in the ALS group (0.138) than the Neurotypical group (0.232; p=0.019 Mann-Whitney, measured in the left leg). The ability of IMC-βγ from the leg to distinguish between ALS and Neurotypical subjects was fair, with an area under the receiver-operating curve of 0.72 for the left leg and 0.76 for the right leg (not significantly different, p=0.715). That IMC measured in the leg is useful to distinguish between patients with early ALS and Neurotypical subjects suggests that ALS might be diagnosable earlier than currently clinically feasible. Because ALS has an asymmetric onset and can start in any limb, this study suggests that IMC might be useful to identify patients with early ALS regardless of in which limb it initially presents.

Disclosures: N.P. Issa: None. **S. Aydin:** None. **B. Soliven:** None. **K. Rezania:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.16/C95

Topic: C.06. Neuromuscular Diseases

Support: NINDS R21NS116385
Arizona Alzheimer's Consortium (AAC)
Barrow Neurological Foundation

Title: Analyzing matrin 3 pathology in cortical and subcortical areas in ALS and dementias

Authors: *G. QUEZADA[1], N. BAKKAR[3], S. E. PEREZ[4], E. J. MUFSON[5], A. BOEHRINGER[2], R. P. BOWSER[6], D. X. MEDINA[5];
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Abstract: Matrin 3 (MATR3) is a highly conserved nuclear DNA/RNA- binding protein that plays an important role in regulating post-transcriptional RNA processing, including alternative splicing, mRNA stability and mRNA export. Mutations in MATR3 have been identified as causal in amyotrophic lateral sclerosis (ALS), a neurodegenerative disorder characterized by the loss of upper and lower motor neurons in the brain and spinal cord. MATR3-positive inclusions have been found in post-mortem brain tissue of both familial and sporadic ALS patients. Additionally, Matrin 3 pathology has been observed in other neurodegenerative disorders, such as frontotemporal dementia (FTD) and Alzheimer's disease (AD). However, the extent of Matrin 3 pathology has yet to be investigated. In this study, we aim to quantify the prevalence of Matrin 3 pathology in cortical and subcortical regions of neurodegenerative subtypes. We hypothesize that Matrin 3 pathology is present in various neurodegenerative disorders. To test our hypothesis, we utilized quantitative immunohistochemistry to measure pathology in different subtypes and regions of the central nervous system (CNS). More specifically, we examined the subcellular localization of Matrin 3 in several areas of the brain, including frontal cortex, motor cortex, and entorhinal cortex. We identified cytoplasmic immunoreactivity and nuclear inclusions in cortical layers 2 and 3 of frontal cortex of FTD and AD cases, but not in the deeper layers. We also observed a stronger degree of expression in the deeper layers of MCI and AD cases compared to controls. Completion of this work will allow us to identify potential common mechanisms among neurodegenerative diseases that are driven by Matrin 3 dysregulation/dysfunction. This work will also further our understanding of how Matrin 3 is altered in response to different neurodegenerative states.

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Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.17/C96

Topic: C.06. Neuromuscular Diseases

Support: NIH/NINDS K08-NS112330KO8
U19NS132303
Alzheimer’s Association AACSF-17-531484
UCSF CTSI TL1 Fellowship 5TL1TR001871-04
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Carol and Gene Ludwig Award for Early Career Research
Larry H. Hillblom Fellowship
Shupin Fellowship from the UCSF Neurology Endowment

Title: Validated assays for the quantification of C9orf72 human pathology

Authors: *S. E. SALOMONSSON[1,2], C. D. CLELLAND[1,2];
[1]Dept. of Neurol., UCSF Weill Inst. for Neurosci., San Francisco, CA; [2]UCSF Memory and Aging Center, San Francisco, CA

Abstract: A repeat expansion mutation in the C9orf72 gene is the leading known genetic cause of FTD and ALS. The C9orf72-ALS/FTD field has been plagued by a lack of reliable tools to monitor this genomic locus and its RNA and protein products. We have validated assays that quantify C9orf72 pathobiology at the DNA, RNA and protein levels using knock-out human iPSC lines as controls. Here we show that single-molecule sequencing can accurately measure the repeat expansion and faithfully report on changes to the C9orf72 locus in what has been a traditionally hard to sequence genomic region. This is of particular value to sizing and phasing the repeat expansion and determining changes to the gene locus after gene editing. We developed ddPCR assays to quantify two major C9orf72 transcript variants, which we validated by selective excision of their distinct transcriptional start sites. Using validated knock-out human iPSC lines, we validated 4 commercially available antibodies (of 9 tested) that were specific for C9orf72 protein quantification by Western blot, but none were specific for immunocytochemistry. We tested 15 combinations of antibodies against dipeptide repeat proteins (DPRs) across 66 concentrations using MSD immunoassay, and found two (against poly-GA and poly-GP) that yielded a 1.5-fold or greater signal increase in patient iPSC-motor neurons compared to knock-out control, and validated them in human postmortem and transgenic mouse brain tissue. Our validated DNA, RNA and protein assays are applicable to discovery research as well as clinical trials.

Disclosures: S.E. Salomonsson: A. Employment/Salary (full or part-time):; University of California, San Francisco. **C.D. Clelland:** A. Employment/Salary (full or part-time):; University of California, San Francisco. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH/NINDS K08-NS112330KO8, U19NS132303, Alzheimer's Association AACSF-17-531484, UCSF CTSI TL1 Fellowship 5TL1TR001871-04, Bright Focus Foundation A20201490F, Carol and Gene Ludwig Award for Early Career Research, Larry H. Hillblom Fellowship, Shupin Fellowship from the UCSF Neurology Endowment.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.18/C97

Topic: C.06. Neuromuscular Diseases

Support: Intramural Research Training Award (IRTA) at the National Institute of Health

Title: Analyzing the skeletal muscle transcriptome in Spinal and Bulbar Muscular Atrophy

Authors: *M. EZUMA-NGWU, C. GRUNSEICH;
NINDS, Bethesda, MD

Abstract: Spinaland Bulbar Muscular Atrophy (SBMA) is a slowly progressive X-linked motorneuron disease caused by a CAG repeat expansion in the androgen receptor gene.The disease results in slow progression of atrophy, weakness, andfasciculations in limb and bulbar muscles. Our goal is to study gene expressionin SBMA patient skeletal muscle to assist in biomarker development andcharacterization of pharmacodynamic targets for future therapeutic studies. Wecollected 5 SBMA patient biopsy samples and 9 control autopsy samples. Controlsamples were collected from the National Disease Research Interchange (NDRI).RNA was extracted from the tissues and prepared for RNA sequencing. From theRNA sequencing analysis, we identified the top 20 genes that were mostdifferentially expressed in the patient and control samples. Western blot analysiswas used to analyze these targets at the protein level. Four control samplesand four SBMA samples were used for western blotting. Six antibodies, Proto-Oncogene BHLHTranscription Factor (MYC), Nicotinamide N-Methyltransferase(NNMT), Angiotensin I Converting Enzyme (ACE), Solute Carrier Family 45Member 3 (SLC45A3), Indolethylamine N-Methyltransferase (INMT), and GrowthArrest and DNA Damage Inducible Alpha (GADD45A), were analyzed. We then performed RT-PCR, westernblotting, and immunocytochemistry of selected targets in treated patient andcontrol fibroblast cells to characterize dihydrotestosterone (DHT) and vehiclecontrol (EtOH) responsivity. Genes were identified with both increased anddecreased expression in SBMA patient samples. INMT, ACE, and SLC45A showed anincreased expression in the SBMA patient samples. NNMT, GADD45A, and MYC showedan increased expression in patient muscle tissue on the protein level anddecreased expression at the RNA level. DHT treated patient fibroblast cells showedan increased expression of MYC and treated control samples showed a decrease inexpression. Expression levels of MYC was increased in patients with higher CAGrepeats. MYC was detected in both the nucleus and cytoplasm and colocalizationwas appreciated. We characterized the gene expression profile in SBMA patientand control samples. This characterization allows us to evaluate the diseasespecific changes in gene expression which may serve as potential targets whenevaluating therapeutic response in the muscle tissue during therapeutic studies.

Disclosures: M. Ezuma-Ngwu: None. **C. Grunseich:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.19/C98

Topic: C.06. Neuromuscular Diseases

Support: Cátedra sobre la Esclerosis lateral Amiotrófica "Gregoria Ramos Gil"

Title: Chaperone-mediated autophagy is deficient in the spinal motor neurons of ALS patients, with the exception of Onuf's nucleus.

Authors: *D. GARRIGÓS[1], M. MARTINEZ[2], E. GEIJO-BARRIENTOS[3], S. MARTINEZ[4]; [1]Univ. Miguel Hernandez (UMH-CSIC), Alicante, Spain; [2]Anatomía y embriología humana, Inst. de Neurociencias, Alicante, Spain; [3]Univ. Miguel Hernandez-CSIC, San Juan, Alicante,, Spain; [4]Inst. de Neurociencias, Inst. De Neurociencias. UMH-CISC, San Juan De Alicante, Spain

Abstract: Amyotrophic Lateral Sclerosis (ALS) is an almost selective motoneuron (MN) neurodegenerative disease in which the factors determining preferential involvement of MN are yet to be fully understood. TDP-43 protein nuclear clearance and cytoplasmic aggregates are identified as pathogenic landmarks of ALS neurodegeneration. TDP43 misfolding protein is the major component of the ubiquitinated neuronal cytoplasmic inclusions deposited in spinal motor neurons both in familiar and sporadic ALS patients (fALS and sALS, respectively). Since TDP43 protein contains the HSC70 recognition motif, demonstrating that TDP43 is a substrate of chaperone-mediated autophagy (CMA), CMA function may represent a cellular mechanism to regulate TDP43 cytoplasmic turnover in cells. We have analyzed the expression of LAMP2A in MN, to explore if CMA disfunction may participate in ALS TDP43 proteinopathy. When 10 sections of human cervical, thoracic, lumbar and sacral segments of the 6 control spinal cords, were processed by immunohistochemistry we have detected strong expression of LAMP2A in control spinal MN of the anterior horn. We analyzed by immunohistochemistry the expression of LAMP2A in the same regions of sALS patients' spinal cord, showing that LAMP2A immunopositivity was very weak in anterior horn MN. Interestingly, in the sacral levels, the MN of Onuf's nucleus showed strong expression of LAMP2A, like the spinal and Onuf´s MN in control spinal cords. These results support the requirement of high activity of CMA to maintain control MN alive, as well as in ALS, and reinforces the possibility that CMA may play a role in the selective pathophysiology of sALS in MN. Next, the neuroprotective effect of LAMP2A expression (CMA function) in Onuf's MN will be analyzed in a mouse ALS model (SOD1-G93A), first by the analysis of the selective increases of LAMP2A expression in control and mutant mice MN, and second, by exploring cell autonomous processes that may confer neuroprotection in Onuf's MN. The objective of this experimental approach is to determine the protective mechanisms operating in Onuf's MN to protect from ALS neurodegeneration, in order to explore new therapeutic avenues.

Disclosures: D. Garrigós: None. **M. Martinez:** None. **E. Geijo-Barrientos:** None. **S. Martinez:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

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Program #/Poster #: PSTR158.20/C99

Topic: C.06. Neuromuscular Diseases

Support: 5T32NS041234-18
R01AG061708-03

Title: Investigation of the Maturation and Network Dynamics of Corticospinal Motor Neurons Using High-Density Microelectrode Arrays and Optical Imaging

Authors: *C. QUINTANILLA[1], Z. FITZGERALD[2], M. S. RADOJICIC[3], E. ULUPINAR[2], D. N. BITLIS[2], M. MARTINA[4], P. R. ANDJUS[3], W. VAN DRONGELEN[5], P. OZDINLER[6]; [1]Northwestern Univ., Chicago, IL; [2]Dept. of Neurol., Feinberg Sch. of Med., Northwestern Univ., Chicago, IL; [3]Inst. of Physiol. and Biochemisty "Jean Giaja", Fac. of Biol., Univ. of Belgrade, Belgrade, Serbia; [4]Neurosci., Northwestern Univ. Med. Sch., Chicago, IL; [5]Section Pediatric Neurol., The Univ. of Chicago, Chicago, IL; [6]Neurol., Northwestern Univ., Chicago, IL

Abstract: Corticospinal motor neurons (CSMN) are one of the key components of the motor neuron circuitry, located in the motor cortex of the brain. They are in part responsible for the initiation and modulation of movement, and their degeneration is the hallmark for numerous diseases, such as amyotrophic lateral sclerosis, hereditary spastic paraplegia, and primary lateral sclerosis. Cortical hyperexcitation followed by hypoexcitation has been proposed as an early event in ALS, suggesting the involvement of dysfunctional cortical activity in ALS pathology. However, the understanding of the high-spatiotemporal resolution of their activity and connectivity in health and disease is lacking. In this study, we combine high-density microelectrode array (HDMEA) system with optical imaging to bring cell-type specific resolution to understand electrophysiological features of healthy CSMN as they mature and form connections with other cortical neurons/cells located in the motor cortex. UCHL1eGFP mice that express eGFP in CSMN were used for motor cortex microdissection at postnatal day 0 (P0) for culturing on HDMEAs, so that CSMN can be distinguished among all other cells/neurons on the HDMEAs. Spontaneous activity was recorded at 12, 15 and 18 days *in-vitro (DIV)* and then individual channels that contained recordings from CSMN were utilized for data analysis. Our study is the first to investigate functional responses of CSMN and our results provide a timeline for CSMN maturation between 15 and 18 DIV based on changes in their firing and burst parameters. Changes in cross-correlations between CSMN and their surrounding neighbors further suggest a dramatic transition of their connectivity patterns from "random" network to "scale-free" network between 12 and 15 DIV. Our results enhance our understanding of CSMN dynamics and pave the way for future cell-type specific and functional analyses of CSMN across various disease models with distinct underlying causes. Such investigations are also essential for assessing target engagement, efficacy, and toxicological profiles during the preclinical phases of drug discovery initiatives.

Disclosures: C. Quintanilla: None. **Z. Fitzgerald:** None. **M.S. Radojicic:** None. **E. Ulupinar:** None. **D.N. Bitlis:** None. **M. Martina:** None. **P.R. Andjus:** None. **W. van Drongelen:** None. **P. Ozdinler:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

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Program #/Poster #: PSTR158.21/C100

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

Support: NIH Grant RO1NS126191

Title: Characterizing the Stathmin-2 containing vesicle population

Authors: *E. J. C. THORNBURG-SURESH, A. ANAGNOSTOPOULOS, D. W. SUMMERS;
Biol., Univ. of Iowa, Iowa City, IA

Abstract: Axon degeneration is an early and sometimes initiating event in many neurodegenerative diseases like ALS. Stathmin-2 (Stmn2) has emerged as a key player in the regulator of axon integrity. Importantly, Stathmin-2 depletion is tied to ALS pathology, emerging as a potential biomarker and therapeutic target. Previous work showed that Stmn2 needs to be localized to a vesicular membrane to enact its axon maintenance functions; however, why this localization is required is unknown. Our work seeks to identify other proteins localized to Stmn2-containing vesicles. We use a combination of live imaging and a proximity labeling assay to identify these proteins. Live imaging analysis was done in sensory neurons derived from the dorsal root ganglia of embryonic (E13.5) CD10 mice. We co-expressed tagged Stmn2 with one of our proteins of interest and analyzed comigration rates. Comigration is determined by another researcher blinded to study conditions. The BioID studies were completed in either human embryonic kidney (HEK) 293 cells or Neuro-2a cells. We found that Stmn2 comigrates with Dual Leucine Zipper Kinase (DLK), a member of a pro-degenerative signaling pathway. We also found that Stmn2 does not comigrate with Nmnat2, another axon survival protein. Our results provide further evidence that Stmn2 localization is directly tied to its regulation by DLK. Interestingly, DLK itself is sensitive to changes in microtubule dynamics, which Stmn2 directly regulates. Understanding other proteins on Stmn2-vesicles will help identify potential interacting partners and provide insight into its mechanism in axon integrity and ALS. Future studies will further use BioID and proteomics to identify other candidate proteins found on this vesicle.

Disclosures: E.J.C. Thornburg-Suresh: None. **A. Anagnostopoulos:** None. **D.W. Summers:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.22/C101

Topic: C.06. Neuromuscular Diseases

Title: Measuring phosphorylated tau as a biomarker for amyotrophic lateral sclerosis

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Abstract: There is an unmet need not only for effective therapies but also for biomarkers to hasten disease diagnosis for amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease for which diagnosis could take up to two years. To date, several studies have demonstrated a significant decrease in the ratio between the microtubule protein tau and tau phosphorylated at T181 (pTau-T181) in cerebrospinal fluid (CSF) derived from people living with ALS, thus suggesting that tau and pTau could serve as potential biomarkers for ALS. Similarly, we have recently reported a significant decrease in pTau-T181:tau ratio in ALS CSF together with an increase in tau levels in CSF derived from people living with bulbar onset-ALS. Importantly, we have also reported that CSF tau levels correlated with faster disease progression, further supporting a role for tau as potential biomarker in ALS. Here, we assessed total tau, pTau-T181 and pTau-T181:tau ratio in a large cohort of plasma derived from people living with sporadic ALS (sALS) as well as in a smaller cohort of C9ORF72 gene-positive asymptomatic individuals (dominant inherited ALS or DIALS) using Quanterix Simoa assay. Our results revealed a significant decrease in plasma tau levels together with a significant increase in plasma pTau-T181 levels and pTau-T181:tau ratio in sALS compared to healthy controls. Additionally, our preliminary results revealed no changes in total tau, pTau-T181 levels and their ratio in matching CSF and plasma samples derived from asymptomatic individuals. Furthermore, there was no correlation between DIALS CSF and plasma tau levels. Collectively, our results suggest that plasma and pTau levels could serve as biomarkers for ALS. Ongoing studies will determine whether plasma tau levels correlate with faster disease progression in sALS as we reported for CSF as well as whether tau levels are altered in people living with familial ALS who have pheno-converted.

Disclosures: A. Castillo-Torres: None. **T. Petrozziello:** None. **S. Huntress:** None. **B. Hammerschlag:** None. **P. Kivisäkk:** None. **S.E. Arnold:** None. **M. Cudkowicz:** None. **M.A. Garret:** None. **J. Berry:** None. **G. Sadri-Vakili:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.23/C102

Topic: C.06. Neuromuscular Diseases

Support: VA Merit review BX002466
R01 AG061729
P30 AG013319
P30 AG044271
Methodist Hospital Foundation
Cure Alzheimer's Fund

Title: Lipidome Insights towards the Amyotrophic Lateral Sclerosis Pathogenesis

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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that primarily affects motor neurons in the brain and spinal cord. Although the spinal cord degeneration has been characterized a hallmark of ALS, the research focuses are relatively shy compares to the research efforts put towards cerebral alterations in ALS. Given the fact that spinal cord exerts unneglectable roles in motor coordination, sensory, and reflex action, understanding the metabolic dysregulation is crucial to understand the disease and mitigate its progression. In this study, we specifically focused on lipid metabolism in ALS. The lumbar spinal cord specimens from 10 neurologically normal and 10 TDP-43 positive sporadic ALS patients were used for lipidomics analysis. The mean ages and standard deviations were 72 ± 9 and 71 ± 9 years for control and ALS patients, respectively, and no statistical difference between the two groups. Total 12 lipid classes with over 200 species were tested in the current study using multi-dimensional mass spectrometry-based shotgun lipidomics (MDMS-SL). Based on the lipidomics results, several lipid classes/species, which majorly involve in oxidative stress, apoptosis, and myelin integrity, showed overt changes. Specifically, plasmalogen was significantly decreased in ALS spinal cords, indicating increased oxidative stress or reduced buffering ability in response to oxidative stress with ALS. Secondly, there was a reduction in phosphatidylserine in ALS group, might indicate increased cellular apoptosis with disease condition. Lastly, the reduction of myelin-specific lipids, i.e., cerebroside and sulfatide species, implied that myelin integrity disruption occurs in ALS spinal cord compared to control. In summary, our study raises evidence from lipidomics perspective to support the current understanding of the ALS disease mechanisms, which involves oxidative stress and apoptosis. In addition, this study is the first ever to reveal that myelin lipid disruption might be involved in ALS pathogenesis. All of these novel findings would help to provide insights on the disease mechanism and potentially inspire new therapeutics. Specimens were provided by the Department of Veterans Affairs Biorepository. VA Merit review BX002466.

Disclosures: Z. Xu: None. **S. He:** None. **X. Han:** None.

Poster

PSTR158: ALS and Motor Neuron Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR158.24/C103

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: VA Merit Award 1I01BX005180

Title: Activation of Akt/mTOR signaling and decreased expression of p27 is linked to motor neuron degeneration in amyotrophic lateral sclerosis patients

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Abstract: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder, characterized by the loss of motor neurons in the motor cortex and spinal cord, resulting in paralysis and death. It is thought that multiple pathological stress mechanisms converge in ALS, triggering the loss of homeostasis and the eventual death of motor neurons. We hypothesize that in response to the cellular stress in ALS, there is an overactivation of metabolic and mitogenic pathways that trigger cell cycle re-entry and apoptosis in terminally differentiated motor neurons.To explore whether alternation of metabolic and mitogenic signaling pathways coupled with cell cycle re-entry is associated with motor neuron cell death, we examined measures indicating the activation of the Akt/mTOR pathway via phosphorylation levels of upstream (p-Akt) and downstream (p-S6) effectors of mTOR, the maintenance of cells in quiescence via the level of cyclin-dependent kinase inhibitor p27 kip1 (p27), the regulation of apoptotic cell death via the level of the anti-apoptotic protein BCL-2, and the differentiated motor neuron population via the level of neurogenin-2 (NGN-2) in the post-mortem lumbar spinal cord tissue from ALS patients compared age-matched controls by Western blot analyses. Our results show that there is increased activation of Akt/mTOR signaling accompanied by decreased protein levels of p27, BCL-2, and NGN-2 in ALS. We detected an inverse linear correlation between p-S6 and p27, linking the Akt/mTor pathway to cell cycle re-entry, and a positive linear correlation between BCL-2 and NGN-2, associating apoptosis with the death of motor neurons.Our data indicate that increased activation of Akt/mTOR signaling coupled with loss of p27 protein expression leads to motor neuron degeneration in the spinal cord of ALS patients. These findings suggest that modulation of Akt/mTOR signaling pathway or the expression of p27 may be relevant targets for improving ALS therapeutic strategies.

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Poster

PSTR159: Neurovirology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR159.01/C104

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

Support: FAPESP Regular Fellowship 2020/05416-4

Title: Cardiovascular and kidney diseases are positively associated with neuroinflammation and reduced Brain-Derived Neurotrophic Factor in patients with severe COVID-19.

Authors: *P. H. C. LIRIO[1], B. L. MARQUES[1], D. S. SCOMPARIN[1], F. F. SCARANTE[1], E. ARRUDA[2], A. C. CAMPOS[1];
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Abstract: Even though respiratory dysfunctions are the primary symptom associated with SARS-CoV-2 infection, cerebrovascular events, and neurological symptoms are described in many patients. However, the connection between the neuroimmune profile and the lung's inflammatory condition during COVID-19 and its association with the neurological symptoms reported by COVID-19 patients still needs further exploration. The present study characterizes the SARS-CoV-2 infectivity profile in postmortem nervous and lung tissue samples of patients who died due to severe COVID-19, and the pro-inflammatory factors present in both nervous and lung tissue samples, via a proteomic profiling array. Additionally, Brain-Derived Neurotrophic Factor (BDNF) levels and intracellular pathways related to neuroplasticity/neuroprotection were assessed in the samples. Out of the 16 samples analyzed, all samples but 1 were positive for the viral genome (genes E or N2, but only 3.9% presented E and N2) in the olfactory brain pathway. The E or N2 gene were also detected in all lung samples, with 43.7% of the samples being positive for the E and N2 genes. In the E/N2 positive brain samples, the Spike protein of SARS-CoV-2 co-localized with TUJ-1+ (neuron-specific class III beta-tubulin) and GFAP+ (glial fibrillary acidic protein) astrocytes. IL-6, but not IL-10, expression was markedly higher in most nervous tissue samples compared to the lung specimens. While intracellular adhesion molecule-1 (ICAM-1), interleukin-8 (IL-8), macrophage migration inhibitory factor (MIF), and plasminogen activator inhibitor 1 (PAI-1) were increased in lung samples from SARS-Cov-2 patients, only MIF and IL-18 were detected in nervous tissue samples. Correlation analysis suggested that high levels of IL-6 are followed by increased levels of IL-10 in the brain, but not in lung samples. Our analysis also demonstrated that the presence of comorbidities, such as cardiovascular disease, hypertension, and hypothyroidism, is associated with neuroinflammation, while chronic kidney conditions predict the presence of neurological symptoms, which correlate with lower levels of BDNF in the brain samples. Our results corroborate the hypothesis that a pro-inflammatory state might further impair neural homeostasis and induce brain abnormalities found in COVID-19 patients.

Disclosures: P.H.C. Lirio: None. **B.L. Marques:** None. **D.S. Scomparin:** None. **F.F. Scarante:** None. **E. Arruda:** None. **A.C. Campos:** None.

Poster

PSTR159: Neurovirology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR159.02/C105

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

Support: Alzheimer's Association/National Academy of Neuropsychology grant ALZ-NAN-22-941933
Dirección General de Políticas de Investigación en Salud (DGPIS)/FPIS2023-INGER-7134

Title: Epigenetic Ages and COVID-19

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Abstract: COVID-19 has been contained; however, the side effects associated with its infection continue to be a challenge for public health, particularly for older adults. On the other hand, epigenetic status contributes to the inter-individual health status and is associated with COVID-19 severity. Nevertheless, current studies focus only on severe COVID-19. Considering that most of the worldwide population developed mild COVID-19 infection. In the present exploratory study, we aim to analyze the association of mild COVID-19 with epigenetic ages (HorvathAge, HannumAge, GrimAge, PhenoAge, SkinAge, and DNAmTL) and clinical variables obtained from a Mexican cohort of older adults. We found that all epigenetic ages significantly differ from the chronological age, but only GrimAge is elevated. Additionally, both the intrinsic epigenetic age acceleration (IEAA) and the extrinsic epigenetic age acceleration (EEAA) are accelerated in all patients. Moreover, we found that immunological estimators and DNA damage were associated with PhenoAge, SkinBloodHorvathAge, and HorvathAge, suggesting that the effects of mild COVID-19 on the epigenetic clocks are mainly associated with inflammation and immunology changes. In conclusion, our results show that the effects of mild COVID-19 on the epigenetic clock are mainly associated with the immune system and an increase in GrimAge, IEAA, and EEAA.

Disclosures: J. Gomez-Verjan: None. **P. Garcia-delaTorre:** None. **N.A. Rivero-Segura:** None.

Poster

PSTR159: Neurovirology

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Program #/Poster #: PSTR159.03/C106

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

Support: R01DA054921
P30DA013429
T32DA007237

Title: Sars-cov-2 spike protein and cocaine increase blood brain barrier permeability through the sigma1 receptor

Authors: *S. E. DAVIS[1], R. PETRILLI FORTUNA[2], J. L. BARR[3], D. LOPEZ[4], W. HO[1], E. BRAILOIU[1], E. M. UNTERWALD[5];
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Abstract: Persons with a history of substance use disorder (SUD) have worsened COVID-19 outcomes (e.g. hospitalization, mortality) following infection with SARS-CoV-2. Previous work has demonstrated that drugs of abuse such as cocaine increase blood brain barrier (BBB) permeability allowing for infiltration of foreign toxins and pathogens to the central nervous system. Recently published work found that the SARS-CoV-2 spike protein internalizes tight junction proteins *in vitro*. Therefore, we *hypothesize* that comorbid SARS-CoV-2 infection in persons with a history of SUD will have increased BBB permeability compared to those without infection, which may be mediated by the sigma1 receptor. Here, sodium fluorescein extravagance was rapidly increased (5, 10, 15 minutes) with intravenous tail injection of spike protein (200 ng/kg) using a *in vivo* miniscope. Expanding upon this finding, this project determines the dose dependent effects of cocaine and spike on BBB permeability. Further, this study determines whether spike protein potentiates cocaine-induced extravasation and whether there is a role for the sigma1 receptor in mediating these outcomes. To define the molecular mechanism of action for changes in BBB permeability due to spike and/or cocaine challenge, we investigate the functional changes in tight junction proteins such as ZO-1 and Claudin-5 using immunohistochemistry and western blot methods. This work will shed light on the combined effects of spike and cocaine on BBB permeability and investigate the role of the sigma1 receptor in spike protein induced effects on the BBB.

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Poster

PSTR159: Neurovirology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR159.04/C107

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

Support: R01NS124065
R01NS124065-02S1
P41 EB027061
1S10OD017974-01

Title: White matter microstructural correlates of cognitive flexibility in long-COVID: findings from the COVID-BRAIN study

Authors: *A. I. SILVA[1], J. JOERS[1], K. GUNDRY[1], J. THOTLAND[1], D. DEELCHAND[1], Y. PARK[1], G. MANOUSAKIS[2], A. METZLER[2], C. LENGLET[1], L. E. EBERLY[3], M. ATIK[4], O. KANTARCI[4], B. ZEYDAN[4], M. JUTTUKONDA[5], J. SHERMAN[5], L. POLLAK[5], S. MUKERJI[5], G. HARROLD[5], S. REHMAN[6], C. KARMONIK[7], T. ASHIZAWA[7], P. BARKER[6], E.-M. RATAI[5], K. KANTARCI[4], G. OZ[1];
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Abstract: Long-COVID is defined by long-term health problems persisting or appearing after acute SARS-CoV-2 infection. Approximately 10% of patients experience long-COVID, with no current treatment. There is an urgent need to understand the biological basis of long-COVID. We investigated long-term consequences of SARS-CoV-2 infection in the brain using multimodal MRI. We collected harmonized clinical, neurological, cognitive, blood and multimodal MRI data across 5 US sites. Diffusion tensor imaging (DTI), presented here, assessed white matter microstructure in long-COVID and was examined for associations with cognitive data. This report includes 148 participants: 84 suffered from long-COVID (65 non-hospitalized and 19 hospitalized during acute infection) and 64 controls. Participants with long-COVID were enrolled if they continued to show at least one neurological symptom, 6 months after confirmed SARS-CoV-2 infection. Baseline visit occurred 23 ± 10 months post-infection. Control participants were enrolled if they did not have a known SARS-CoV-2 infection. We used general linear models to compare each long-COVID group to controls, while accounting for age and sex differences, and to identify associations between diffusion metrics and cognitive function. DTI acquisition parameters and processing were based on the Human Connectome Project. Fractional anisotropy (FA), and axial-, radial- and mean diffusivity (AD, RD, and MD, respectively) maps were used in a voxel-wise permutation analysis and in a regional analysis (atlas-based segmentation) of group differences. Participants with long-COVID show AD and MD differences relative to controls. Lower AD was found in both non-hospitalized and hospitalized participants, while lower MD was only found in non-hospitalized cases. Differences in AD were strongest in the cerebral peduncle (CP) and internal capsule. Participants with long-COVID showed deficits in cognitive processing speed (Symbol Digit Modalities Test) and in cognitive flexibility (Stroop Color and Word Test, interference score). Lower AD in CP, which may influence response inhibition, was associated with poorer performance on the Stroop test ($t=2.5$, $p=0.01$). DTI revealed differences in white matter microstructure in long-COVID that are associated with deficits in cognitive flexibility. These may be driven by neuroinflammation or axonal swelling. Future work will involve neurite orientation dispersion and density imaging (NODDI) to gain further insights into the biological basis of DTI abnormalities in long COVID.

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Poster

PSTR159: Neurovirology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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

Support: Gilead: The study was supported by Gilead Sciences (LIN-US-983-6131).
NIH (K23HL157610)
NINDS (K23NS121628)

Title: Delayed Cognitive Dysfunction in COVID-19 Pneumonia Patients

Authors: *J. B. BRISCOE[1], J. KIM[2], S. KHANDUJA[2], J. KANG[2], I. CHINEDOZI[2], H. RANDO[3,2], K. WILDI[4], H. CHOI[5], A. GUSDON[5], G. WHITMAN[2], S.-M. CHO[2];
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Abstract: Objective To evaluate long-term neurocognitive impairment after intensive care unit (ICU) admission in patients diagnosed with coronavirus disease (COVID-19). **Background** Post-discharge cognitive impairment is a well-recognized complication of COVID-19. We examined long-term cognitive and psychiatric outcomes in a multicenter prospective trial of patients admitted to the ICU with diagnoses of COVID-19. **Design/Methods** Twenty-six COVID-19 adult intensive care unit (ICU) patients from 2 tertiary hospitals diagnosed with pneumonia between 1/1/21 and 10/30/23 were categorized into two groups by severity of illness: Group 1: Severe (hypoxia and respiratory rate > 30) and Group 2: Critical (signs from severe, plus acute respiratory distress syndrome [ARDS], sepsis, shock, or multi-organ failure). Impaired cognitive testing (BrainCheck score > 1 standard deviation below normal) was determined at three and nine months. **Results** Of 26 patients (group 1, n=9, 35% vs. group 2, n=17, 65%), 17 (65%) completed three, and 19 (73%) completed nine-month BrainCheck testing. There was no difference in average age (49 vs. 51; p=0.78) or sex (females in group 1, 44% vs. group 2, 53%; p=1.0). At three months, no difference in BrainCheck scores was seen between groups. However, in those completing 9-month testing, group 1 had worse BrainCheck scores than group 2 (79 [66.8, 90] vs. 110 [94, 113], p=0.045). Specifically, group 1 had worse performance in the domains of mental flexibility (67 [51, 84] vs. 110 [91, 116], p=0.03), Stroop (67 [60, 87] vs. 100 [92, 105], p=0.02), and immediate recognition (85 [64, 96] vs. 106 [102, 115],

p=0.02).**Conclusions** Severe but not critical COVID-19 infection, as defined in this study, is associated with cognitive impairments in attention, executive function, and memory at nine months but not at three months post-discharge. These unexpected results deserve validation but suggest that severe disease, as determined by pulmonary involvement, does not fully capture the target organs of COVID-19.

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Poster

PSTR159: Neurovirology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR159.06/C109

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

Support: R01NS123445

Title: Changes in brain structure and function in mild COVID-19: change from pre-pandemic assessment

Authors: *M. L. LIPTON[1], K. HO[2], R. FLEYSHER[1], K. YE[3], M. ZIMMERMAN[4], S. SIEGEL[2], F. SHALIKA[5], R. LIPTON[5], J. DAILY[5];
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Abstract: *SARS-CoV-2 infection causes severe acute brain effects and less severe persistent neuropsychiatric manifestations as part of the clinical syndrome described as Post-acute Sequelae of SARS CoV-2 (PASC). Most reports of PASC neuropsychiatric effects have been in severe COVID-19, are nonspecific and could be consequences of critical illness and intensive care, confounding casual association with SARS-CoV-2 infection. The UK biobank assessed a cohort of older individuals with mild COVID-19 identifying neuroimaging marker change from prior to COVID-19. We studied 70 young previously healthy individuals without severe COVID-19 (none hospitalized). Each was previously studied prior to February 2020, using a standardized imaging and neurocognitive protocol. Participants returned a mean of 5.7 years after their original visit for identical neuroimaging [3.0 Tesla structural and diffusion MRI (dMRI: 2mm^3 109 directions @ b-values 300, 800 and 2000)] and computerized cognitive assessments (Cogstate battery). dMRI were fit to diffusion tensor imaging (DTI) and neurite orientation dispersion density imaging (NODDI) models to extract fractional anisotropy (FA,*

axial (AD), radial (RD) and mean (MD) diffusivities, orientation dispersion index (ODI), neurite density index (NDI) and isotropic volume fraction (ISO). Each parameter was averaged over 171 regions of interest (ROI) defined using FreeSurfer. We confirmed SARS-CoV-2 infection using laboratory and clinical criteria in 49 and determined 21 to be free of prior SARS-CoV-2 infection at the time of assessment. Statistical significance of the effect of SARS-CoV-2 infection status on each imaging measure was assessed via ANOVA to determine the incremental contribution of COVID-19 status, with adjustment for age and sex. Significance was defined based on the Bonferroni method to address multiple comparisons. We identified a significant effect of SARS-CoV2 status on multiple dMRI metrics, most prominently in frontal, limbic and parietal regions, both gray and white matter. The pattern of findings, with greater FA, ODI, NDI and MD are consistent with inflammation. Lower AD and higher ODI also suggest axonal loss. Performance on the international shopping list task assessing verbal learning was adversely associated with SARS-CoV-2 status. These findings suggest that previously healthy individuals with mild or asymptomatic SARS-CoV-2 infection exhibit persistent structural brain effects and adverse impact on cognitive function which require further follow up and investigation.

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Poster

PSTR159: Neurovirology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR159.07/C110

Topic: F.04. Neuroimmunology and Neurovirology

Title: Covid-19 vaccine-associated optic neuritis: systematic review

Authors: *M. RAMIREZ DIONISIO[1], J. MARTINEZ-LAZCANO[2];
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Abstract: The recent SARS-CoV-2 pandemichas modifiedmethods for conductingclinicaltrialsincludingpreventivemeasures and safety strategies.So far there isno evidenceof themechanismof the vaccinesand theirsideeffects. However, neuro-ophthalmologicalsymptoms have been reported after theirapplicationagainstSARS-CoV2. In the presentwork, we present a systematicreviewof the case reports of mono- and bilateralopticneuritisafter administrationof SARS-CoV-2 vaccines.Opticneuritis(ON) isa multifaceteddiseaseinvolvinginflammationof one or both opticnerves, although adult-onset ON isusuallyidiopathic.A bibliographicsearch was carriedout inPUBMED usingthe keywords"COVID-19 vaccinationand opticneuritis",the search involvedinclusioncriteriaon thecomplete clinicalcharacterizationof ON, associatedvaccines,timeof appearance of clinicalsymptoms, age, and only articleswritteninEnglishwere included.A total of 126

articleswere recovered, whichincludedonly 33 that corresponded to cases of ON, among which44patientswere shown, of which15 articles(13 patients)were excluded due to observedcomorbidities(autoimmunediseases,NeuromyelitisOptica,mainlyencephalomyelitis.Ther esults show between 1 day to 4 weeks of the onset of symptoms after immunization,meanage 42 years, the incidenceinwomen 79% vs 29% inmen, 65% unilateralvs 35 bilateral.Onphysicalexamination,greater involvementwas observed inthe righteye 35%, left eye 32%,and bilateral32%; On fundoscopy examinationreport infour groups, normal 13%,papilledema16%, papillitis45%, and unreported (NR) 26%. OCT imagingstudiesshow 13%normal 26% opticnerve inflammationand 61 NR. A full laboratory workup reported AQP4(+) 3%, (-) 55 and 42 NR; MOG IgG (+) 10%, (-) 39% and 52 NR. Steroidtreatment is90%steroids,3% steroidswithplasmapheresis,and 6% other treatments. Associatedvaccines;vector type 39% and gene type 55%, inactivatedtype 6%, the attenuated and proteintype donot present an associationwiththe condition.As inthe case reported by our group, thebibliographicevidencesuggested highlightsthe need for prompt plasmapheresisas an adjunctto intravenousmethylprednisolone(IVMP) inpatientswithNMOSD-associatedopticneuritis.

Disclosures: M. Ramirez Dionisio: None. **J. Martinez-Lazcano:** None.

Poster

PSTR159: Neurovirology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR159.08/C111

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

Support: CAPES
CNPq
FAPERJ

Title: Gabaergic interneurons development is disrupted in a mouse model of zika virus infection.

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Abstract: Zika virus (ZIKV) infection during pregnancy is known to impair brain development and cause several brain abnormalities. Besides the severe reduction of the brain, children exposed to ZIKV during gestation can present hyperexcitability signs such as seizures. GABAergic interneurons are responsible for regulating hyperexcitability within the brain and an

imbalanced inhibitory system is associated with psychiatric disorders. In this study, we aim to understand the effects of Zika virus infection on cortical GABAergic interneurons development. We used *in vivo* infection via in-utero intracerebroventricular (i.c.v.) injection on embryonic Swiss mice. Also, i.c.v. or intraperitoneal (i.p.) injections on neonate mice. In *vitro* infection was also used by infecting embryonic medial ganglionic eminence (MGE) dissociated cells. Vero cells supernatant (MOCK) was used as a control. First, we evaluated neonatal animals' seizure susceptibility induced by heat. Mice infected via i.c.v. at postnatal (P) day 0. and tested at P10 have a 50% lower latency to seizure compared to the control. Also, c-FOS staining, a marker of neuronal activity, is higher in the cortex of infected animals indicating an imbalance in the excitation-inhibition system. Then, we evaluated whether ZIKV infection could impair medial ganglionic eminence (MGE) progenitors' proliferation. Using immunohistochemistry, we found that MGE progenitor cells show a reduction of 40% proliferation quantifying the number of Ph3-positive and EdU-positive cells after 7 days of infection *in vitro*. Next, we evaluated if ZIKV infection could impair GABAergic interneuron position in the cerebral cortex. Animals were infected in utero at embryonic (E) day 13 and harvested at P0. We found that calbindin-positive neurons in the cerebral cortex of infected animals were misplaced. 50% of those cells were located on the inferior cortical layers in comparison to 35% in controls, suggesting an impairment in cell position. Furthermore, we investigated if the calbindin remains mislocated in adults (P60) when animals were infected at P0. 15% of calbindin cells were positioned in inferior cortical layers in comparison to 30% in controls at P60. In addition, using qPCR, we checked the expression of a key regulatory protein of inhibitory synapse gephyrin and found that it is increased in animals infected at P0 and analyzed at P7 and P60. Our results suggest that ZIKV infection impacts interneuron development by interfering with progenitor proliferation and disturbing cell positioning. Also, infected animals are susceptible to temperature induced seizures, indicating a possible perturbance in inhibitory/excitatory balance.

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Poster

PSTR159: Neurovirology

Location: MCP Hall A

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Program #/Poster #: PSTR159.09/C112

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

Support: NIH Grant K08NS119882

Title: Starvation-induced neuronal autophagy dysresgulates Zika virus replication and neurodevelopment

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Abstract: The factors influencing diverse neurodevelopmental outcomes during prenatal viral infection remain poorly understood. Viral infections during this critical period can lead to severe neurodevelopmental disorders, though the viral mechanisms responsible for brain injury remain unknown. Preliminary clinical and genome sequencing data have identified autophagy as a candidate pathway involved in the pathogenesis of congenital Zika syndrome (CZS). Autophagy is a nutrient-sensing catabolic process that traps and degrades viral cargo, like Zika, within lysosomes. Closely related viruses can exploit autophagic machinery to promote viral replication. Furthermore, maternal nutrition directly impacts fetal nutritional status, potentially influencing prenatal susceptibility to viral infection.
To characterize the relationship between Zika infection, pre-infection autophagy status, and neuronal injury, we develop a starvation-dependent model of autophagy by altering nutrient supplementation before and during ZIKV infection. Induced pluripotent stem cell (iPSC)-derived neural stem cells were infected with ZIKV and collected at time points spanning early, mid, and late infection and autophagy activation (6, 12, 24, 48 hours). Autophagic flux and viral replication were quantified via RT-qPCR, Western Blot analysis, and immunocytochemistry. Cerebral organoids were followed for up to four weeks after infection to elucidate the impact of aberrant autophagy on neurodevelopment in a viral context. Cell proliferation, neural differentiation, and apoptotic cell death were monitored via immunofluorescence.
Our findings suggest that starvation-induced autophagy enhances Zika virus replication in neural stem cells. Increased ZIKV envelope protein (ZIKV E) levels were observed with greater nutrient deprivation. This consequently resulted in abnormal cell proliferation (Ki67) and differentiation of neural stem cells (SOX2) and cerebral organoids toward neurons (TUJ1), astrocytes (GFAP), and oligodendrocytes (OLIG2).
Together, these findings suggest that pre-infection autophagy induction can modulate susceptibility to virally induced neural injury. We are currently developing targeted pharmaceutical approaches against the different stages of autophagy to decrease viral replication, boost neuronal survival, and ultimately reduce injury.

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Poster

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

Support: JNC-ISN Trainee Merit Award

Title: Immunosuppression-induced Zika virus reactivation causes brain inflammation and behavioral deficits in mice

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Abstract: Zika Virus (ZIKV) is a flavivirus capable of generating microcephaly and other neonatal abnormalities. In addition to its great tropism for the nervous system, different studies highlight the presence of ZIKV for long periods after the acute phase of infection in various tissues. However, the late consequences of ZIKV persistence and further rounds of active replication that may occur have never been explored. Here, we investigated whether 3-day-old Swiss mice infected with ZIKV (10^6 PFU via s.c.) are susceptible to viral reactivation in adulthood. We found that when ZIKV-infected mice are immunosuppressed with Dexamethasone (DX; 50 mg/kg/day i.p.) or Cyclosporine (Ciclo; 30 mg/kg/day i.p.), they have increased susceptibility to chemically-driven seizures. . Levels of subgenomic flavivirus RNAs (sfRNAs) were increased, relative to the amounts of genomic RNAs, in the brains of mice following immunosuppression and were associated with changes in cytokine expression. We investigated the impact of immunosuppression on the testicles and found that ZIKV genomic RNA levels are increased in mice following immunosuppression, which also caused significant testicular damage. These findings suggest that ZIKV can establish new rounds of active replication long after acute stages of disease, so exposed patients should be monitored to ensure complete viral eradication. All procedures performed in the present study were approved by the Institutional Committee for Animal Care and Use of the Federal University of Rio de Janeiro (protocol nº 093/19) and followed the ARRIVE guidelines

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Poster

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

Support: 1ZIANS009426

Title: A biomarker-based model for survival prediction in the first year from symptom onset in progressive multifocal leukoencephalopathy

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Abstract: Progressive multifocal leukoencephalopathy (PML) is an infectious complication with high mortality arising in immunosuppressed patients. Survival in PML requires clearance of the infection and limitation of ongoing neurodegeneration before a critical threshold of CNS injury has been attained that would not be compatible with survival. Here we assess the ability of two models trained on five candidate biomarkers to predict long-term survival in a cohort of 122 PML patients. The biomarkers tested were log JC (Human polyomavirus 2) viral copy number, brain volume and PML lesion volume normalized to total intracranial volume, and age and BMI adjusted cerebrospinal fluid neurofilament and glial fibrillary acidic protein. We implemented two predictive models, one using Euclidean distance from a local polynomial regression average model for our five biomarkers, and another in which we additionally included gender, race, ethnicity, and underlying disease through the use of a Gower distance**.** For each model, we tested the initial and final time point for each biomarker during the first year from symptom onset. The Euclidean distance model tested on the initial time points had a positive predictive value (PPV) of 41% and negative predictive value (NPV) of 55% while the Gower distance model had a PPV of 45% and NPV of 52%. Our Euclidean distance model tested on final time points had a PPV of 11% and NPV of 96% and the Gower distance version had a PPV of 15% and NPV of 96%. Our results show that while first available biomarker measurements may not be useful in this modeling, measurements obtained close to a year from symptom onset can be used by both models to accurately identify people likely to survive PML, with demographic data adding little predictive power to the model. Our results suggest possible future application of these models to the clinical trial setting either for patient stratification or readout of therapeutic efficacy.

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Poster

PSTR159: Neurovirology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR159.12/C115

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

Title: Genetic diversity influences neural stem cell responses to inflammatory cytokines

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Abstract: Viral infections of the brain are a common cause of human illness and exhibit profound variation in disease outcomes in the population. At the cellular level there are multiple factors that contribute to disease severity including but not limited to the virus, viral entry, the cell type, and the host immune response. Our genetics contribute to the disease by governing several steps of the infection to disease pathway. However, there are wide gaps in our knowledge of the complex interplay between human genetic variation and brain cell type-specific vulnerabilities across the many viruses that pose a risk to human health. Elucidating these relationships, especially in CNS infections could lead to the identification of risk biomarkers, disease mechanisms, and improved antiviral therapeutics. Thus, to better understand outcomes of neurotropic infections, we used a novel experimental platform called a **"cell village"** to dissect the relationship between genetic heterogeneity and responses to infections and the immune response. To analyze these cell villages we use transcribed SNPs using an algorithm called *dropualtion* in conjunction with single-cell RNA (scRNA) seq to assign transcriptome to cells and individual cells to the donors. These approaches are coordinated with computational pipelines such as *Census-seq* that uses genomic DNA (gDNA) sequencing to assign cellular phenotypes to natural genetic variation. Using cell villages as a model, embryonic stem cell (ESC) derived neural progenitor cells (NPCs) from 40 different donors were cultured in a shared *in vitro* environment. The 40-donor **NPC village** was then perturbed with a pleiotropic anti-viral cytokine interferon-gamma (IFNg) either 48 hours or 5 days after constructing the village for gDNA and scRNA sequencing respectively. Using *Census-seq* algorithm, we noted that compared to control, some donors showed increased proportion in the village, some donors remain unaffected, while other donors showed dropout from the village. Our scRNA analysis also revealed a drastic difference in NPCs after treatment and donor dependent gene expression profiles. These results highlight that there are unique donor dependent responses to inflammatory cytokines showcasing that genetic composition plays a role in the outcomes to inflammation. Future studies involve determining risk variants to several neurotropic viruses with a goal to identify biomarkers that make the host either vulnerable or resistant to disease.

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Poster

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

Support: R01 NS5103212
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Title: The skull harbours an expanded pool of antigen-specific CD8+ T cells during acute brain viral infection

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Abstract: The skull bone marrow (BM) is increasingly recognized as a pivotal immune reservoir, with a strategic position to provide prompt immunological response to the underlying brain tissue. However, in the era of emerging human viral infections, questions about the immunological contribution of the skull BM during neurotropic viral infections are yet to be answered. In this study, we used C57BL/6 mouse model of CNS infection with the neurotropic Theiler's Murine Encephalomyelitis Virus (TMEV) to determine the phenotype and expansion of skull BM-derived and antigen-specific CD8+ T cells during acute brain infection using spectral flow cytometry. Both wildtype (wt) and single MHC class I (Db loxP/ Kb loxP) expressing mice exhibited an increased frequency of cells positive for H-2D^{b} -viral VP2 epitope tetramer (D^{b}:VP2 $_{121\text{-}130}$), at 7 days following intracranial TMEV infection in skull and peripheral BM. Although, the frequency of CD8+ T cells was higher in femur and tibia BM, skull BM showed significantly higher percentage of virus-specific CD8+ T cells, indicating that the site of infection can influence the extent of immune response in neighboring BM compartments. This response was accompanied by altered frequencies of other cell types including CD45+NK1.1+, CD45+TCRb-CD19+CD21+, CD45+TCRb-CD19-Ly6G-CD11b+CD11c+MHCII+. Finally, distinct cellular changes were seen in central BM vs peripheral BM. These findings implicate the skull marrow environment in the generation of heightened virus-specific immune response during acute viral infection of the brain.

Disclosures: A. Hassani: A. Employment/Salary (full or part-time):; Mayo Clinic. **J. Thelwell:** A. Employment/Salary (full or part-time):; Mayo Clinic. **M. Pedra Seady:** A. Employment/Salary (full or part-time):; Mayo Clinic. **M. Maynes:** A. Employment/Salary (full or part-time):; Mayo Clinic. **F. Jin:** A. Employment/Salary (full or part-time):; Mayo Clinic. **M. Hansen:** A. Employment/Salary (full or part-time):; Mayo Clinic. **A.J. Johnson:** A. Employment/Salary (full or part-time):; Mayo Clinic.

Poster

PSTR159: Neurovirology

Location: MCP Hall A

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Topic: F.04. Neuroimmunology and Neurovirology

Support: K00NS118713
P30MH075673
R01DA052859
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Title: Active infection and antiretroviral therapy impacts brain lipid distribution across in SIV-infected rhesus macaques

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Abstract: Human immunodeficiency virus infection (HIV) still results in higher indices in neurocognitive impairment, mood disorders, and brain atrophy in the modern era of viral suppression (VS) achieved using antiretroviral therapies (ARTs). Despite VS, HIV infection results in considerable bioenergetic strain to the brain. Brain lipids are vulnerable to HIV-associated energetic strain due to their high abundance and unique composition compared to other tissues. Dysfunctional lipid metabolism has been linked to many neurological disorders. Specifically for HIV, studies show viral proteins increase expression of lipid breakdown genes and decrease the abundance of key structural lipids in brain. However, brain lipid metabolism and hallmarks of HIV brain pathology are region dependent. Regional metabolic changes after HIV infection may impact neurologic function and quality of life. Therefore, it is critical to spatially characterize the impact of HIV infection on the brain lipid profile. To address this gap, we evaluated brain lipid distribution using matrix laser desorption/ionization imaging mass spectrometry (MALDI-IMS) using brain regions across four groups of rhesus macaque models of HIV infection (via simian immunodeficiency infection (SIV)) with differences in infection status and ART regimen: 1) SIV-infected, ART-treated, 2) SIV-infected, untreated, 3) SIV-infected, ART-withdrawn, and 4) uninfected, untreated.While these data are still undergoing analysis, we have determined several patterns across the different groups of infection status and ART regimen. Principal Component Analyses (PCAs), which assess the similarity or dissimilarity between groups, was performed comparing infection/ART treatment groups for each tissue. In these PCAs across groups for each brain region, the full ion profiles of SIV only groups were more similar to SIV, ART-withdrawn groups while uninfected, untreated groups were more similar to SIV, ART-treated groups. Overall, these data thus far suggest that SIV-infection has a great impact on the full ion profile of multiple brain regions.

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Poster

PSTR159: Neurovirology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR159.15/C118

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

Title: Dysregulation of human endogenous retroviruses in Alzheimer's disease: insights from single-cell transcriptomics

Authors: *J. CORTÉS SILVA, M. SINGH;
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Abstract: Dysregulation of human endogenous retroviruses in Alzheimer's disease: insights from single-cell transcriptomics

Authors J.A. Cortés Silva[1], M. Singh[1]; [1]Clinical Neuroscience, Max Planck Institute for Multidisciplinary Sciences, Gottingen. Germany. **Disclosures J. A. Cortés Silva:** None. **M. Singh:** None.

Abstract The intricate dysregulation of specific Human Endogenous RetroViruses (HERV) loci represents a novel avenue of exploration in understanding Alzheimer's Disease (AD) pathogenesis, particularly concerning neuroinflammation. Employing a multifaceted investigative strategy, our study aims to elucidate the interplay between HERV expression and susceptibility to AD, with a specific focus on how these factors intersect with neuroinflammation. Transcriptome-Wide Association Studies (TWAS) provide a precise examination of neurological HERVs, allowing us to discern their impact on relevant cell types affected by neuroinflammation in AD brains. Preliminary data from single-cell transcriptomic analysis of pre-frontal cortex samples reveal dysregulated genes and HERV subfamilies associated with AD pathology, emphasizing their potential involvement in neuroinflammatory processes.Moreover, our investigation extends beyond observational analysis to encompass functional validation, exploring how dysregulated HERV loci contribute to neuroinflammation and subsequent neuronal dysfunction in AD. Through comprehensive epigenomic and epitranscriptomic profiling, we scrutinize the regulation of HERV expression, shedding light on the disruption of HERV-gene networks exacerbated by neuroinflammation during AD progression. By utilizing induced neurons, we delve into the biological mechanisms through which HERV loci exacerbate neuroinflammatory responses, further elucidating their role in AD neurobiology.Additionally, we explore the therapeutic potential of pharmacological inhibitors targeting pathogenic HERVs, presenting a promising avenue for intervention in AD progression by mitigating neuroinflammatory processes. In conclusion, our findings underscore the intricate relationship between HERV dysregulation and neuroinflammation in AD pathogenesis, providing valuable insights into potential therapeutic targets for mitigating disease progression and alleviating cognitive decline in affected individuals.

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Poster

PSTR159: Neurovirology

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Title: Hmg i/y and tdp43 are ubiquitinated by herpes simplex virus (hsv) to regulate outcome of infection in primary adult sensory neurons

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Abstract: Herpes simplex virus (HSV) enters sensory neurons with the potential for productive or latent infection. For either outcome, the virus must curtail the intrinsic immune response, regulate viral gene expression, and remove host proteins that could restrict viral processes. Infected Cell Protein 0 (ICP0), a virus-encoded E3 ubiquitin ligase, supports these processes by mediating the transfer of ubiquitin to target proteins to change their location, alter their function, or induce their degradation. To identify ubiquitination targets of ICP0 during productive infection in sensory neurons, we immunoprecipitated ubiquitinated proteins from primary adult sensory neurons infected with wild type HSV (HSV-1 KOS), a recombinant HSV that expresses a truncated non-functional ICP0 (HSV-1 *n*212), and uninfected control neurons using anti-ubiquitin antibody FK2 (recognizing K29, K48, K63 and monoubiquitinated proteins), followed by LC-MS/MS and comparative analyses. We identified 40 unique proteins ubiquitinated by ICP0 and 17 ubiquitinated by both ICP0 and host mechanisms, of which High Mobility Group protein I/Y (HMG I/Y) and TAR DNA Binding Protein 43 (TDP43) were selected for further analysis. We show that HSV-encoded ICP0 ubiquitinates HMG I/Y and TDP43, altering protein expression at specific time points during productive HSV infection, demonstrating that ICP0 manipulates the sensory neuronal environment in a time-dependent manner to regulate infection outcome in neurons.

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Poster

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

Support: NIH Grant NS104351
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Virginia-Maryland College of Veterinary Medicine Internal Research Competition Grant
Center for Emerging, Zoonotic, and Arthropod-borne Pathogens Grant

Title: Glial-cell derived neurotrophic factor maintains HSV2 latency through RET and NCAM in sensory neurons

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Abstract: Herpes simplex viruses 1 and 2 (HSV1 and HSV2) preferentially establish latency in different types of neurons. Neurotrophic factors (NTFs), which support survival and homeostasis of neurons, contribute to the maintenance of HSV latent infections. Nerve growth factor (NGF) maintains HSV1 latency in sympathetic neurons, which are dependent on NGF. However, we previously showed that deprivation of neurturin (NTN) or glial-cell derived neurotrophic factor (GDNF) induces reactivation of latent HSV1 or HSV2, respectively, in primary adult sensory neurons. Upon binding to GDNF family receptors (GFRs), GDNF and NTN activate Ret, a receptor tyrosine kinase that regulates numerous intracellular signaling pathways involved in cell proliferation and differentiation. One of these signaling pathways, PI3K/Akt, has previously been implicated in maintaining HSV1 latency in embryonic sympathetic neurons and is regulated by Ret phosphorylation at Tyr^{1062}. Our data shows that in uninfected sensory neurons RET is phosphorylated at Tyr^{981}, activating the Src pathway, and Tyr^{1096}, activating the Grb2 pathway, but not Tyr^{1062}, when deprived of neurotropic factors for 15 minutes. This suggests that adult sensory neurons possess different intrinsic mechanisms regulating viral latency and neurotrophic factor signaling when compared to embryonic sympathetic neurons. GDNF can also signal through neural cell adhesion molecule (NCAM). An NCAM-blocking antibody in the presence of GDNF caused HSV2 to reactivate, but not HSV1, as shown by a 10-fold increase in viral DNA following treatment. Our results suggest that HSV2 maintains latency in sensory neurons using Ret and NCAM signaling pathways compared to sympathetic neurons that maintain HSV1 latency via the PI3K/Akt pathway. Thus, different types of neurons use alternative mechanisms to regulate the outcome of viral infections, including the maintenance of HSV latency.

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Poster

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

Support: PRIN_20179JHAMZ_006
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Title: Hsv-1 infection induces phosphorylated tau propagation among neurons via extracellular vesicles

Authors: *V. PROTTO[1], M. MITEVA[2], F. IANNUZZI[3], D. D. LI PUMA[4,5], M. MARCOCCI[2], C. GRASSI[4,5], A. PALAMARA[1,6], G. DE CHIARA[3];
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Abstract: Extracellular vesicles (EV), key players in cell-to-cell communication, are known to promote the dissemination of neurotoxic proteins among neuronal cells in different neurodegenerative diseases, including Alzheimer's disease (AD). Interestingly, growing evidence supports the role of herpes simplex virus-1 (HSV-1) infection in the pathogenesis of AD. Indeed, viral replication is known to induce the accumulation of AD-related neurotoxic proteins in the brain, such as phosphorylated tau protein (ptau) and amyloid beta peptide. Moreover, recent studies reported that aggregated forms of tau can be transmitted among neurons and that exogenous aggregates of tau could enter inside cells acting as seeds for the aggregation of the endogenous protein. In the present study we investigated if HSV-1 infection could promote the spread of ptau among neurons via EV, thus propagating HSV-1-induced tau-dependent damage within the brain. To this aim, EV were purified and characterized following mock- or HSV-1 infection (ctr-EV and HSV-EV, respectively) from supernatants of human neuroblastoma SH-SY5Y cells and murine primary neurons. Western blot (WB) analysis of their protein content revealed that HSV-EV were loaded with many isoforms of high molecular weight ptau, particularly those phosphorylated at T205, T181 and T217. We also found a significant increase of total tau in the supernatant from HSV-1-infected primary neurons, indicating that following HSV-1 infection tau is released outside the cells. To verify if EV-tau from HSV-1-infected neuronal cells could be transferred to recipient cells, we performed a recipient assay taking advantage of human tau tagged with GFP ($htau^{GFP}$), that we transiently transfected in N2A neuroblastoma cells, and tau KO neurons as recipient cells. EV isolated from N2A-$htau^{GFP}$-trasfected cells were treated with UV rays to deactivate the viral particles that could be hidden inside EV prior to layer them on tau KO neurons. We found that recipient tau KO neurons layered with HSV-EV uptook significantly higher levels of $htau^{GFP}$ compared to those layered with ctr-EV. Finally, we exploited an *in vivo* model of acute infection and assessed that cerebral HSV-1 infection promotes the release of ptau via EV in the brain of infected mice. Overall, our data suggest that, following HSV-1 infection, EV play a role in tau burden spreading within the brain, thus contributing to neurodegeneration.

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Poster

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Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR159.19/C122

Topic: F.04. Neuroimmunology and Neurovirology

Title: Aav production protocol to optimize all critical viral attributes using dual densitygradients

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Abstract: Adeno-associated virus (AAV) is a versatile tool in neuroscience to identify and analyze neuronal circuits. However, the AAV production process can be lengthy and produce variable results in titer, empty to full capsid ratio, and protein contamination depending on the type of purification strategy used. We have developed a new AAV production protocol that combines the low contaminating protein levels of an iodixanol and the high full to empty virus particle ratio of a cesium chloride (CsCl) density gradient to leverage the positive attributes of both methods. We purified AAV vector preparations (preps) using multiple combinations of density gradients including one to three rounds of CsCl only, iodixanol followed by CsCl (idx-cs), and CsCl followed by iodixanol (cs-idx). We also obtained AAV preps of the matching capsid/payload combination produced by outside sources who purified using iodixanol only or iodixanol followed by affinity column (idx-col). The quality of each viral prep was measured for a set of common critical attributes. Our cs-idx dual density gradient purification strategy yielded a 1.7-4.0 fold increase in titer (GC/ml) and a 5-15% reduction in empty capsids compared to other gradient combinations. We also observed a visible reduction in contaminating proteins by protein gel and improved in vivo labeling with the cs-idx prep. AAV is a proven vital tool for neuroscience research, and the procedures used to produce AAV vectors directly correlate to its functionality in vivo. Here we present a highly optimized AAV purification protocol that maximizes efficiency, quality, and in vivo performance for neuroscience applications.

Disclosures: A.J. O'Dell: None. **S. Saraswathi Prasannakumari:** None. **M.O. Bohlen:** None. **M.A. Sommer:** None. **K. Ritola:** None.

Poster

PSTR159: Neurovirology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR159.20/C123

Topic: F.04. Neuroimmunology and Neurovirology

Title: Novel protocol for production of high purity recombinant rabies viral vectors by Ultracentrifugation in CsCl Density gradient

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Abstract: Rabies virus is a non-segmented negative-strand RNA virus that infects axon terminals and travels in the retrograde direction from the post- to pre-synaptic neuron. Rabies

retrograde neuronal circuit labeling has been used for decades to help understand neuronal networks, however, its utility has been limited by the toxicity observed in the starter and retrograde labeled neuronal populations. This toxicity has been attributed to expression of rabies proteins: nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), transmembrane glycoprotein (G), and viral RNA polymerase (L) elicit a host immune response. The presence of rabies proteins within targeted neurons originates from either de novo protein expression via the rabies expression construct or as contaminants in the rabies viral vector prep. Currently, rabies vectors used in neuroscience applications are purified using a sucrose cushion and/or gradient. This method concentrates and retains rabies proteins produced during vector production with the final rabies vector prep. We hypothesized a rabies purification strategy resulting in significantly fewer contaminating proteins while maintaining experimentally relevant titers would lead to increased starter cell and pre-synaptic neuron survival. To this end, we developed an improved rabies buoyant density purification strategy using small plate numbers and limited supernatant collections. Rabies purification using a continuous cesium chloride density gradient resulted in six-fold reduced level of rabies protein contamination. Additionally, experimentally relevant titers (E8 IU/ml) can be obtained for CVS-N2c-dG-TdTomato (EnvA) vectors originating from a single supernatant collection (60ml) of four p150 plates in a final volume of 200 ul. This highly efficient rabies vector production and purification strategy allows for the generation of rabies vectors with decreased contaminants and high titers.

Disclosures: S. Saraswathi Prasannakumari: None. **M.O. Bohlen:** None. **M.A. Sommer:** None. **K. Ritola:** None.

Poster

PSTR160: Stroke: Clinical Study

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR160.01/C124

Topic: C.09. Stroke

Support: NIH Grant R01 HD059783
Dorothy Foehr Huck and J. Lloyd Huck Distinguished Chair of Kinesiology and Neurology awarded to R.L. Sainburg

Title: Hemisphere-specific virtual reality training of the ipsilesional arm in chronic, severely impaired stroke survivors with right-hemisphere damage

Authors: *N. M. KITCHEN[1,2], J. YUK[3], C. MAENZA[1], T. E. MURPHY[4], C. J. WINSTEIN[5], R. L. SAINBURG[3,6];
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Abstract: Unilateral sensorimotor stroke often produces hemisphere-specific motor deficits in the ipsilesional arm, that vary with the amplitude of contralesional arm impairment and are functionally limiting. Furthermore, stroke survivors with severe paresis must rely on the ipsilesional arm, with demonstrable deficits in coordination, to carry out activities of daily living (ADLs). Accordingly, remediation of the ipsilesional arm motor deficits can improve performance of ADLs. Here, we present data from 9 chronic (over 6 months post-stroke), right-hemisphere damaged (RHD) stroke survivors who were recruited as part of a larger clinical intervention study. All participants were right-handed prior to their stroke (Edinburgh Inventory) and severely impaired based on inclusion criteria of an Upper-Extremity Fugl-Meyer score of < 28. These participants performed 5 weeks (3 x 1 hour sessions per week) of ipsilesional (right) arm motor training, which included a virtual reality (VR) task (20 min) and real-world dexterity tasks (35 min). The real-world dexterity training included a large variety of tasks such as opening and closing bottles and rapidly stacking cups, whereas the VR training involved a shape tracing task aimed at addressing deficits in limb impedance control that have previously been demonstrated to result specifically from RHD. Functional motor performance was assessed using the Jebsen-Taylor Hand Function Test (JTHFT) - a clinical assessment intended to imitate ADLs through manipulation of everyday objects (e.g. paperclips, cans). We then assessed the potential association of changes in ipsilesional arm performance on the hemisphere-specific VR training task with the change in time taken to perform the JTHFT. RHD stroke survivors demonstrated significant improvements in VR tracing task performance over the 5-week training period. Furthermore, this group showed improvements in ipsilesional arm motor function through reduced time to complete the JTHFT (-6.83 ± 5.36 secs). There was also a moderate strength correlation (r = 0.52) between improvement in measures of virtual tracing performance and reduction in overall JTHFT time, indicating that VR training is partially associated with improvements in functional performance of simulated ADL tasks. This suggests a potential benefit of a targeted, hemisphere specific training paradigm as a therapeutic adjuvant to mitigate functional motor deficits of the ipsilesional arm for chronic, severely impaired RHD stroke survivors.

Disclosures: N.M. Kitchen: None. **J. Yuk:** None. **C. Maenza:** None. **T.E. Murphy:** None. **C.J. Winstein:** None. **R.L. Sainburg:** None.

Poster

PSTR160: Stroke: Clinical Study

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR160.02/C125

Topic: C.09. Stroke

Support: 5R01HD059783

Title: Hemisphere-specific virtual reality training of the ipsilesional arm in severely impaired and chronic stroke survivors with left-hemisphere damage

Authors: *J. YUK[1,2,3,4], N. KITCHEN[5], C. MAENZA[5], T. MURPHY[6], C. J. WINSTEIN[7], R. L. SAINBURG[1,8];
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Abstract: While rehabilitation efforts in stroke survivors typically focus on addressing motor impairments in the arm opposite to the side of brain injury (contralesional arm), emerging evidence highlights the presence of motor deficits in the arm on the same side as the brain lesion (ipsilesional arm). Stroke survivors with severe paresis in the contralesional arm must rely on the ipsilesional arm as their primary or sole manipulator for activities of daily living (ADLs). Thus, intervention aimed at addressing motor deficits in this limb have the potential to enhance functional performance in daily activities. Here, we present data obtained from right-handed (prior to stroke) 12 stroke survivors in a chronic stage, over 6 months post-stroke, who had damage to the left hemisphere (LHD) and who had an Upper-Extremity Fugl-Meyer score of less than 28. Over 5 weeks, all patients completed 3 sessions of ipsilesional (left) arm training per week (total 15 sessions). A training session was composed of a virtual reality (VR) task (20 mins) and real-world dexterity tasks (35 mins). The VR task was a virtual shuffleboard task which requires specification of movement direction and speed, early in movement. The dexterity training included a variety of tasks such as opening and closing bottles and rapidly stacking cups. The Jebsen-Taylor Hand Function Test (JTHFT), a clinical assessment that includes simulated ADLs through manipulation of everyday objects, was used to characterize dexterity in the ipsilesional arm before and after training. After the training, participants showed statistically significant improvement on task accuracy and in the number of VR trials performed in a given period of time. Furthermore, participants demonstrated statistically significant improvement in JTHFT (reduced the completion time by 3.69 secs on average). However, we did not find a significant correlation between improvements in VR task and JTHFT. This implies that improvements in simulated ADL tasks (measured by JTHFT) in LHD participants benefitted more from the real-world dexterity training than the hemisphere-specific VR training. In contrast, a separate analysis of RHD stroke participants showed a moderate association between improvement in measures of hemisphere specific VR training and reduction in overall JTHFT time in the ipsilesional arm. Taken together, these results suggest that the side of the brain lesion might play a factor on the efficacy of hemispheric specific VR training in the ipsilesional arm.

Disclosures: J. Yuk: None. **N. Kitchen:** None. **C. Maenza:** None. **T. Murphy:** None. **C.J. Winstein:** None. **R.L. Sainburg:** None.

Poster

PSTR160: Stroke: Clinical Study

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR160.03/C126

Topic: C.09. Stroke

Support: NIH Grant R21HD108585

Title: Exercise training with a motorized, split-crank pedaling device improves interlimb coordination in stroke survivors; effects depend on exercise tolerance and severity of disability

Authors: G. KOWAL[1], T. RUOPP[1], A. SCHUSTER[2], O. NETTESHEIM[2], S. PANTHAKI[2], L. BEARDSLEY[2], N. ORSZULAK[2], B. D. SCHMIT[1], *S. SCHINDLER-IVENS[3];
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Abstract: This abstract summarizes the preliminary results of an open-label clinical trial designed to support or quickly refute the safety, tolerance, and therapeutic effects of a new exercise intervention for lower limb rehabilitation after stroke. The intervention uses a motorized, split-crank pedaling device called CUped to compel the use of the paretic limb and improve interlimb coordination. To date, four participants with chronic stroke have completed exercise training with CUped. Each participant was asked to engage in up to 24 training sessions over 6 weeks and complete up to 864 minutes of exercise with CUped. Participants were instructed to pedal while maintaining a 180° interlimb phase relationship. When phasing was not maintained, motors applied torque to assist the lagging limb and/or resist the leading limb. Safety and tolerance were evaluated by number of adverse events and time spent exercising, respectively. Therapeutic effects were assessed by changes in mean interlimb phase error during split-crank pedaling (μE). Comparison was made from baseline to posttest. Results revealed no adverse events. Tolerance was widely distributed with values of 276, 288, 720, and 864 minutes spent pedaling. In the two participants with "high" tolerance, μE decreased from 73° to 24° and from 78° to 41°, respectively. In one participant with "low" tolerance, μE decreased from 70° to 68°. In the other, μE increased from 82° to 113°. Stroke survivors with high tolerance were independent, community ambulators whose walking velocity at baseline was 0.64 and 0.77 m/sec, respectively. Both attended the maximum number of training sessions. Participants with low tolerance were wheelchair users, walked 0.30 and 0.09 m/sec at baseline, and did not have perfect attendance. These observations suggest that exercise involving CUped is safe for people with stroke. Effectiveness may be highly variable and related to exercise tolerance. Moreover, tolerance may be a function of stroke-related disability. Stroke survivors with a higher level of disability may have low exercise tolerance and obstacles to participation that make it difficult to improve tolerance.

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Poster

PSTR160: Stroke: Clinical Study

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Topic: C.09. Stroke

Support: NIH Grant R21HD108585
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Advancing Healthier WI Grant
Rev. John P. Raynor, SJ Fellowship

Title: Reinforcement matters: visual feedback to improve interlimb coordination during a robotic, split-crank pedaling task

Authors: *T. S. RUOPP[1], S. M. SCHINDLER-IVENS[2], B. D. SCHMIT[3];
[1]Biomed. Engin., Marquette Univ., Milwaukee, WI; [2]Physical Therapy, Marquette Univ., Milwaukee, WI; [3]Dept. of Biomed. Engin., Marquette Univ. Dept. of Biomed. Engin., Milwaukee, WI

Abstract: Our prior work used split-crank pedaling to identify distinct yet interrelated contributors to lower limb movement dysfunction after stroke: abnormal interlimb coordination (ILC) and reduced paretic muscle output (Cleland et al., 2019). We then developed a robotic, split-crank pedaling device called CUped to address dysfunction. People with stroke were asked to pedal both limbs at a 180° phase relationship. When 180° was not achieved, torque assisted the lagging limb and resisted the leading limb proportional to the error. CUped improved ILC, but movement strategy was suboptimal. Participants learned to increase the velocity of the downstroke limb while slowing the upstroke limb such that mean velocity remained the same, but standard deviation of velocity increased. The present study tested the hypothesis that the addition of visual feedback (VF) would further improve ILC by reducing these variations in movement velocity. VF was comprised of keeping a virtual cyclist on the centerline of a road. The cyclist moved left or right depending on phase error and which pedal was leading. In one condition, called VF simple (VFs), only the cyclist and road were displayed. In the other condition, called VF bounded (VFb), positive (R+) and negative (R-) reinforcement were added. R+ included a green border on the screen and a vertical indicator bar which increased with increasing with phase accuracy. R- included an invisible left/right phasing boundary which, when exceeded, would result in the cyclist falling over and the screen border turning red. The border remained red, and the cyclist remained fallen until the participant returned to the centerline for two seconds. The fall boundary decreased over time. Twenty chronic stroke survivors participated. One group pedaled with VFs and no VF (VFoff), and a second group pedaled with VFb and VFoff. ILC was assessed by mean (μE) and standard deviation (σE) of phasing error. Pedaling strategy was assessed by mean (μVel) and standard deviation (σVel) of velocity. Values were compared across tasks. Consistent with our hypothesis, VF improved ILC, but it depended on the VF type. Results showed that VFb reduced μE ($p<0.001$) and σE ($p=0.009$) while VFs did not. Importantly, σVel was decreased with VFb ($p=0.007$), but not VFs. Lower μVel was observed with VFb ($p=0.008$) whereas no change in μVel was found with VFs. These results suggest that VF improves ILC when pedaling with CUped, however it is not

enough to simply show participants their error. It is necessary to include aspects of R+ when the goal is being met, and/or R- when the goal is not met. Future work will test whether VFb can promote lasting ILC improvement via sustained training.

Disclosures: T.S. Ruopp: None. **S.M. Schindler-Ivens:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership Interest. **B.D. Schmit:** None.

Poster

PSTR160: Stroke: Clinical Study

Location: MCP Hall A

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Program #/Poster #: PSTR160.05/C128

Topic: C.09. Stroke

Support: NIDILRR RERC 90REGE0010
NIDILRR RERC 90REGE005

Title: Idle innovations: field observations reveal most rehabilitation technology goes unused

Authors: *C. CELIAN[1], H. REDD[1], P. RYALI[2], K. SMALLER[1], J. L. PATTON[3], D. J. REINKENSMEYER[4], M. R. RAFFERTY[5];
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Abstract: Each year, significant funds are allocated to support the advancement of rehabilitation technology (RT) through research and development grants. Despite this investment, there remains a notable gap between technology developed and that which is used by clinicians. Our objective was to provide insights into RT-use trends among occupational and physical therapists to guide the development process and facilitate a more successful uptake of RT. We conducted direct field observations of clinicians using RT during patient care across inpatient and outpatient settings within a technology-friendly neurorehabilitation hospital. We also inventoried the RT in the observed settings. Our analysis revealed 329 instances of rehabilitation technology uses across 90 distinct devices. Notably, clinicians used technology for interventions (72%) more than for measurement purposes (30%). Among the intervention devices, RT was used the most for balance/gait interventions (39%), followed by strength/endurance (30%) and transfer/mobility training (16%). Measurement devices were primarily used for monitoring vital signs (83%) and assessing grip strength (7%) and upper extremity function (5%). Common characteristics among observed devices include AC power reliance (56%), actuation mechanisms (57%), absence of monitors (53%), multi-functionality (68%), and minimal training requirements (57%). Setup times were generally brief, with intervention RT requiring more time (mean ± SD = 3.8 ± 4.21) than measurements (0.8 ± 1.3). Verbal instructions were nearly ubiquitous (72%), with clinicians

offering more performance feedback (59.7%) than result-oriented feedback (30%). Therapists split their attention evenly between direct patient care (49.7%) and administrative tasks such as documentation (50%). Despite the technological resources available, a significant portion of RT remained underused. This study sheds light on the practical dynamics of RT implementation, informing strategies for maximizing its clinical efficacy and integration.

Disclosures: C. Celian: A. Employment/Salary (full or part-time):; Shirley Ryan AbilityLab. **H. Redd:** A. Employment/Salary (full or part-time):; Shirley Ryan AbilityLab. **P. Ryali:** None. **K. Smaller:** A. Employment/Salary (full or part-time):; Shirley Ryan AbilityLab. **J.L. Patton:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institute on Disability, Independent Living, and Rehabilitation Research (90REGE005). **D.J. Reinkensmeyer:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institute on Disability, Independent Living, and Rehabilitation Research (90REGE005), National Center for Advancing Translational Sciences (UL1 TROO1414). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Hocoma, Flint Rehabilitation Devices. F. Consulting Fees (e.g., advisory boards); Flint Rehabilitation Devices, Aretech. **M.R. Rafferty:** A. Employment/Salary (full or part-time):; Shirley Ryan AbilityLab. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institute on Disability, Independent Living, and Rehabilitation Research (90REGE0010), National Institute on Aging (P30AG059988), U.S. Department of Defense (W81XWH-20-1-0231).

Poster

PSTR160: Stroke: Clinical Study

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR160.06/C129

Topic: C.09. Stroke

Support: NIDILRR Grant 90REGE0010

Title: Towards a clinically applicable measurement of spastic limbs - Development of a Modified InTelligent Scale for Spasticity (MITSS)

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Abstract: Clinically utilized measures of spastic extremities are often severely underpowered and imprecise. The Modified Ashworth Scale, Modified Tardieu Scale, and Parkinson's Rigidity are all examples of clinically used measures, the scales of which are ordinal in the range of 0-4, leading to coarse classifications of spastic responses. The range of responses to rapid movements is larger than these scales capture, the Modified Tardieu Scale is a slight improvement over the Modified Ashworth Scale insofar as it also notes the "Angle of Catch" of a moderately spastic limb. Clinicians, when performing these assessments, often complete a battery of limb assessments in a matter of a few minutes. Here, we aim to design a simple, yet powerful, tool to quickly and accurately quantify the level of spasticity in a manner that can easily be understood and used by clinicians. There are many research-grade tools that can very precisely measure spastic muscle thresholds, passive stiffnesses, muscle electromyographic responses, velocity-dependent responses, rigidity, and oscillatory responses. While these are unarguably precise measures of neuromechanical features of an injured limb, they are both prohibitively expensive and time-intensive. Clinical adoption is severely limited by these factors, which have halted any implementation. The device described here, dubbed the Modified InTelligent Scale for Spasticity (MITSS) is an easily don-able, clinician-worn, instrumented cuff. Included is a 9-DOF IMU, to assess the range of motion of the limb, as well as acceleration peak detection for catch response. The Accelerometer and Gyroscope allow for accurate classification of limb kinematics at the time of assessment. On each side of the device are custom-built force plates that utilize hall effect sensors and permanent magnets to measure the torque response of the tested joint, this can be used in tandem with the acquired joint angles to generate torque-angle response curves. This is all collected and analyzed by an on-board Teensy Microcontroller Unit, which can calculate and output clinically relevant values, which can be directly used in practice, onto an integrated LCD screen. The housing for this all is a custom molded silicone mitt, akin to a silicone pot gripper which is simple to don and instantly ready to use. We worked extensively with clinicians who regularly measure spasticity to refine the design and outputs to match what they found important.

Disclosures: L. Oshea: None. **A.J. Barry:** None. **N.L. Suresh:** None. **W.Z. Rymer:** None.

Poster

PSTR160: Stroke: Clinical Study

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Program #/Poster #: PSTR160.07/C130

Topic: C.09. Stroke

Support: NIH UG3NS12313501

Title: Effects of Epidural Spinal Cord Stimulation on Posterior Root-Muscle Reflexes Evoked in Antagonistic Muscles

Authors: *L. BORDA[1], E. SORENSEN[2], E. CARRANZA[3], N. VERMA[4], R. DE FREITAS[2], A. BOOS[5], G. F. WITTENBERG[6], P. GERSZTEN[7], L. E. FISHER[8], E. PIRONDINI[9], M.

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Abstract: Every year approximately 800,000 people are affected by stroke in the United States. Everlasting symptoms include weakness, impaired motor control/planning and spasticity, the latter resulting from abnormal or hyperexcitable spinal reflexes. The origin of this upregulation is still unknown, however both supraspinal and intraspinal mechanisms regulate reflex arc excitability. Theories on the origin of abnormal intraspinal processing after stroke include reduced pre- and/or post-synaptic inhibition on Ia-afferents which could in turn lead spinal motoneurons to spontaneous firing. Our group has shown how delivering electrical stimulation primarily targeting the large-diameter sensory afferents in the dorsal roots of the cervical spinal cord, led to striking motor improvements in individuals with post-stroke hemiparesis. Given that epidural spinal cord stimulation (SCS) mainly recruits the Ia sensory afferents, we designed a study with the aim of investigating whether SCS can also facilitate inhibitory mechanisms of the intraspinal reflex circuit thus reducing α-motoneuron hyperexcitability. We hypothesized that SCS targeting facilitation of an agonist muscle (e.g. triceps brachii (TB)) would simultaneously suppress recruitment of antagonistic muscles (e.g. biceps brachii (BB)) through reciprocal inhibition. We tested this hypothesis by measuring changes in the amplitude of the posterior root muscle (PRM) reflex evoked in the BB while tonic SCS was delivered to facilitate activation of the TB. Two 8-contact SCS leads were implanted percutaneously in the dorsal epidural space of the cervical spinal cord ipsilateral to the paretic arm of two individuals with a unilateral subcortical stroke. Electromyography (EMG) sensors were placed on the antagonistic muscle pair, biceps brachii (BB) and triceps brachii (TB). EMG recruitment curves were measured to identify and select SCS electrodes that evoked responses in biceps or triceps. We measured the amplitude of the evoked responses while participants underwent voluntary and passive elbow stretch. We show that, in both participants, evoked monosynaptic responses in antagonistic muscles are suppressed in presence of SCS. Adding to our previous findings which highlighted the potential benefits of SCS to restore motor functions after stroke, our results suggest the important role that epidural SCS could also have in reducing hyperexcitability of α-motoneurons. Such effects may prove effective for reducing post-stroke spasticity.

Disclosures: L. Borda: None. **E. Sorensen:** None. **E. Carranza:** None. **N. Verma:** None. **R. de Freitas:** None. **A. Boos:** None. **G.F. Wittenberg:** None. **P. Gerszten:** None. **L.E. Fisher:** None. **E. Pirondini:** None. **M. Capogrosso:** None. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Reach Neuro,inc, Bionic Power inc, IOTA Biosciences inc. F. Consulting Fees (e.g., advisory boards); Neuronoff inc, Neuroone inc.

Poster

PSTR160: Stroke: Clinical Study

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Program #/Poster #: PSTR160.08/C131

Topic: C.09. Stroke

Support: NIH UG3NS123135-01A1

Title: A Functional Model of the Effects of Epidural Cervical Spinal Cord Stimulation on the Control of 2D Reaching Tasks in Stroke Participants

Authors: *J. HSU[1], O. REFY[2], L. BORDA[2], E. CARRANZA[3], R. DE FREITAS[4], E. SORENSEN[4], A. BOOS[5], N. VERMA[2], G. F. WITTENBERG[5], P. GERSZTEN[6], L. E. FISHER[7], E. PIRONDINI[8], M. CAPOGROSSO[9], D. J. WEBER[10];
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Abstract: People with chronic hemiplegia post-stroke experience muscle weakness and spasticity, which are common but opposing deficits in motor control. Recent work from our group has shown that these deficits can be reduced using spinal cord stimulation (SCS)[1], but the mechanisms underlying these effects are not well understood. Here, we present a simple biomechanical model and feedback controller for estimating the effect of SCS in improving strength and dexterity during a reaching task. We first show that SCS leads to improvement of 2D center-out reaching tasks as measured by path efficiency, smoothness, reach time and success rates among multiple participants. To explain this improvement, we use a biomechanical model with four muscles simulating the flexor and extensor actions of muscles spanning the shoulder and elbow joint. Activation levels for each muscle are set by a proportional-derivative (PD) controller: None. based on an ideal reference trajectory and the angular position and velocity states for the shoulder and elbow joint. The controller gains (K_P and K_D) are optimized for each trial to minimize the difference between the simulated joint torques and actual torques measured during the experiment. Optimization was carried out using Bayesian optimization with a Gaussian process prior. The simple model generates torque and motion trajectories that match the experimental data collected with neurotypical and paretic subjects. We further show that healthy participants exhibit a stereotypical gain pattern that differs from those observed in participants with stroke. Motor deficits, such as muscle weakness and spasticity are indeed reflected in a lower proportional gain (K_D) and higher derivative gain (K_P), respectively. However, when SCS is applied with properly tuned stimulation parameters that lead to functional improvement, the fitted control gains are ‘tuned’ back closer to healthy ranges. The model results therefore suggest that SCS causes the normalization of abnormal circuitry gains towards healthy ranges. The model sheds the light on muscle-specific motor system circuitry changes that underlie functional recovery under SCS, or lack thereof. The model can further be used to more

objectively tune SCS parameters to achieve muscle-specific goals.
References[1] Powell, M.P., Verma, N., Sorensen, E. et al. Epidural stimulation of the cervical spinal cord for post-stroke upper-limb paresis. Nat Med 29, 689-699 (2023). https://doi.org/10.1038/s41591-022-02202-6

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Poster

PSTR160: Stroke: Clinical Study

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR160.09/Web Only

Topic: C.09. Stroke

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Title: Waist circumference and other variable factors, plasma proteins, and ischemic stroke: a multivariable mediation mendelian randomization study and genetic analyses

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Abstract: Background: Obesity, blood lipids, and certain lifestyle factors are implicated in ischemic stroke (IS) risk, but causal relationships are not well-established. **Methods:** Univariable MR and multivariable MR (MVMR) assessed 19 individual risk factors. Mediation MR was used to identify potential mediators. Molecular docking evaluated compound-protein interaction patterns. Moreover, assessed heritability and genetic correlation specific to WC and IS, explored tissue-specific SNPs heritability enrichment, performed cross-trait meta-analysis, and conducted colocalization analysis to understand the association between WC and IS risk.**Results:** The univariable MR analyses unveiled that positive correlation between WC and IS risk, while HDL was linked to decreased IS risk and LDL to increased IS risk. MVMR investigations further delineated these associations, highlighting WC's positive association with IS risk, while HDL exhibited a protective effect, and LDL was positively linked to IS risk.(all $P < 0.05$) Intriguingly, MVMR assessing the combined effects of WC, HDL, LDL and other modifiable indicators on IS

risk revealed nuanced associations. Specifically, years of schooling, HDL, and coffee consumption showed negative associations, whereas LD and WC were positively associated with IS risk.(all $P < 0.05$) Mediation analysis uncovered that WC indirectly influenced IS risk through 11 human plasma proteins: LRRTM2, NRP1, PILRA, TNFRSF19, BMPR1A, SLC35B2, SLITRK3, TCN2, TINAGL1, TMED10 negatively and PTGR1 positively associated with IS. (all $P < 0.05$) The indirectly effects through 11 proteins account for 5%, 3%, 9%, 3%, 6%, 4%, 6%, 2%, 6%, 3% and 4% of the total effect, respectively. Molecular docking identified several potential therapeutic compounds, including Ethinyl Estradiol, Acetaminophen, Cocaine, Diclofenac, Diethylstilbestro, Methotrexate, Caffeine, Dexamethasone, Progesterone, Warfarin, Amphetamine, Methamphetamine, Nicotine, Progesterone, and Resveratrol, targeting these proteins for IS treatment. Bivariate LDSC analysis revealed a significant genetic correlation between WC and IS. Tissue-specific enrichment analysis identified brain-related tissues enriched in SNPs associated with WC, highlighting potential neural mechanisms underlying the observed genetic correlations. Cross-trait meta-analysis identified 742 significant SNPs shared between WS and IS.**Conclusion:** WC was identified as the primary causal factor for IS risk, with 11 proteins may involved in the WC-IS pathway. These findings contribute to our understanding of IS pathogenesis and treatment development.

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Poster

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Support: IITP grant of Ministry of Science and ICT (2021-0-00742)
RS-2023-00262005
HR22C1605

Title: Early Functional Predictors for Outcome of Independency in Daily Living in Stroke Patients: A Decision Tree Analysis

Authors: *H. KIM[1,2], E. CHUNG[1], H. JEON[1,2], S. SHIN[1,2,3], M. KIM[1,2,3];
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Abstract: Abstracts In the realm of stroke rehabilitation, the prediction of activities of daily living (ADL) is crucial for establishing effective therapeutic goals. Accurate prediction of functional outcomes during the early recovery phase is of great consequence in stroke

rehabilitation, facilitating evidence-based clinical decision-making and the establishment of realistic therapeutic goals. The purpose of this study is to investigate the predictive functional factors influencing the acquisition of basic activities of daily living (ADL) performance abilities during the early stages of stroke rehabilitation using classification and regression analysis trees (CART). We retrospectively collected and analyzed the clinical data of 289 stroke patients who underwent rehabilitation during hospitalization (164 males; mean age: 62.2 ± 13.9 years). The follow-up period between admission and discharge was about six weeks. Extracted medical records included demographic characteristics and various functional assessments with item scores. The modified Barthel Index (MBI) at discharge served as the target outcome for analysis. A 'good outcome' was defined as an MBI score ≥ 75 at discharge, while an MBI score < 75 was classified as a 'poor outcome'. As a result, two CART models were developed. The first model, predicting ADL outcomes based on early motor functions, achieved an accuracy of 92.4%. Among patients with a 'good outcome,' 70.9% exhibited 1) ≥ 4 points in the 'sitting-to-standing' category in the motor assessment scale and 2) ≥ 32 points in the Berg Balance Scale score. The second model, predicting ADL outcome based on early cognitive functions, achieved an accuracy of 82.7%. Within the 'poor outcome' group, 52.2% had 1) ≤ 21 points in the 'visuomotor organization' category of Lowenstein Occupational Therapy Cognitive Assessment (LOTCA), 2) ≤ 1 point in the 'time orientation' category of mini-mental state examination. The ability to perform 'sitting-to-standing' and visuomotor organization functions at the beginning of rehabilitation were revealed as the most significant predictors for achievement of successful basic ADL at discharge in stroke patients. Our analysis using CART models suggests key considerations in early motor and cognitive rehabilitation. **Acknowledgement** This research was supported by the IITP grant of Ministry of Science and ICT (2021-0-00742 Development of Core Technology for Whole-body Medical Twin) and supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: RS-2023-00262005, HR22C1605)

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Poster

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Support: NIH Grant UG3 NS123135-01A1

Title: Characterization of motor improvements enabled by cervical epidural spinal cord stimulation in individuals affected by chronic hemiparesis after stroke

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Abstract: Stroke stands as one of the leading causes of motor disabilities worldwide, entailing significant societal and economic burdens. Upper-limb motor impairments after stroke are stereotypically manifested with unilateral loss of motor (*i*) *strength* and (*ii*) *dexterity*, as well as intrusion of abnormal (*iii*) *spasticity* and (*iv*) *motor synergies*. While these behavioral components characterizing hemiparesis are solidified in the chronic phase after stroke, emerging neurostimulation technologies are a promising approach for assisting residual motor functions and promoting therapeutic recovery. Our research group has pioneered an effective method for improving upper-limb motor function in chronic post-stroke individuals by using cervical epidural spinal cord stimulation (SCS). Specifically, we have demonstrated that SCS can enable both (*a*) *assistive effects*, while facilitating residual supraspinal drive through stimulation of large diameter sensory fibers innervating paretic muscles of the arm and hand; as well as consolidate (b) *therapeutic effects* over time in the absence of stimulation. In this study, we characterized motor improvements enabled by SCS on upper-limb motor recovery in 6 participants with chronic motor deficits caused by stroke. A pair of lead electrodes implanted in the epidural space spanning from the C3 to T1 spinal segments were used to deliver SCS. The lead electrodes were lateralized towards the paretic side of each participant, and stimulation parameters were customized to selectively activate afferent fibers innervating different groups of upper-limb muscles. Both assistive and therapeutic effects of cervical SCS were quantified through motor assessments conducted throughout 4 weeks, while stimulation was administered. Specific motor assessments were used to characterize improvements across the behavioral components of hemiparesis after stroke (*i- iv*). For instance, the Fugl-Meyer Assessment (FMA) was used to quantify improvements in motor (*i*) *strength* and (*iv*) *synergies*, whereas a planar reach task using an exoskeleton robot for anti-gravity arm support was used for quantifying improvements in (*ii*) *dexterity*. Our results showed that assistive effects anticipated therapeutic effects retained across time, as significant motor improvements enabled by SCS were generally shown in all participants. Overall, these results detail the effects of SCS as a powerful emerging method for motor rehabilitation of individuals affected by stroke.

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Poster

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Topic: C.09. Stroke

Support: ANR Grant 22-CE19-0008

Title: Cortical plasticity induced by movement-patterned focal muscle-tendon vibration in subacute stroke

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Abstract: INTRODUCTION: After stroke, patients have sensory-motor alterations resulting in motor function loss and sensory deficits. In the subacute phase, patients often struggle to move their hemiparetic limbs, maintain balance, and walk. Consequently, neuronal pathways, such as the proprioceptive one, undergo alterations, potentially impairing function until rehabilitation begins. Focal muscle-tendon vibration (FMV) is recognized for its ability to stimulate proprioceptive pathways, aiding in the maintenance of sensory-motor brain network activity in healthy subjects (Roll et al., NeuroImage, 2012). In stroke patients, plantar-FMV therapy improved balance over weeks (Önal et al, Physiotherapy, 2022), although its specific impact on cortical activity remains insufficiently explored. We hypothesized that, in subacute stroke, connectivity is significantly change with FMV use over a few weeks. The objective of the study was to investigate whether synchronized FMV, mimicking lower limb movement feedback, induces changes in cortical activity in the mid-term. METHOD: We developed a double-blind randomized controlled study (n°IRB 2021-A00822-39) in 56 hemiparetic patients in post-stroke subacute phase, unable to ambulate independently. Over a period of 5 weeks, participants underwent three 30-minute sessions of FMV or placebo therapy targeting lower limb muscles each week. Brain activity was recorded using high-density electroencephalography (HD-EEG) during two sessions of 5-minute resting state. Data acquisitions were assessed before, during, and after the FMV therapy or placebo, as well as clinical evaluations (Berg Balance Test, 10-meter walk test). Following preprocessing, electrodes on frontal, central, and parietal cortex were selected to cover sensory-motor areas. Subsequently, phase amplitude connectivity analysis was performed to calculate the weighted Phase Lag Index (wPLI). RESULTS: Results in the first 12 patients (FMV=6; Placebo=6) indicate that there were no significant differences in age, gender, stroke type and side, or stroke onset delay. After 5 weeks of intervention, patients in the FMV group demonstrated higher progression on the Berg Balance Test score compared to the placebo group (FMV = 14.8 m/s (2.91); Placebo = 4 m/s (2.91); p=0.025). Moreover, connectivity between frontal and central areas in the alpha band increased, while it decreased in the beta band between central and parietal areas. CONCLUSION: These preliminary results suggest that FMV therapy improve functional recovery associated with cortical connectivity changes.

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Poster

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Title: Blood flow modulation to improve motor and neurophysiological outcomes in individuals with stroke: a scoping review

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Abstract: Current stroke rehabilitation techniques explore adjunct therapeutic strategies, supported by neurophysiological mechanisms, to enhance standard training methods and improve motor function. Blood Flow Modulation (BFM) refers to strategies aimed at intentionally altering the dynamics of blood circulation to specific tissues or organs for therapeutic, protective, or performance-enhancing purposes. Ischemic Conditioning (IC), a BFM procedure involving brief occlusion and reperfusion in stationary limbs, is emerging as a potential adjunct to enhance functional improvements in stroke rehabilitation. A similar BFM technique known as Blood Flow Restriction with Exercise (BFR-E) comprises blood flow restriction during aerobic or resistance exercise. IC and BFR-E are two BFM strategies that have shown promise across various health domains and are clinically relevant for stroke rehabilitation. However, the neurophysiological mechanisms underlying these techniques remain largely unexplored, particularly in stroke. Leveraging evidence from related modalities that share analogous underlying principles such as ischemic nerve block, intermittent hypoxia, and exercise, these techniques hold potential to bolster neural activation and motor recovery post-stroke. Clinical and preclinical studies suggest that BFM may promote neuroplasticity, facilitating motor improvements by mitigating intracortical inhibition and elevating neurotrophic factors and proteins that foster central nervous system connectivity. Despite their potential benefits, our knowledge on the application and efficacy of IC and BFR-E in stroke is limited. We performed a scoping review to synthesize the current evidence regarding the impact of IC and BFR-E on motor and neurophysiological outcomes in individuals post-stroke. Moderate to strong evidence from five studies displayed enhancements in paretic leg strength, gait speed, and paretic leg fatiguability after IC and improvements in clinical performance and gait parameters after BFR-E. While trends toward motor function improvement were observed post-interventions, results for neurophysiological outcomes were inconclusive. IC augmented electromyography magnitude and BFR-E increased serum lactate levels but had limited effects on cortical and peripheral stimulation parameters. Our review suggests that IC and BFR-E are promising clinical approaches in stroke, however additional research is imperative to establish standardized protocols, elucidate underlying mechanisms, and validate efficacy of these interventions, paving the way for their informed integration into stroke rehabilitation.

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Poster

PSTR160: Stroke: Clinical Study

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Topic: C.09. Stroke

Title: Multimodal platform combining VR and TENS for stroke rehabilitation - a parallel study

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Abstract: Stroke is a disabling medical condition that affects 15 million individuals per year, leaving 70% of survivors without functional independence. This condition results in upper-limb impairments, involving motor and sensory deficits, alongside distortions in body representation. The latter expands from misjudging upper-limb dimensions to a diminished sense of ownership of the affected limb, and alterations in planning and execution of movements. Despite initial evidence of the crucial role of sensory-motor integration and body representation integrity in promoting effective rehabilitation, conventional approaches tend to prioritize motor recovery while disregarding stroke-induced sensory and self-representation deficits. Moreover, existing rehabilitation methods critically lack intrinsic objective assessment of the patient's motor, sensory, and body representation recovery. To these aims, we developed a multimodal platform combining Virtual Reality (VR) and Transcutaneous Electrical Nerve Stimulation (TENS) to deliver task-oriented upper-limb rehabilitation while measuring objective kinematic and body representation indicators of patients’ recovery. We conducted a parallel study to evaluate the rehabilitative efficacy of the VR+TENS platform compared to conventional rehabilitation for chronic stroke patients. Participants underwent twelve one-hour rehabilitation sessions over three weeks, during which they performed personalized task-oriented games targeting diverse upper-limb muscular components. Patients in the VR+TENS group demonstrated clinically significant functional improvements in the Action Research Arm Test (ARAT) and reductions in sensory-motor impairments (Fugl-Meyer Upper Extremity). Notably, they increased the number and rate of completed movements throughout the rehabilitation sessions, alongside enhancements in velocity, smoothness, and movement efficiency. These functional and motor improvements were supported by a significant improvement in body representation metrics, highlighting how the platform enabled patients to accurately perceive the dimensions of their impaired limb. These findings demonstrate the striking impact of the platform on the multidimensionality of stroke symptoms and pave the way for a holistic intervention easily transferable to home settings.

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Poster

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Title: Neural and Behavioral Mechanisms of Visuo-Spatial Prism Adaptation in Older Adults

Authors: *F. O. ALOBA[1], M. MUTHUKUMAR[2], J. M. HOPE[3], J. SPENCER[5], M. R. BORICH[3], T. M. KESAR[4];
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Abstract: Background: Prism Adaptation Therapy (PAT) is a non-invasive effective treatment that targets the visuospatial-motor neural pathway and helps reduce pathological post-stroke Spatial Neglect (SN). Non-invasive somatosensory electrical stimulation (Stim) to the affected limb is used as an adjunct to motor training and enhances cortical motor excitability. However, neural and behavioral mechanisms underlying PAT are poorly understood. Furthermore, the effects of healthy aging versus post-stroke SN on the behavioral and neural processes underlying the effects of PAT and Stim are not well studied. We hypothesize that Stim will modulate input-associated cognitive processing complementing the effects of PAT on the dorsal visual stream pathway.
Purpose: To evaluate the effects of combining PAT and Stim on (1) upper limb motor performance and (2) corticomotor and intracortical excitability.
Methods: N=15 older adult (OA) able-bodied individuals participated in the study. We evaluated the effects of a single session of PAT +Stim versus PAT + Sham Stim on behavioral and neurophysiologic outcomes, and associations between neurophysiologic effects and PAT-induced sensorimotor adaptation. Before and after PAT, we evaluated visuospatial-motor behavior using upper limb pointing and computerized line bisection tasks; corticomotor and intracortical excitability using single and paired pulse motor evoked potentials (MEPs) amplitudes elicited in response to transcranial magnetic stimulation (TMS) delivered to M1 hotspots of bilateral upper and left lower limb.
Results: To date, our results on 15 OA participants (8 females, 7 males) provide pertinent information about the neural mechanisms of PAT in the unimpaired aging nervous system. We observed that both PAT + Stim and Sham Stim showed significant after-effects with visuoproprioceptive (eyes open) ($p<0.001$), proprioceptive pointing (eyes closed) ($p<0.001$). However, PAT + Stim ($p=0.012$) induced a larger increase in MEP amplitude in the left upper limb compared to PAT + Sham Stim ($p=0.057$).
Discussion: Our results suggest that combining PAT and Stim may target neural substrates and SN mechanisms that neither therapeutic intervention can target alone leading to more robust outcomes with treatment. Our current evaluation of the effects of PAT and Stim in older adults is

an important step for parsing out the effects of stroke versus aging on the neural and behavioral effects of novel visuospatial motor treatments. Our long-term goal is to address knowledge gaps in our understanding of neural mechanisms of SN and develop clinical interventions for the rehabilitation of people post-stroke with SN.

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Poster

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Title: Upper limb reaching kinematics, hand dexterity and neurophysiological outcomes from the enhancing spontaneous recovery after stroke study (ESPRESSo)

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Abstract: ESPRESSo is a registered, single-site randomised, assessor-blind, controlled Phase IIa clinical trial (#ACTRN12620000871943p), that aimed to determine whether a 3-week programme of high-intensity, high-dose neuroanimation therapy (NAT) can improve upper limb recovery and outcomes early after stroke. Eligible patients were recruited on admission if baseline upper extremity Fugl-Meyer (UE-FM) score < 51 and positive motor-evoked potential (MEP) status. Exclusion criteria included cerebellar stroke and inability to sit to perform upper limb therapy. All participants began therapy within 2 weeks of stroke. Sixty-four participants were randomised with minimisation into NAT or conventional therapy (COT) groups (n=31,33). In addition to standard care upper limb therapy, both groups received 90 minutes of therapist time per weekday for three weeks to complete high-dose upper limb therapy. The NAT group used the Mindpod Dolphin platform under therapist supervision, to engage in high-intensity, high-dose spontaneous exploratory arm and hand movements, focused on movement quality in a game-based virtual environment. The COT group received task-orientated training. Post-intervention assessments were obtained 1, 3 and 6 months post-stroke. Several secondary outcomes were obtained to elucidate mechanisms of recovery from upper limb motor impairment and explore differences between groups. A measure of hand dexterity normalised for strength

was assessed using a custom-made instrumented pressure device where participants performed pinch versus grasp and release tasks with the paretic and non-paretic sides. Upper limb kinematics during functional reaching tasks included quantifying movement smoothness using Spectral Arc Length metric (SPARC) across all post-intervention time points. Transcranial magnetic stimulation was applied across a wide range of intensities to generate MEPs from hand and forearm muscles (FDI, ADM, ECR and FCR). These data were used to construct stimulus-response curves and threshold matrices across all post-intervention time points. These secondary outcomes are expected to provide mechanistic understanding into upper limb motor recovery early after stroke and shed light on differences between participants who underwent high-dose, high intensity NAT compared to therapist time time-matched COT. The ESPRESSo trial outcome(s) will be reported for the first time at the Neuroscience 2024 meeting.

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Poster

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Title: At-home Stroke NeuroRehabilitation with the NeuroEXO Brain-Machine Interface System

Authors: *L. SÁNCHEZ RODRÍGUEZ[1,2], J. GONZALEZ ESPANA[3,2], M. A. PACHECO RAMÍREZ[3,2], K. NEDLEY[4], S.-H. CHANG[5], G. FRANCISCO[4], J. L. CONTRERAS-VIDAL[6,2]; [1]Univ. of Houston, Katy, TX; [2]Noninvasive Brain-Machine Interface Systems Laboratory, NSF Industry—University Cooperative Research Center for Building Reliable Advances and Innovations in Neurotechnology (IUCRC BRAIN) Center, Houston, TX; [3]Univ. of Houston, Houston, TX; [4]The Inst. for Rehabil. and Res. (TIRR) Mem. Hermann Hosp., Houston, TX; [5]

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Abstract: The Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) identify stroke is a leading cause of long-term disability and reduces mobility in more than half of stroke survivors of age 65 and older. To improve mobility, stroke survivors receive rehabilitation therapy with varying outcomes. Emerging potential solutions to augment conventional rehabilitation include brain-computer interface (BCI). Most of those solutions focus on strength training notwithstanding reward, engagement, and motivation, which are crucial for inducing the cortical plasticity phenomena are not considered. To provide an affordable solution accessible to the population, the NeuroEXO was conceived. The NeuroEXO upper-limb stroke robotic home-based rehabilitation BCI system uses Movement Related Cortical Potential (MRCP) to detect movement intent. The system includes 5 electroencephalography (EEG) electrodes, 3 electrooculography (EOG) electrodes combined with a wireless robotic arm, a Graphical User Interface (GUI), and a processing unit. This feasibility study focused on assessing usability, changes in MRCP, electrode impedance, and user' compliance with the NeuroEXO system. It also aimed to understand the impact of at-home stroke rehabilitation in upper limb movement and concomitant cortical activity. Five adults with chronic stroke (2F/3M, 35 to 60 years old, FMA-UE $\in$ [13-28]) participated in the study. Comprehensive data regarding clinical and work status, time since injury, handedness, and travel was collected for a thorough analysis. The protocol included pre-clinical assessments, a week of in-clinic EEG recordings for BCI calibration, system training, and 6-weeks of at-home neurorehabilitation sessions accompanied by post-clinical assessments. Assessments included Grip and Pinch Strength, Joint Position Sense, Manual Muscle Test (MMT), and the Fugl-Meyer Assessment for upper extremity (FMA-UE). Results showed compliance from the participants, who completed from 20.56% to 100% of at-home sessions. As participants became familiar with the system, the BCI rate, defined as the duration spent on performing each block, showed improvement ($p < 0.05$). An increase in MRCP amplitude was also observed when early versus late cortical activity was compared across some participants. A usability survey showed that the participants' assessment was that the system was highly usable, thus, demonstrating the feasibility of the impact of at-home rehabilitation devices.

Disclosures: L. Sánchez Rodríguez: None. **J. Gonzalez Espana:** None. **M.A. Pacheco Ramírez:** None. **K. Nedley:** None. **S. Chang:** None. **G. Francisco:** None. **J.L. Contreras-Vidal:** None.

Poster

PSTR160: Stroke: Clinical Study

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR160.18/C140

Topic: C.09. Stroke

Support: NIH Grant DP5OD029571

Title: Assessing and rehabilitating the fine sensorimotor hand function of stroke patients using an instrumented fragile object.

Authors: *M. ADKINS[1], M. K. BUCZAK[2], C. D. OLSEN[1], M. A. TROUT[1], N. TOTH[3], T. S. DAVIS[4], M. M. IVERSEN[5], J. A. GEORGE[1];
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Abstract: Stroke is a leading cause of long-term disability worldwide and is most often associated with reduced motor control and diminished tactile sensation in the hand on one side of the body. Today's hand rehabilitation programs primarily focus on gross motor function, often overlooking deficits in tactile feedback and fine sensorimotor function. This is despite evidence that integrated sensory and motor training can be more effective than conventional approaches relying primarily on motor rehabilitation. To help improve stroke hand rehabilitation, we developed a wireless handheld instrumented object that mimics a fragile object, dubbed the Electronic Grip Gauge (EGG). The EGG allows patients to practice regulating their grip force, a task that integrates both tactile feedback and fine motor function. The EGG consists of a 3D-printed cube embedded with sensors that track applied grip force, load force, acceleration, and relative object position. A cohort of four hemiparetic stroke patients practiced transferring the EGG over a vertical barrier under two distinct modes while an app running on a tablet automatically converted the data received into quantitative performance metrics for hand dexterity (e.g., average transfer speed, average grip force, average peak grip force, and number of EGG breaks, etc.). The two EGG transfer modes consisted of: 1) Non-Fragile: any grip force may be used to transfer the EGG and no sound is emitted. 2) Fragile: grip force must stay below a set "break" threshold. If the threshold is exceeded, the EGG emits a break sound. Participants completed 20 transfers per mode per hand (paretic vs non-paretic). In 3 of 4 patients, transferring the EGG in fragile mode took a significantly longer time with the paretic hand than with the non-paretic hand. The average peak grip force while transferring the fragile mode EGG was also significantly larger when using the paretic hand in 3 of 4 patients. The average peak grip force for fragile transfers using the paretic hand strongly correlated with motor function (box and blocks test; $R = 0.88$) and the average transfer duration moderately correlated with sensory function (monofilament and 2-point discrimination test; $R = 0.60$ & 0.66 respectively). These early results help validate the EGG as a potentially novel method for assessing sensorimotor function. Future work will explore the long-term use of supplemental audio-visual feedback on grip force to rehabilitate sensorimotor function after stroke.

Disclosures: M. Adkins: None. **M.K. Buczak:** None. **C.D. Olsen:** None. **M.A. Trout:** None. **N. Toth:** None. **T.S. Davis:** None. **M.M. Iversen:** None. **J.A. George:** None.

Poster

PSTR160: Stroke: Clinical Study

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR160.19/C141

Topic: C.09. Stroke

Support: Caltech Housner Student Discovery Fund

Title: Personalized acupoint massage method for rehabilitation in patients with facial paralysis

Authors: *K. WAN;
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Abstract: Facial paralysis is an increasingly prevalent condition characterized by the loss of voluntary muscle activity mainly on one side of the face, resulting from various causes such as Bell's palsy, viral infections, stroke, and trauma. This condition manifests as asymmetry in the mouth and eyes, often causing significant cosmetic and functional impairments, including oral dysfunction, muscle contractures, nasal obstruction, and synkinesis. The psychological and neurological impact on patients can also be profound, affecting interpersonal relationships.Recent advancements in personalized rehabilitation have highlighted the effectiveness of facial acupoint massage, which significantly enhances clinical outcomes by improving recovery times and reducing complications. However, research on the precise localization of massage acupoints tailored to the individual's condition and rehabilitation stage is limited, leading to a gap in personalized treatment strategies.This study aims to address these gaps by developing a personalized assisted rehabilitation method based on acupoint massage for patients with facial paralysis. Utilizing traditional Chinese medicine principles, we analyze the relationship between specific facial acupoints and clinical symptoms of facial paralysis. By targeting specific acupoints, the massage aims to restore muscle and nerve function on the affected side. Additionally, we employ image technology and a transformation matrix method for six primary massage acupoints (Yangbai, Zanzhu, Sibai, Yingxiang, Jiache, and Dicang) to achieve precise acupoint localization relative to key facial contours (eyes, nose, mouth) on the affected side. Our findings indicate changes in main acupoint positions such as the Sibai and Dicang, necessitating tailored massage methods. For instance, a case study involving a photograph of an adult male with right facial paralysis shows rotational deviations of the right eye and the corner of the mouth counterclockwise by 10° and 15°, respectively. Thus, the research reveals that in massage rehabilitation for facial paralysis patients, the positions of the Sibai and Dicang acupoints shift horizontally by 4.5mm and 1mm, and vertically by 0.4mm and 8.1mm, respectively. By comparing the shape similarity between healthy and affected faces, we also propose a segmented massage strategy to adjust the intensity and frequency of massages at different stages of rehabilitation. Our approach provides targeted training in facial acupoint massage rehabilitation. It not only offers clinical guidance for the treatment of facial paralysis but also helps minimize the sequelae associated with the condition.

Disclosures: K. Wan: None.

Poster

PSTR160: Stroke: Clinical Study

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR160.20/C142

Topic: C.09. Stroke

Title: Feasibility of telemedicine for stroke in Africa : case of northern Benin in 2023

Authors: *M. AGBETOU, G. COSWALDINE, S. TOKONNONTO, T. ADOUKONOU; Univ. of Parakou, Parakou, Benin

Abstract: Background : Information on patient and caregiver acceptance and satisfaction with telestroke is limited in Africa. Objective: We carry out an exploratory study of telemedicine and stroke in Africa, and then demonstrate the feasibility of telestroke in Benin, particularly in the north of the country. Methods: The study was twofold. First, an exhaustive review of the literature in six electronic databases (Pub Med, Science Direct, Cochrane, African Index Medicus, Embase and Google scholar) and grey literature (theses,congress and conference abstracts) was carried out. Systematic review methodology was used. Then, as part of a pilot study, we set up a system for informing and video telephone calls to neurologist referents at the CHUD Borgou Alibori in northern Benin by physicians who had performed a video-assisted neurological examination for stroke patients. Results: For the exhaustive literature review, of the 1564 articles identified in the 06 databases, 05 met the inclusion criteria (03 in Ghana, 1 in Nigeria and 1 in Zambia). The use of telemedicine is feasible, acceptable and appropriate for the control of blood pressure and other vital parameters in stroke survivors, and it’s also possible to deliver a rehabilitation intervention via m-health in sub-Saharan Africa. For the pilot study, 17 participants were included. 75% of patients and 88.88% of caregivers were fairly or very satisfied. In 77.78% of cases, telemedicine's contribution to rapid patient care was highly satisfactory and 100% of patients found teleconsultation acceptable in northern Benin. Discussion : In most developing African countries, access to healthcare services is uneven, due to geographical barriers and limited resources. The telestroke model was accepted by patients and caregivers in northern Benin because it was free and less expensive in terms of travel costs from outlying regions to the only neurological care center in northern Benin, but also for the speed of care, despite the fact that our country does not have an optimal internet connection system. Conclusion: Telestroke is a feasible, acceptable, appropriate and satisfactory way of enabling stroke teams to support several community hospitals without pre-existing neurological services, using new technologies in Africa.

Disclosures: M. Agbetou: None. **G. Coswaldine:** None. **S. Tokonnonto:** None. **T. Adoukonou:** None.

Poster

PSTR160: Stroke: Clinical Study

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR160.21/C143

Topic: C.09. Stroke

Title: The effect of stochastic resonance stimulation on sensorimotor performance among stroke survivors: a feasibility study

Authors: *S. KNOTT[1], O. ROLIN[2], V. W. CHU[1];
[1]Occup. Therapy, [2]Physical Med. and Rehabil., Virginia Commonwealth Univ., Richmond, VA

Abstract: A stroke can lead to decreased sensation and motor performance (Nelles et al., 1999). Although research has shown the importance of touch for dexterous hand movements (Augurelle et al., 2003; Johansson & Westling, 1984; Monzée et al., 2003), typical therapy for stroke focuses on improving motor performance without addressing sensation (Enders et al., 2013). Stochastic Resonance (SR) is a phenomenon where sensation can be enhanced with the presence of noise in a nonlinear system (McDonnell and Abbott, 2009). It is theorized that the application of an imperceptible vibrotactile stimuli to the skin can increase the firing synchrony of afferent neurons to the somatosensory cortex. This increases the excitability of the mechanoreceptors, leading to enhanced tactile sensation (Enders and Seo., 2011). Previous studies found improvements in sensorimotor performance when SR was applied to the wrist among stroke survivors (Kurita et al., 2013; Seo et al., 2014). The primary aim of this study is to determine the feasibility of wearing an SR device for all waking hours for 1 week. A secondary aim of this study is to determine if sensorimotor performance changes after wearing the SR device for 1 week.The study took place over 2 weeks, where the participants completed 3 assessment visits (baseline, pretest, posttest). The Nine Hole Peg Test (NHPT) and Semmes Weinstein Monofilament Test (SW) were completed twice (sham vs SR) during each visit. Participants were blinded to the order of the conditions. The participants served as their own control (week 1 without SR device). During week 2, participants were instructed to wear the device for all waking hours at 60% or 90% of their detection threshold. The participants' baseline scores were compared to the posttest scores.5 out of 8 participants completed the study. All participants who completed the study stated that they wore the device at home for one week without experiencing discomfort. 4 out of 5 participants stated that they would wear a similar device if the design was slimmer. 3 participants stated that they noticed a difference with fine motor movements while playing the piano, video games, and gardening. NHPT times improved from 23.47s to 20.68s ($p=0.006$) after using SR for one week. However, SW scores did not change significantly ($p = 0.391$) after using SR for one week.SR is an inexpensive technology that is feasible to wear during the day without discomfort. There is preliminary evidence showing improved fine motor movement after wearing the device for 1 week. However, this does not seem to stem from improved sensation in the thumb and index fingers. Future research should focus on the long-term effects of SR on sensorimotor performance.

Disclosures: S. Knott: None. **O. Rolin:** None. **V.W. Chu:** None.

Poster

PSTR160: Stroke: Clinical Study

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR160.22/C144

Topic: C.09. Stroke

Title: Enhancing Ankle Motor Performance Post-Stroke: Exploring the Influence of Transcranial Direct Current Stimulation (tDCS) and Visual Feedback

Authors: *A. DOSHI[1], A. CHANDHOK[2], S. MADHAVAN[3];
[1]Neurosci., [3]Physical Therapy, [2]Univ. of Illinois Chicago, Chicago, IL

Abstract: Background: While anodal transcranial direct current stimulation (tDCS) has shown promise in enhancing corticomotor pathways and motor function among chronic stroke patients, its efficacy can vary significantly among individuals. The impact of tDCS on skill acquisition is influenced by task specificity, especially in visuomotor tasks where extrinsic feedback plays a crucial role. This could potentially moderate the neuromodulatory effects of tDCS. The objective of the current study was to explore if tDCS over the primary lower limb motor cortex (M1) differentially facilitates motor performance during an ankle motor control task based on the availability of visual feedback. **Methods:** 20 participants with chronic stroke will perform plantarflexion and dorsiflexion movements using their paretic ankle to track a computer-generated target sine wave on a custom-built ankle tracking device. The study session was divided into three blocks: Pre-tDCS, With-tDCS, and Post-tDCS. Each block included two randomized visual feedback (VF) settings: 1) FullVF, complete visual feedback of the ankle and sine wave, and 2) NoVF, participants were blindfolded during the task. During the With-tDCS block, participants received 20 minutes of continuous 1mA anodal tDCS over their primary motor cortex. **Results:** Our preliminary results revealed an expected difference in motor performance between the FullVF and NoVF trials during the Pre-tDCS block. Participants demonstrated higher spatiotemporal accuracy, averaging 81%, when provided with full visual feedback compared to 56% accuracy in trials without visual feedback. During the FullVF trials, participants showed a marginal increase in error (1.35%) from the pre-tDCS to the with-tDCS block and a similar increase in error (1.40%) from the with-tDCS to the post-tDCS block. In contrast, in NoVF trials, participants exhibited an increase in error (6.6%) from pre-tDCS to with-tDCS block, followed by a decrease in error (4.2%) from with-tDCS to post-tDCS block. **Conclusion:** These preliminary results suggest that tDCS may have a more pronounced effect on motor performance when visual feedback is absent, potentially indicating a compensatory mechanism in response to the stimulation.

Disclosures: A. Doshi: None. **A. Chandhok:** None. **S. Madhavan:** None.

Poster

PSTR160: Stroke: Clinical Study

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR160.23/C145

Topic: C.09. Stroke

Support: NIH NIDCD R21DC017787 (PI Riley)

Title: Physiological evidence for tDCS-mediated improvement in post-stroke fatigue and attention impairment

Authors: *H. N. REMBRANDT[1], E. A. RILEY[2];
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Abstract: Transcranial direct current stimulation (tDCS), a non-invasive, painless method of applying direct current electrical stimulation to specific areas of the brain, is an effective method for enhancing attention and post-stroke fatigue, as evidenced by behavioral improvements in post-stroke populations. While behavioral evidence supports this method, there is a paucity of physiological data corroboration of improvement; the current study is designed to investigate if a single session of tDCS will improve attention and fatigue as evidenced by relevant physiological methods in persons with post-stroke aphasia. Attention can be measured physiologically through electroencephalography (EEG), a test that measures the electrical signals of brain activity, and pupillometry, a measurement of pupil dilation due to cognitive activity. This study is a within subjects design wherein all participants receive both the experimental (active tDCS) and control (sham tDCS) conditions across two sessions with a three-day washout period and a randomized order of administration. Sessions included a subjective fatigue rating scale and a sustained attention task conducted with simultaneous EEG and pupillometry data collection both before and after a 25-minute attention training task with simultaneous tDCS administration. The sustained attention task consisted of an oddball paradigm with visual stimuli wherein the participant is asked to press a button when the standard stimulus (i.e., every letter except "X") is presented. Data collection for this study is currently ongoing, with an enrollment goal of 10 participants, all with a diagnosis of post-stroke chronic aphasia. Preliminary analysis of the participants' post-treatment reaction time of correct responses to the standard stimuli reveals no significant differences in a Wilcoxon Signed Ranks Test ($n = 6$; $p = .463$). Following the completion of data collection, pupil dilation and EEG data will be analyzed to determine potential treatment effects. The preliminary analysis of reaction time demonstrates that a single session of tDCS is not sufficient to counteract any fatigue related increases in reaction time during a sustained attention task; however, this will be reexamined following the completion of data collection. If the lack of significance is also found at that point, the comparison of this information to the physiological data will reveal a relationship between behavioral and physiological outcomes of a single session of tDCS. In turn this will inform future research on tDCS as a treatment for attention, and the physiological impacts thereof.

Disclosures: H.N. Rembrandt: None. **E.A. Riley:** A. Employment/Salary (full or part-time):; Syracuse University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH NIDCD R21DC017787 (PI Riley).

Poster

PSTR160: Stroke: Clinical Study

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR160.24/C146

Topic: C.09. Stroke

Support: NIDILRR RERC COMET 90REGE0005-01-00
NIH T32 Training grant for funding MSTP at UICOM

Title: Icosahedron Partitioning to Differentiate Arm Movement Deficit Tendencies Post-Stroke

Authors: *A. SRIVATSA[1], P. RYALI[2], C. CELIAN[3], L. OSHEA[4], J. L. PATTON[5];
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Abstract: Upper extremity rehabilitation in stroke survivors with hemiplegia have targeted improving range of motion and the abilities of patients to perform daily activities. With new robotic innovations being introduced as potential tools in therapy such as anti-gravity exoskeletons, sensors in electromyography and IMUs have been explored to evaluate their effectiveness. However, current movement ability assessments can consume valuable therapy time and only partially help in understanding motor capabilities such as range, speed and coordination.

Here, we present a markerless joint tracking analysis to explore movement impairments in stroke survivors with hemiplegia. We hypothesized that these participants would exhibit reduced movement in specific spatial regions compared to neurotypical participants. To test this, we analyzed the percentage of time spent in icosahedron-defined zones. Five participants who were neurotypical and five participants with post-stroke hemiplegia performed a *free exploration* task (asked to move everywhere with varying speeds without repeating patterns) for two minutes. We recorded wrist and shoulder kinematic data with *Xbox Kinect II* and *Body2Basics* markerless joint tracking system. While previous work used rectilinear partitioning that was not anatomically relevant, here we identified the quantities of wrist position data in an icosahedron with 20 faces of a triangular pyramidal zone converging at the glenohumeral joint. We compared the percent time spent in each zone between the two groups.

Participants post-stroke spent 79.37% of the time in six of the 20 zones, each of which were either posterior to the coronal plane or inferior to the glenohumeral joint, while neurotypical participants spent 48.07% ($p < 0.001$) of the time in these same six zones. Among higher proportions of time in the other fourteen zones, the neurotypical group spent significantly more time in the anterior-superior zone (6.67% in the neurotypical group vs 2.09% in post-stroke group, $p=0.004$) and the anterior-inferior zone (3.30% in neurotypical group vs 0.22% in post-stroke group, $p=0.045$) than the participants post-stroke.

These results suggest that icosahedron partitioning can offer anatomically differentiable *distribution analysis* of arm movement deficits from the markerless joint tracking. This novel approach can help identify upper extremity kinematics during unstructured tasks that measure and track progress in rehabilitation.

Disclosures: A. Srivatsa: None. **P. Ryali:** None. **C. Celian:** None. **L. Oshea:** None. **J.L. Patton:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.01/C147

Topic: C.10. Brain Injury and Trauma

Support: Indiana CTSI

Title: Alterations in Neuronal Network Dynamics, Astrocyte Reactivity, and Oxidative Stress Post-Traumatic Brain Injury

Authors: *S. MUFTI[1], C. ADAM[1], E. A. ROGERS[2,1], J. MARTINEZ[1], T. B. BEAUCLAIR[1], N. KRISHNAN[1], M. GONZALEZ[1], R. SHI[1];
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Abstract: Traumatic brain injury (TBI) can induce critical changes in neuronal network dynamics and alter the structural and functional connectivity of affected brain regions, potentially leading to serious sequalae, such as seizures. Epileptogenesis results from a shift in network dynamics towards a hyperexcitable and highly synchronized state, and one of the leading factors that can cause this pathological shift is TBI. While *in vivo* TBI models offer crucial insight into pathophysiological changes occurring in the brain post-injury, it is often difficult to precisely control the extent of internal brain injury in animals (e.g., degree of tissue deformation) even with the ability to command injury application (e.g., rate of rapid acceleration injury or weight-drop), which could account for the variation produced between approaches and impact the precise modeling of subsequent symptoms, like seizures. In response, we utilized our unique TBI-on-a-chip model to investigate seizure-like activity (SLA) and changes in network dynamics post-TBI, with minimal systemic confounding variables. This *in vitro* system simulates the pathophysiology of concussive TBI by applying clinically relevant, rapid acceleration injuries to murine cortical networks on microelectrode arrays, while providing real-time, cell-scale monitoring of electrophysiological and morphological changes. Extracellular recordings of spike activity revealed that networks exposed to 10 rapidly (4-6 sec) administered 30 g impacts displayed heightened network synchronization, a hallmark of SLA. Additionally, cross-correlation analysis revealed significant changes in network dynamics and burst leadership post-impact. Furthermore, immunocytochemical studies on networks exposed to single impacts of 30, 100, and 200 g showed that fluorescence intensities of the astrocyte reactivity marker, GFAP, and the lipid peroxidation product and oxidative stress marker, acrolein, progressively increased with larger g forces. Astrocytes have been shown to regulate concentrations of ions and neurotransmitters in the extracellular space at the synapse, and their dysfunction through increased reactivity can lead to neuronal hyperexcitability and increased susceptibility for

seizures. Moreover, we show that acrolein alone is capable of inducing increases in GFAP without impact injury, which signifies its role in astrocyte reactivity. In summary, our TBI-on-a-chip model could provide vital insights into functional and morphological changes post-TBI, while enabling the investigation of underlying SLA mechanisms, which could lead to the identification of potential therapeutic targets.

Disclosures: S. Mufti: None. **C. Adam:** None. **E.A. Rogers:** None. **J. Martinez:** None. **T.B. Beauclair:** None. **N. Krishnan:** None. **M. Gonzalez:** None. **R. Shi:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.02/C148

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

Authors: *T. BALENA[1], K. J. STALEY[2];
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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, fluorescein-dextran, and BioTracker NucView 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.J. Staley:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.03/C149

Topic: C.10. Brain Injury and Trauma

Support: Burn and Shock Trauma Research Institute

Title: Cellular senescence contributes to the neuropathology and vestibular dysfunction associated with repetitive mild traumatic brain injury

Authors: *M. VOLYANYUK[1,2], K. M. LOTESTO[3,2], W. SHIN[4,2], S. C. BYRAM[5,6,2,7], E. M. FOECKING[8,9,10,11,2];
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Abstract: Vestibular symptoms from mild traumatic brain injury (rmTBI) are one of the most common and persistent complaints following injury. Cellular senescence has been identified as a prominent contributor to rmTBI pathology. Senolytics, a class of drugs that eliminate senescent cells, have effectively reduced cognitive impairments associated with rmTBI. However, the presence of cellular senescence in the vestibular nucleus and its contribution toward rmTBI-related vestibular dysfunction remains unknown. This research aimed to identify the role of cellular senescence in rmTBI and explored the utility of ABT-263 (Bcl-2 inhibitor) as a senolytic

treatment for mitigating vestibular impairments associated with injury. Eight-week-old male Long-Evans rats received 5 closed-head mild TBIs spaced 48 hours apart. Sham rats received identical treatment and anesthesia but with no impacts. Vestibular nuclei were collected 1,3, 7, and 14 days post-final injury (DPI) for RT-qPCR analysis. Expression of senescence markers *Cdkn2a*, *Tp53*, and the anti-apoptotic markers, *Bcl-2*, and *Bcl-xl*, were maximally upregulated in the vestibular nucleus 3 DPI. *IL-1β* and *IL-18*, markers of the senescence-associated secretory phenotype, were maximally upregulated 7 DPI. *Cdkn2a* and *IL-18* continued to demonstrate upregulations 14 DPI. These data demonstrate the acute role of cellular senescence in the vestibular nucleus following rmTBI. A second cohort of rats received a single injection of ABT263 or vehicle directly after the final injury to determine if the cellular senescence in the vestibular nucleus contributes to rmTBI-associated vestibular dysfunction. Vestibular impairment was assayed using a vestibular battery (VB) and open field test (OFT). Animals subject to rmTBI demonstrated profound vestibular deficits 14 DPI, and treatment with ABT263 significantly reduced these deficits. This research showcases cellular senescence as a prominent pathological process in the vestibular nucleus following rmTBI that contributes to vestibular function. These exciting implications suggest that targeting cellular senescence may be a useful therapeutic approach to alleviate vestibular symptoms associated with a variety of neuropathologies, including traumatic brain injury, neurodegenerative diseases, and aging.

Disclosures: M. Volyanyuk: None. **K.M. Lotesto:** None. **W. Shin:** None. **S.C. Byram:** None. **E.M. Foecking:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.04/C150

Topic: C.10. Brain Injury and Trauma

Support: NIH grant RO1NS107853
NIH grant RO1NS132794
NIH grant RO3AG077460

Title: Tspo regulates acute brain damage post-intracerebral hemorrhage in male, but not female, mice

Authors: *F. BONSACK[1], S. SUKUMARI RAMESH[2];
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Abstract: Intracerebral Hemorrhage (ICH) is a subtype 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 from our laboratory demonstrated profound TSPO expression in microglia and

infiltrating macrophages following ICH in the ipsilateral brain striatum and a possible role of TSPO in neuroinflammation. Based on these, employing transgenic animal models, herein, we test the novel hypothesis that TSPO could regulate acute brain damage post-ICH. Mice were subjected to a preclinical model of ICH. Male TSPO knockout mice exhibited significantly increased neurobehavioral deficits at day 3 post-ICH in comparison to control. These deficits were associated with augmented neurodegeneration and brain cell death, as evidenced by Fluorojade-B and TUNEL staining. Concomitantly, there was a significant increase in the RNA expression of pro-inflammatory microglia/macrophage marker iNOS and a significant decrease in an anti-inflammatory marker, CD206, in TSPO knockout mice in comparison to controls. Mechanistically, TSPO knockout mice exhibited a significant reduction in the gene expression of major cholesterol trafficking protein, ABCA1 and cholesterol efflux in the striatum at 3 days post-ICH. Interestingly, TSPO-mediated effects were observed in male mice but not in females. Altogether, the data implicate that TSPO induction after ICH could be an intrinsic regulatory mechanism to prevent exacerbated brain injury in male subjects.

Disclosures: F. Bonsack: None. **S. Sukumari Ramesh:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.05/C151

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R35NS097283

Title: Rho-associated protein Kinase 2 or ROCK-2 inhibition aids the periodic arrangement of membrane-associated periodic skeleton (MPS) along the regenerating axons post-injury.

Authors: *A. BASU[1], I. INGABIRE[2], S. M. STRITTMATTER[3];
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Abstract: Studying axon-regeneration at an ultrastructural level is important, which aids in a clear understanding of anatomical and functional regeneration. The axonal cytoskeleton of a fully matured central nervous system neuron is composed of a highly organized periodic structure called membrane-associated periodic skeleton (MPS) which is indispensable to maintain the structure and function of the axon. The MPS comprises a periodic lattice of actin rings interconnected by spectrin tetramers. Despite extensive research on MPS distribution in axons during development, the mechanism by which the MPS gets arranged in regrowing axons remains largely unknown. We investigated whether and how this MPS (specifically Spectrin) localization gets regulated in the regrowing axons when challenged by physical insult or injury. For this, we injured the primary cortical neuron culture from P1 mice pups at DIV11 and the human cortical sensory neuron (i^3N) culture derived from CRISPRi (interference) induced

human pluripotent stem cells (hiPSc) at DIV40. The super-resolution STED microscopic imaging helped us to identify the ultrastructure of the axonal cytoskeleton. At first, we observed that β II and αII Spectrin tetramers (MPS) form periodic ring structures at a regular interval of ~190nm in the uninjured axons of fully developed mouse and human cortical neurons. Then we checked the MPS arrangement in the regrowing axons post-injury. Strikingly, we found that the periodicity of the Spectrin tetramers was completely lost near the growth cone region of the regenerating axons after 3-5 days post-injury. Later this MPS periodicity partially comes back after 15-20 days of injury. This phenomenon infers that the regrowing axons during the initial days of regeneration are mostly structurally dynamic which correlates with the absence of periodic arrangement of MPS. With time, the parts of the regrowing axons get stabilized which in turn helps in attaining the MPS periodicity. Further, we investigated the molecular mechanism behind the MPS periodic arrangement during regeneration. For this, we blocked Rho-associated Kinase 2 (ROCK-2) gene expression either pharmacologically by Y-27632 drug in mouse neurons or by CRISPRi of ROCK-2 gene in human iPSCs-derived cortical neurons. We found that ROCK-2 inhibition in both mice and human neurons significantly enhanced the regrowth length of the regenerating axons. Later, we observed that the periodicity of MPS distribution is significantly regained back in just 3-4 days post-injury in ROCK-2 inhibited background. Therefore, in this study, we have provided a detailed nanoscale analysis of MPS arrangement in the regrowing axons post-injury.

Disclosures: A. Basu: None. **I. Ingabire:** None. **S.M. Strittmatter:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.06/C152

Topic: C.10. Brain Injury and Trauma

Title: The novel tissue Mesher that dominate Meningeal Space Hemorrhage repair

Authors: *C. YUN[1], *Y. CHEN[2], G. XIAO[1], J. WU[1], Q. DAI[1];
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Abstract: The brain is enveloped by the meninges, which are classically known as dura, arachnoid, and pia mater. As a membranous covering that contains the cerebrospinal fluid (CSF), lymph- and blood-vessels, and immune cells The brain meninges system in the homeostatic of brain environments, neuro-immunity, and pathogen invasion defense is of paramount importance. In the case of pathology or trauma, the brain blood vessels rupture, which leads to a large amount of blood rushing into the meningeal space, which consists of extradural/subdural hemorrhages and subarachnoid hemorrhage (SAH), collectively namely as meningeal space hemorrhage (MSH) that threatening many basic life safeties of humans and animals. One of the most MSH common types is SAH. Worldwide, the annual number of incidents of SAH is nearly

one million, approximately a quarter of patients with SAH die before hospital admission and half of the surviving patients develop serious sequelae the annual number of incidents of MSH is nearly four million. Although overall outcomes are improved in those admitted to the hospital, the underlying cellular and molecular mechanism of MSH repair remains poorly understood. Combined a labeling-free imaging strategy, single-cell RNA sequence, living animal large-scale imaging, and 2p-SAM, we found that when mechanical injury resulted in voluntary extensive blood accumulating in meningeal space, new tissue is generated in the meningeal space, that has a unique tissue characteristic of fast tissue shape changes and overall tissue movement occurs at the same time as contraction, though the vigorous tissue contraction the large blood clots are fast broken down into small. Furthermore, through the Validation of transgenic mice, we found the TG1 gene can specifically label the tissue and inhibit pathway A leading the tissue to stop contracting and the blood clot could not be removed in the meningeal space. In summary, we found a novel tissue that dominates Meningeal Space Hemorrhage repair.

Disclosures: C. Yun: None. **Y. Chen:** None. **G. Xiao:** None. **J. Wu:** None. **Q. Dai:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.07/C153

Topic: C.10. Brain Injury and Trauma

Support: CIRM

Title: Targeting TLR4 Signaling to Modulate Adult Neurogenesis and Excitability Following Traumatic Brain Injury

Authors: *R. JABERI[1,2], A.-T. NGUYEN[3], E. CONTRERAS[3], V. SANTHAKUMAR[3]; [1]Univ. of California, Riverside, Riverside, CA; [2]Molecular Cell and Systems Biology, University of California, Riverside, Riverside, CA; [3]Mol. Cell and Systems Biol., Univ. of California, Riverside, Riverside, CA

Abstract: Mammalian hippocampal neurogenesis continues into adulthood and is regulated by environmental cues, learning, and response to neurological insults like Traumatic Brain Injury (TBI). In previous studies, we demonstrated that blocking TBI-induced an increase in neuronal expression of Toll-like receptor 4 (TLR4), an innate immune receptor, reduced excitability, and improved memory deficits and risk of epilepsy after TBI. Curiously, limiting posttraumatic increase in neurogenesis can also reduce seizure susceptibility. Since TLR4 is expressed in the dentate neurogenic niche, we hypothesize that TLR4 signaling could also mediate injury-induced increases in dentate neurogenesis and serve as a central node for diverse neuropathological changes after TBI. We examined mice receiving moderate fluid percussion injury (FPI, 2 atm) and sham control to determine TLR4 regulation of excitability and adult neurogenesis. TLR4 signaling was antagonized *in vivo* using CLI-095 (a.k.a Tak242 or Resatorvid, i.p), a small-

molecule TLR4 inhibitor, or vehicle. Following FPI, there was an increase in medial perforant path (MPP)-evoked local field potential (LFP) amplitude which was reduced by CLI-095 (0.5 mg/kg i.p) administered 2 hours after injury. Pulse labeling with EdU (5-Ethynyl-2-deoxyuridine), a thymidine analog administered after FPI, identified an increase in neural stem cell (NSC) proliferation in the dentate gyrus. CLI-095 treatment reduced NSC proliferation in FPI animals without altering proliferation in sham controls. To evaluate if the post-FPI increase in proliferation leads to the generation of adult-born granule cells (abGCs) and to evaluate their function in the circuit, we used Nestin-CreERT2: TdT treated with tamoxifen immediately after FPI, to selectively induced reporter (Tdt) expression in abGCs born after injury. We found a significant increase in the number of Tdt labeled abGCs after FPI. Whole-cell patch clamp recordings from labeled abGCs and unlabeled granule cells from mice one month after FPI demonstrate that abGCs born after injury were excitable and received excitatory and inhibitory synaptic currents. Morphological analysis of biocytin fills of labeled abGCs and unlabeled granule cells identified posttraumatic increases in dendritic length and complexity selectively in abGCs born after FPI. Our data identify that TLR4 signaling enhances adult neurogenesis after brain injury and that abGCs born after injury have abnormal dendritic arborization and integrate into the circuit. These studies demonstrate a multifaceted role for TLR4 signaling in posttraumatic neuropathology which can be targeted for therapeutics.

Disclosures: R. Jaberi: None. **A. Nguyen:** None. **E. Contreras:** None. **V. Santhakumar:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.08/C154

Topic: C.10. Brain Injury and Trauma

Support: National Natural Science Foundation of China (no. 81925031 and 81820108026)

Title: Monocyte-derived dendritic cells as orchestrators of $CD8^+$ T cell-mediated cytotoxicity in radiation-induced brain injury

Authors: *S. LI, Y. TANG;
Neurol., Sun Yat-Sen Mem. Hospital, Sun Yat-Sen Univ., Guangzhou, China

Abstract: Introduction: Radiation-induced brain injury (RIBI) remains the most common medical complication of cranial radiotherapy with poorly understood pathological mechanisms. The critical roles of T cells-mediated adaptive immunity in the pathogenesis of RIBI and other neurodegenerative diseases have been shown previously. However, the involvement of innate immune system, especially antigen-presenting cells (APC), during the disease progression of RIBI remains underexplored. **Methods:** Single-cell RNA sequencing was used to identify the

disease-associated APC populations in the lesioned brain tissues of patients, followed by lineage tracing and pharmacological intervention in a mouse model of RIBI, to assess their tissue origin and pathological roles in mediating $CD8^+$ T cell activation, brain injury, and cognitive dysfunction. **Results:** The type 2 conventional DC-like (cDC2-like) cell cluster was identified as the main APC population in RIBI. In combination with in silico pseudotime analysis and lineage tracing using CX3CR1-CreER: Ai9 mice, we showed that these cDC2-like cells were derived from peripheral monocytes, whose depletion resulted in a significant reduction in cDC2-like cells and $CD8^+$ T cells in the brains of RIBI mouse model, together with improved cognitive functions. Transcriptomic and TCR analyses further suggested a two-step activation process for cytotoxic $CD8^+$ T cells post irradiation. Importantly, blockage of the co-stimulatory signals between cDC2-like cells and $CD8^+$ T cells with CD80/CD86 antagonizing antibodies also resulted in $CD8^+$ T cell reduction and mitigated radiation-induced necrotic lesions. **Conclusions:** Monocyte-derived cDC2-like cells play a detrimental role on RIBI progression by activating $CD8^+$ T cells. Therapeutic intervention by targeting cDC2-like cells and their co-stimulatory signals with $CD8^+$ T cells may hold promise for clinical treatment of RIBI and other neurodegenerative diseases.

Disclosures: S. Li: None. **Y. Tang:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.09/C155

Topic: C.10. Brain Injury and Trauma

Support: Kentucky Spinal Cord and Head Injury Research Trust grant 22-4
University of Kentucky Neuroscience Research Priority Area
National Center for Research Resources and the National Center for Advancing Translational Sciences
National Institutes of Health Grant UL1TR001998

Title: Temporal dynamics of B cell infiltration after contusive traumatic brain injury in mice.

Authors: *A. FRANKLIN[1,2], C. MADDOX[3], A. J. DESANA[1], B. WILLIAMS[3], J. MILLER[3,4], A. M. STOWE[5], K. E. SAATMAN[2];
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Abstract: Traumatic brain injury (TBI) is a leading cause of mortality and morbidity for young adults. TBI survivors face persistent cognitive and neurobehavioral deficits. Repeated unsuccessful clinical trials targeting neuronal injury mechanisms have motivated investigations of other cell types in the complex secondary injury cascade following trauma. The roles of astrocytes and microglia in driving neuroinflammation are well established, as are contributions

of systemic innate immune cells such as neutrophils and monocytes. Much less is understood about the adaptive immune response to TBI. B cells are a critical component in the secondary line of defense and injury progression, interacting with T cells, secreting cytokines, and producing antibodies. After TBI, various central nervous system proteins can act as antigens to activate B cells and stimulate antibody production. Although clinical studies describe systemic adaptive immunity engagement through peripheral autoantibody production, knowledge of the timing and regional extent of B cell diapedesis into the brain following TBI is limited, as previous studies in experimental TBI were largely restricted to a single timepoint. We hypothesize that contusion TBI triggers delayed B cell diapedesis into the cortex. Adult male mice received a lateral controlled cortical impact (CCI) TBI or sham injury and brains were collected at 1, 3, 7, 14 or 28 days (n = 6-8 CCI and 4 sham/time point). Series (1:10) of coronal brain sections were immunolabeled with the B cell antibody B220. Regional cell counts were performed, excluding B cells within hemorrhagic regions. Compared to sham mice, CCI-injured mice exhibited increased numbers of B220+ B cells within the injury epicenter of the cortex at 1 and 3 days, which peaked at 7 days before declining at 14 and 28 days. A significant increase in B cells was also observed at 7 days postinjury in the cortex adjacent to the contusion and in the cortex of the contralateral (non-impacted) hemisphere. Small numbers of B cells were observed in deeper brain regions as well, with increased cell numbers in the ipsilateral and contralateral hippocampi at 3 and 7 days post injury, respectively, suggesting migration or delayed diapedesis. Though overall B cell numbers outside hemorrhage areas are low, the potential for production of autoantibodies by B cells could have implications in injury progression and neuroinflammation. Furthermore, B cell activation and inflammatory status could influence pathology. Future studies will characterize morphological and phenotypic characteristics of B cells within the injured brain to gain insight to their potential function and impact on injury pathology.

Disclosures: A. Franklin: None. **C. Maddox:** None. **A.J. DeSana:** None. **B. Williams:** None. **J. Miller:** None. **A.M. Stowe:** None. **K.E. Saatman:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.10/C156

Topic: C.10. Brain Injury and Trauma

Support: NIH UL1TR001998

Title: Modulating B-Cell Response with Glatiramer Acetate Attenuates Traumatic Brain Injury in Mice

Authors: *P. YANEV[1,2], A. FRANKLIN[3], H. WILLIAMS[4], D. NTHENGE[3], T. A. UJAS[5], K. E. SAATMAN[4], A. M. STOWE[2];
[2]Neurol., [3]Physiol., [4]Spinal Cord and Brain Injury Res. Ctr., [5]Neurosci., [1]Univ. of Kentucky, Lexington, KY

Abstract: Background: Traumatic Brain Injury (TBI) continues to be a major concern, with an estimated 5.3 million individuals living with permanent disabilities, underscoring persistent challenges in clinical management. Despite efforts targeting neuronal injury, unsuccessful clinical trials have shifted focus towards other cell types. While the roles of astrocytes, microglia, and systemic innate immune cells in secondary injury cascades are increasingly understood, the adaptive immune response to TBI, particularly B-cell involvement, remains understudied. Preclinical studies in ischemic stroke and spinal cord injury suggest that modulation of B-cell phenotypes affects tissue damage. We previously demonstrated delayed B-cell diapedesis in the contused cortex of mice following cortical impact TBI, prompting an investigation into modulation of B-cell phenotypes for potential therapeutic benefit. Glatiramer Acetate (GA), an FDA-approved drug for multiple sclerosis, shows efficacy by increasing anti-inflammatory, IL-10-producing regulatory B-cells while decreasing pro-inflammatory TNFα levels and numbers of antibody-secreting plasmablasts. We hypothesized that daily systemic administration of GA post-TBI would modulate B-cell populations to promote recovery.
Methods: Young male mice (2 mos. old) received controlled cortical impact (n=8/treatment) or sham (n=4/treatment) injuries and were randomized to daily subcutaneous injections of 5 mg/kg GA or vehicle. At day 14, brains and spleens were collected for histology and flow cytometry respectively, to assess contusion volume, splenic B- and T-cell numbers and their phenotypic subsets.
Preliminary results: GA administration resulted in a modest reduction in contusion volume compared to vehicle controls (p=0.059) 2 weeks post-TBI. Flow cytometry analysis showed no alteration in total numbers of splenic T- or B-cells by either TBI or GA treatment. However, within the GA treated mice who received a CCI, the number of total splenic B-cells was inversely correlated with contusion damage (p=0.03). This significant correlation was only in the GA-treated group, with no correlation to injury in vehicle-treated mice. Analysis of brain tissue from these mice is ongoing.
Conclusion: This study aims to identify a therapeutic strategy for B-cell immunomodulation post-TBI to mitigate brain damage. The findings suggest that systemic administration of GA in the weeks following TBI may serve as a therapeutic option for treating cerebral contusion.

Disclosures: P. Yanev: None. **A. Franklin:** None. **H. Williams:** None. **D. Nthenge:** None. **T.A. Ujas:** None. **K.E. Saatman:** None. **A.M. Stowe:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

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Program #/Poster #: PSTR161.11/C157

Topic: C.10. Brain Injury and Trauma

Support: R01NS107853
R03AG077460
R01NS132794

Title: Brain proteomic changes after intracerebral hemorrhage in aged males and females: insights from a mouse model

Authors: *S. KUPPUSWAMY[1], N. WATSON[2], S. SUKUMARI RAMESH[3];
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Abstract: Sivaraman Kuppuswamy, Noah Watson, Sangeetha Sukumari-Ramesh
Brain proteomic changes after intracerebral hemorrhage in aged males and females: insights from a mouse model
Aging is an independent predictor of adverse outcomes after intracerebral hemorrhage (ICH), a stroke subtype with no effective treatment. Also, the incidence of ICH is expected to have doubled by 2050 due to aging and the spreading use of anticoagulants. However, the role of aging in the pathophysiology of ICH remains largely understudied. Though preclinical animal models of ICH are potent tools for characterizing the disease pathology, the studies on aged animal subjects are largely lacking, which limits our understanding of the intricate molecular mechanisms of ICH-induced brain injury. Herein, we attempt to determine the brain proteomic changes after ICH using an unbiased quantitative proteomics approach and bioinformatics. To this end, aged male and female mice (18-24 months old) were subjected to sham/ICH. Mice were euthanized on day 3 post-surgery, and ipsilateral brain tissue was collected and subjected to mass spectrometry. Considering sex as a biological variable, the data derived from males and females were separately analyzed. The quantitative proteomics analysis revealed 133 differentially expressed proteins (DEP) between the sham and ICH groups in male subjects. Among the DEPs, 98 proteins were downregulated, and 35 proteins were upregulated after ICH, compared to sham. In aged female mice, 315 DEPs were identified, of which 221 proteins were downregulated, and 94 proteins were upregulated after ICH compared to sham. Some of the key DEPs in both aged male and female mice were 14-3-3 isoforms and S100-A9. The quantitative mass spectrometry data was validated using immunohistochemistry or western blot analysis, and the bioinformatics analysis revealed the critical pathways associated with ICH. Overall, the study identifies several novel candidates for further exploration after ICH.
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Disclosures: S. Kuppuswamy: None. **N. Watson:** None. **S. Sukumari Ramesh:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.12/C158

Topic: C.10. Brain Injury and Trauma

Support: NIH/NINDS R01NS123162
NIH/NINDS/NIMH R21NS130541

Title: Increased synaptic and network excitability in female mice after traumatic brain injury

Authors: *M. A. EDMOND, P. M. SAWANT;
Dept. of Neurosci. & Exptl. Therapeut., Texas A&M Univ., Bryan, TX

Abstract: Traumatic brain injury (TBI) is increasingly recognized as a source of long-term neurological dysfunction in military and civilian populations. In patients with moderate to severe TBI, many experience somatosensory deficits and network hyper-activity that can even lead to prevalent forms of toxic neural excitability, including seizures and spreading depolarizations (SD). We recently found that swelling in neurons at 48 hours post-TBI, a period of maximal edema, is associated with reduced cellular and network excitability. Moreover, cell excitability is increased when we eliminate edema with bumetanide or mannitol, suggesting a causative link between neuronal edema and excitability. Interestingly, we observed a resolution of neuronal edema 1 week after TBI; leading us to hypothesize that without the protective effects of edema, like those we observed 48 hours after TBI, network excitability will be enhanced at 1 week post-TBI. We assessed synaptic and network activity at 1 week post-TBI, as well as the mechanisms responsible for those changes. We used two-photon microscopy, with fluorescent indicators of calcium and glutamate, in the barrel cortex of awake, head-fixed, female mice. Our results show a significant increase in the frequency of whisker-evoked neuronal calcium transients but a decrease in the peak amplitudes in CCI animals compared to sham. Moreover, we see less adaptation to frequency-dependent evoked responses in CCI mice in contrast to the normal expected adaptation in sham animals. We also see a trend toward increases in the frequency of spontaneous calcium activity after CCI. These results suggest cortical network excitability is enhanced at 1 week post-TBI when edema-associated protection is resolved. Next, when we induce SD, a known agent of clinical worsening after TBI, SD-associated calcium transients have a significantly greater area under the curve in CCI compared to sham animals, further suggesting a network under strain, leading to long-term effects on synaptic functions. These differences in calcium activity may be a result of impaired excitatory neurotransmission or altered neuronal calcium buffering capacity. Surprisingly, when we examine glutamate transients as a proxy to excitatory neurotransmission at 1 week post-TBI, we observe no significant changes during spontaneous and whisker-evoked activity, demonstrating that glutamate is not a key mediator influencing the changes in calcium signaling that we observe. Our future studies will examine the role of neuronal inhibition on hyperexcitability since we know GABAergic signaling can influence synaptic and network excitation and is known to be altered after TBI.

Disclosures: M.A. Edmond: None. **P.M. Sawant:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.13/C159

Topic: C.10. Brain Injury and Trauma

Title: The Role of Microglia in Blast Traumatic Brain Injury: Polarization State After Exposure to Secondary Injury Factor Acrolein

Authors: *N. KRISHNAN[1], C. ADAM[1], J. MARTINEZ[6], T. B. BEAUCLAIR[2], S. MUFTI[3], A. BOWERS[1], R. SHI[4], E. A. ROGERS[5], S. SHARMA[1];
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Abstract: Blast traumatic brain injury (bTBI) can result in debilitating pathologies like neurodegenerative disease years after the injury is sustained, making the increasing prevalence of bTBI a concern. The key to preventing the long-term consequences of bTBI likely lies in targeting secondary injury, which is damage due to chemical factors like inflammatory molecules and lipid peroxidation products like acrolein. Even after mild injury, secondary injury can persist for years and promote pathological consequences, making untangling these mechanisms a necessity. One key cell type with the ability to attenuate secondary injury factors is microglia. Microglia activate after injury and can assume one of two polarization states: M1, which promotes inflammation, or M2, which reduces inflammation. Understanding how secondary injury factors like acrolein affect post-injury microglial polarization could help us understand and mitigate chronic neuroinflammation to mitigate long-term pathological consequences of bTBI. To this end, in this study, BV2 mouse microglia were exposed to a clinically-relevant concentration of 50 µM of acrolein for 4 hours then incubated in acrolein-free medium for 24 hours. Microglial polarization state was then evaluated via immunocytochemistry. Acrolein treatment resulted in a 131.75% increase in the levels of inducible nitric oxide synthase (iNOS), indicating increased M1 polarization relative to untreated controls. Interestingly, acrolein treatment did not result in a notable change in expression of M2 polarization marker CD206. Levels of CD206 in acrolein-treated cultures were only 1.32% lower than those of untreated controls. These results suggest that acrolein promotes inflammatory phenotypes in microglia and this could be a critical mechanism by which chronic neuroinflammation develops after injury. Moreover, this suggests that mitigating the promotion of M1 polarization after injury may be an important therapeutic avenue to prevent long-term consequences of bTBI and that further exploration of the effects of secondary injury factors on microglial polarization over time is needed.

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Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

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Program #/Poster #: PSTR161.14/C160

Topic: C.10. Brain Injury and Trauma

Support: DOD Grant HT9425-23-1-1003

Title: Moderate prefrontal cortex injury remodels the corticostriatal circuit: a potential role for microglia

Authors: *E. CHU, K. PECHACEK, C. ARNHOLD, S. JAMI, L. WANGLER, J. PACKER, E. GOODMAN, J. P. GODBOUT, K. M. MARTENS, C. VONDER HAAR;
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Abstract: Traumatic brain injury leads to a variety of chronic psychiatric symptoms, including increased impulsivity and attentional deficits. The specific mechanisms are unclear. However, circuit remodeling in the ventral striatum, which is responsible for connecting the limbic and motor systems and facilitating decision-making and learning, may play a role. Prior research suggested abnormal neuroinflammation along the Corticostriatal circuit could alter signaling. Microglia are one of the crucial glial cells that regulate neuroinflammatory responses after TBI, therefore we hypothesized abnormal microglial responses and neuroinflammation would impact circuit remodeling after TBI. To examine this, we used a model of moderate prefrontal cortex brain injury in young adult male and female Long-Evans rats. Then, at 7 days and 14 days post-injury, expression of neuroinflammation and neuroplasticity genes were measured at the injury site and nucleus accumbens. In addition, nuclei were isolated from the nucleus accumbens for unbiased sequencing. To examine the role of microglia, we pharmacologically eliminated them and allowed for repopulation. Impulsivity was then assessed using the five-choice serial-reaction time task. Our results indicated that TBI altered the expression of numerous inflammatory, apoptotic and neuroplasticity genes at the injury site. Short microglia turnover/repopulation was unsuccessful in alleviating impulsive deficits. Ongoing experiments will determine neuroplastic and inflammatory changes in the nucleus accumbens at the time point relevant to impulsive changes (14 days post-injury). In conclusion, moderate, focal injury to the prefrontal cortex in rats induced inflammatory changes at the cortex as expected. However, TBI would induce neuroplasticity changes amongst the regions responsible for impulsivity and decision-making, which may explain the chronic psychiatric symptoms observed in human patients. However, further work will be performed to fully understand the cellular mechanisms that contribute to circuit remodeling and psychiatric disorders, which may ultimately reveal potential therapeutic targets to mediate these chronic consequences.

Disclosures: E. Chu: None. **K. Pechacek:** None. **C. Arnhold:** None. **S. Jami:** None. **L. Wangler:** None. **J. Packer:** None. **E. Goodman:** None. **J.P. Godbout:** None. **K.M. Martens:** None. **C. Vonder Haar:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.15/D1

Topic: C.10. Brain Injury and Trauma

Support: W81XWH1810166
W81XWH1810167
W81XWH2210461
W81XWH2210462

Title: Pharmacological Treatment of Existing Posthemorrhagic Hydrocephalus of Prematurity Improves Adult Executive Function

Authors: J. ROBINAUGH[1], A. ODUKOYA[4], K. BELDAY[1], V. O. OMONIYI[7], X. JIA[1], B. VIJAYAKUMAR[1], R. PATEL[2], H. HELMBRECHT[8], Y. KITASE[5], T. HECK[6], *L. JANTZIE[1], S. ROBINSON[3];
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Abstract: Posthemorrhagic hydrocephalus affects children and adults who were born very preterm (PHHP). After more durable treatment of hydrocephalus, improved cognition is the highest research priority for individuals living with PHHP and their families. We hypothesized that a neuroreparative cocktail using prolyl hydroxylase inhibitor roxadustat (FG-4592) and melatonin would augment the health of the CNS microenvironment and improve neurological performance, including executive function. We induced PHHP with bilateral intracerebroventricular injection of littermate lysed red blood cells on postnatal day 1 (P1) in rats of both sexes with in utero exposure to chorioamnionitis. MRI was performed at postnatal day 18 (P18) to P20 to confirm ventriculomegaly. Subsequently, PHHP rats were randomly allocated to repair cocktail or vehicle at P21. The repair cocktail started at P21 and continued through completion of behavioral testing. Specifically, this cocktail consisted of Roxadustat (10mg/kg) plus melatonin (20 mg/kg) qMWF intraperitoneal. At P60, rats started a Visual Discrimination (VD) task using a touchscreen platform. A priori criteria for analysis were used, including passing and failure rate. Data was tested for normality and statistical analyses were performed with a Mann-Whitney or two-tailed t test as appropriate with $p<0.05$ considered statistically significant. In VD, 42% (6/14) of adult vehicle-treated PHHP rats passed, while 89% (17/19) of PHHP rats treated with MLT+ROX repair cocktail passed ($p<0.05$). Rats with PHHP and treated with the repair cocktail tended to make fewer errors (238 ± 137) than vehicle-treated PHHP rats (345 ± 171, Mann-Whitney $p=0.08$). Repair-treated PHHP rats also required fewer correction trials (406 ± 241) than vehicle-treated PHHP rats (667 ± 396, two-tailed t-test $p=0.02$) for successful completion. In conclusion, PHHP impairs visual discrimination and executive function in adulthood. Importantly, our results suggest that delayed administration of a pharmacological ROX+MLT cocktail directed at brain repair in a preclinical model of PHHP improves cognition into adulthood. Immunomodulation offers a pharmacologic strategy to reduce shunt dependence and significant sequelae like deficits of cognition and executive function. Further studies will elucidate the mechanisms.

Disclosures: J. Robinaugh: None. **A. Odukoya:** None. **K. Belday:** None. **V.O. Omoniyi:** None. **X. Jia:** None. **B. Vijayakumar:** None. **R. Patel:** None. **H. Helmbrecht:** None. **Y. Kitase:** None. **T. Heck:** None. **L. Jantzie:** None. **S. Robinson:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.16/D2

Topic: C.10. Brain Injury and Trauma

Support: Department of Veterans Affairs RR&D IK2-RX003376 (O'Donnell)
Department of Veterans Affairs VISN4 CCDF (O'Donnell)
Department of Veterans Affairs [BLR&D I01-BX005017 (Cullen)
Department of Neurosurgery, Perelman School of Medicine, University of Pennsylvania (Petrov)

Title: Intracranial hypertension and changes in astrocytic endfeet in a large animal model of rotational acceleration traumatic brain injury

Authors: D. J. HAN[1], K. D. BROWNE[2], K. WOFFORD[1], D. CULLEN[3], *J. C. O'DONNELL[2]; [1]Neurosurg., Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of Pennsylvania, Philadelphia, PA; [3]Neurosurg., Univ. of Pennsylvania, Media, PA

Abstract: Traumatic brain injury (TBI) precipitates a cascade of pathophysiological events that significantly influence patient prognoses and survival outcomes. An important focus of acute neurocritical care is evaluating and minimizing the risk of cerebral ischemia caused by intracranial hypertension (ICH). Preclinical stroke and cerebral contusion studies in rodents have shown that ATP-binding cassette protein, sulfonylurea receptor-1 (SUR1), is upregulated in response to injury, initiating a rapid increase in oncotic pressure through its interaction with aquaporin-4 (AQP4) in astrocytic end feet, resulting in cytotoxic edema. The relationship between edema and intracranial hypertension is assumed, but largely untested. In part, this is due to the fact that our rodent models of TBI do not produce ICH. Indeed, increased intracranial pressure, like loss of consciousness, appears to require forces loaded via rotational acceleration of the head. This force loading is dependent on brain mass (acceleration x mass = force) and architecture (gyrencephalic cortex, high white-to-grey matter ratio), and therefore cannot be replicated in small animal models. However, these properties in pig brains are similar enough to human to allow for replication of injurious forces via scaled up acceleration, resulting in key manifestations of human TBI such as ICH and coma. Using the porcine closed-head rotational acceleration TBI model, we hypothesized that ICH and vascular SUR1/AQP4 expression will be detected post-TBI. Following multimodal neuromonitoring in our swine neurointensive care unit, immunohistochemistry was conducted to assess changes in GFAP, SUR1, and AQP4. TBI resulted in ICH and increases in SUR1 and AQP4 in astrocytic endfeet ensheathing vasculature. Our study highlights the relevance of using a large animal closed-head rotational acceleration TBI model that recreates the mechanisms and manifestations (e.g. ICH, loss of consciousness) of human TBI, while integrating comprehensive neuromonitoring and histology. Mechanistic study in a model that produces ICH from TBI offers the opportunity to develop treatments targeting ICH at the source, instead of waiting to react to spikes with symptom-targeted treatments.

Disclosures: D.J. Han: None. **K.D. Browne:** None. **K. Wofford:** None. **D. Cullen:** None. **J.C. O'Donnell:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

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Program #/Poster #: PSTR161.17/D3

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 1F31EY036280-01
Adelson Medical Research Foundation

Title: Lens injury mechanisms of retinal ganglion cell protection and axon regeneration

Authors: *M. FINNERAN[1], Q. FENG[2], X.-F. ZHAO[3], R. KAWAGUCHI[4], D. H. GESCHWIND[5], L. I. BENOWITZ[6], R. J. GIGER[7];
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Abstract: In the adult mammalian central nervous system (CNS), regeneration of severed axons is extremely limited and, depending on the severity of the injury, results in permanent functional deficits. Retinal ganglion cells (RGCs) are CNS projection neurons in the inner retina that extend long axons through the optic nerve to the brain for visual information transmission and are essential for vision. In adult mice, a retro-orbital optic nerve crush (ONC) injury induces apoptosis of most RGCs, and the surviving RGCs fail to extend their axons beyond the site of injury. Recent studies have uncovered that a conditioning lesion to the ocular lens (cLI), prior to ONC, promotes RGC protection and axon regeneration following ONC, enabling regenerating RGC axons to reach the optic chiasm. Thus, cLI is a novel and robust experimental model to protect injured CNS neurons and trigger long-distance axon regeneration *in vivo*. Yet, the cellular and molecular mechanisms underlying cLI-elicited neuroprotection and axon regeneration remain poorly understood. To better understand how RGCs respond to cLI following ONC and how this promotes survival and axon regeneration, we employed a multi-OMICs approach to explore RGC intrinsic and extrinsic mechanisms associated with improved regenerative outcomes. In a longitudinal study, we used proteomics of the vitreous humor of mice subjected to ONC only or ONC with cLI. From the same animals, RGC nuclei were labeled using adeno-associated viral vectors to isolate by FACS and were subsequently subjected to single-nuclei RNA-sequencing (snRNAseq). We used intersectional approaches of proteomics and transcriptomics studies under RGC regenerative and non-regenerative conditions for the identification of candidate gene products and signaling pathways regulated by cLI. Preliminary studies identified specific RGC subsets that respond to cLI. To this end, we identified genes and

signaling pathways that are regulated by cLI, revealing candidate genes that mediate neuroprotective and pro-regenerative effects. To explore the functional significance of candidate genes and pathways identified, we use adeno-associated viral vector mediated gene transfer for gain-of-function and loss-of-function studies.

Disclosures: M. Finneran: None. **Q. Feng:** None. **X. Zhao:** None. **R. Kawaguchi:** None. **D.H. Geschwind:** None. **L.I. Benowitz:** None. **R.J. Giger:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.18/D4

Topic: C.10. Brain Injury and Trauma

Support: EY032908
EY002687
EY013360

Title: Mitochondrial calcium homeostasis influences retinal ganglion cell survival in neurodegeneration following optic nerve axon injury

Authors: *S. MCCRACKEN[1], K. SQUIRRELL[1], M. ZHAO[1], P. R. WILLIAMS[2]; [1]Washington Univ. in St. Louis, St. Louis, MO; [2]Ophthalmology and Visual Sci., Washington Univ., Saint Louis, MO

Abstract: Retinal Ganglion Cells (RGCs) are the sole projection neurons from the retina to the brain and are highly susceptible to injury and disease. Using chronic in vivo imaging of an intracellular calcium (Ca2+) biosensor, Twitch2b, we recently discovered that intracellular mitochondrial and ER Ca2+ set-points are differential across RGCs within individual mouse retinas. We also found that high baseline mito-Ca2+ levels, but not ER-Ca2+, predicts survival to optic nerve crush (ONC) axon injury. We hypothesize that homeostatic Ca2+ can be altered by manipulating proteins responsible for mitochondria and ER Ca2+ transport mechanisms to influence RGC survival to ONC injury. Using AAV2 based cell-type specific targeting strategies for RGCs, we overexpressed proteins critical for mitochondrial homeostasis, specifically proteins that are part of the mitochondrial calcium uniporter (MCU) complex (MCU, MICU1/2/3, MCUR1), alongside regulators of ER Ca2+ influx (Serca2b, Serca3, and STIM1). We performed ONC following overexpression of these proteins in RGCs specifically and assessed survival and axon regeneration 14 days after ONC. From this screen, we have identified so far one protein that increased survival, the Mitochondrial Calcium Uniporter (MCU) protein, which is a subunit of the MCU complex that allows for Ca2+ to pass through the inner mitochondrial membrane. We confirmed expression of MCU in RGCs with antibody staining following AAV2-overexpression of the construct. We also verified that MCU overexpression leads to higher mitochondrial Ca2+ levels across the RGC population *in-vivo* with two-photon imaging. We are

further investigating the role of aerobic glycolysis and mitochondrial calcium regulation by altering the Lactate Dehydrogenase (LDH enzyme) alongside MCU. Thus, our results indicate that mitochondrial calcium regulation is involved in RGC survival after ONC injury and that MCU could play a mechanistic role in neuronal resilience to CNS axon injury.

Disclosures: S. McCracken: None. **K. Squirrell:** None. **M. Zhao:** None. **P.R. Williams:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.19/D5

Topic: C.10. Brain Injury and Trauma

Support: NINDS R01-NS110905-05S1
International Center for Responsible Gaming
Ohio State's Chronic Brain Injury Program

Title: Frontal traumatic brain injury drives unique decision-making deficits in gambling-related behaviors

Authors: *J. E. MCCLOSKEY[1], M. ELEID[2], S. WAMPLER[3], C. VONDER HAAR[1]; [1]Neurosci., Ohio State Univ., COLUMBUS, OH; [2]Ohio State Univ., Columbus, OH; [3]Neurosci., The Ohio State Univ., Columbus, OH

Abstract: Traumatic brain injury (TBI) is a leading cause of disability, often resulting in cognitive and behavioral impairments. Deficits in decision-making are a clinically prevalent, chronic consequence that may contribute to addictive disorders such as gambling disorder (GD). Gambling-like decisions can be modeled using the Rodent Gambling Task (RGT). The RGT recapitulates clinical impairments from TBI and a win-paired cue version (cRGT) drives risky decision-making. However, to better understand a complex behavior like GD, the individual elements driving decision-making deficits must be isolated. These studies investigated how TBI affects 1) sensitivity to probabilistic outcomes and 2) the acquisition of incentive salience and the role of cues. Rats (N=79, half female) received severe bilateral, frontal controlled cortical impact or sham surgery. In both experiments, rats were first assessed on one dimension of gambling-related behavior (probability or cue sensitivity), and then went on to complete either the RGT or cRGT. The RGT offered four choices with varying probabilities and magnitudes of reinforcement or punishment. The cRGT paired audiovisual cues with winning trials; these increased in complexity & variability with reinforcer magnitude. In Experiment 1, rats were tested on a two-option probability discounting task where they chose between a guaranteed small reinforcer or risky large reinforcer that degraded in probability. TBI rats were sensitive to probability, but demonstrated lower overall preference for risky outcomes, even when large reinforcement was guaranteed. Paradoxically, TBI rats increased their preference for risky options on the RGT. In Experiment 2, rats underwent Pavlovian conditioning to evaluate the

ability of cues to influence behavior. A light and lever (CS^+) were paired with sucrose (US^+) over 10 sessions and approach behaviors recorded. Conditioned reinforcement was for the CS^+ was then probed. TBI decreased sign-tracking behavior and reduced conditioned reinforcement presses, suggesting less influence by cues. In contrast, TBI reduced optimal decision-making on the cRGT with shifts toward cue-associated risky options. These data highlight discrepant relationships with cues and probabilistic decision-making after TBI. Stains of ΔFosB, a transcription factor associated with learning and addiction, suggest dysregulation of the nucleus accumbens after TBI. Continued research will evaluate the physiological basis of disordered decision-making and identify novel mechanisms by which addiction distinctly develops after TBI.

Disclosures: J.E. McCloskey: None. **M. Eleid:** None. **S. Wampler:** None. **C. Vonder Haar:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.20/D6

Topic: C.10. Brain Injury and Trauma

Support: Department of Defense #N00014-21-S-F002

Title: Developing a living brain phantom model using biomaterials and cerebral organoids to study the neurophysiology of traumatic brain injury

Authors: *N. SMITH[1], A. BAKER[2], M. TARTIS[2], Z. R. LYBRAND[1];
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Abstract: The mechanical response of brain tissue to traumatic brain injury (TBI) is poorly understood but incredibly important for understanding the neurophysiology of brain injury. To understand these neurophysiological changes, we have designed a biofidelic organ-on-a-chip system to embed cerebral organoids to integrate into a polyacrylamide (PAA) hydrogel to combine both the biological and mechanical properties of the brain. PAA hydrogels are useful for this application as they have similar shear stiffness properties to that of the human brain. Hydrogel formulations of 7 and 10% w/v 60-1 were characterized and represent gray and white matter, respectively, to mimic the mechanical properties of the brain. Human cerebral organoids are 3D tissue cultures that mimic the cell composition and neurophysiological function of the brain. A key design factor for a living brain phantom model is long-term culturing of embedded organoids that successfully attach to the PAA gel and maintain cell viability comparable to an non-embedded organoid. This allows for shear deformation testing to be performed on these organ-on-a-chip systems to recreate damage conditions seen in TBIs. Preliminary data has shown that these organoids can stay embedded through shear testing conditions of up to 2 mm of displacement at 70 Hz for 10 cycles. This creates an organ-on-a-chip model that can be

embedded for extended periods of time and can be exposed to simulated TBI insults.To measure the viability of embedded organoids into our biofidelic chip, organoids (n=5) were infected with AAV-hSyn-GFP to visualize neurons, embedded 7 and 10% PAA chips for 14 days, dissociated using a papain treatment, then analyzed with a trypan blue assay for cell viability. After 14 days in both 7 and 10% PAA, the percentage of live cells were normalized to non-embedded organoids and no significant difference was observed in either formulation. This demonstrates the feasibility using PAA as a substrate for cerebral organoids over extended durations. Additionally, after embedding cerebral organoids in these chips they were placed on a shear device under 10% compression to prevent slipping. These chips were then sheared at 2 mm of displacement at 70 Hz for 10 cycles. Preliminary tests show that after being embedded for extended durations the cerebral organoids can stay embedded in these chips through deformations at these scales.The goal of this project is to design an in vitro model that recapitulates both the biological and mechanical properties of the brain to study the effects of shear deformation from brain trauma on the physiological functions of the brain.

Disclosures: N. Smith: None. **A. Baker:** None. **M. Tartis:** None. **Z.R. Lybrand:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.21/D7

Topic: C.10. Brain Injury and Trauma

Support: Chancellor’s Research Fellows (Z.R.L)
Graduate Research Assistantship (GRA) Support (N.Y)

Title: Cryab expression is altered in cerebral organoids following mtbi

Authors: *N. YASIN, T. VU, Z. R. LYBRAND;
Biol., Texas Woman's Univ., Denton, TX

Abstract: Mild Traumatic Brain Injury (mTBI) is prevalent, accounting for 80% of all TBI's. While a single mTBI is considered mild, the cumulative effects of repetitive mTBI, especially among athletes, are associated with long-term consequences. Repetitive mTBIs result in the development of Chronic Traumatic Encephalopathy (CTE), a neurodegenerative disease only diagnosed postmortem. Our limited understanding of CTE and injury-induced neurodegeneration stems from postmortem tissue degeneration, variations in postmortem intervals (PMIs), and inadequacies in rodent models to replicate injury response mechanisms of human cells. In our lab, we use human cerebral organoids, which are 3-D cultures produced from human-induced pluripotent stem cells (IPSCs), mimicking the development of the forebrain in vitro. The elegant organoid model is more relevant to human cell composition, allows for longitudinal studies and genetic manipulation, and offers high throughput screening. We previously modeled parameters of mTBI in vitro by exposing organoids to mechanical pressure via a tabletop blast chamber.

Using the same mTBI parameters and single-cell RNA sequencing (scRNA-seq), we investigated molecular mechanisms underlying mTBI in our organoid model. scRNA-seq revealed crystallin alpha B (CRYAB) mRNA expression is increased following mTBI. We then investigated whether CRYAB mRNA expression is reciprocated into protein expression and if the expression is altered after repetitive mTBI. We performed single and repetitive mTBI on organoids and examined CRYAB expression using immunofluorescence (IF). Findings indicate CRYAB protein expression is upregulated following single mTBI compared to control and that its increased in repetitive compared to single and control. Using the organoid model, we have gathered compelling data showing that CRYAB expression is altered after a repetitive mTBI. CRYAB has many roles in the central nervous system (CNS) but is mainly known as a molecular chaperone that prevents protein aggregation. However, its role in TBI remains unknown. Our next question is whether CRYAB displays neuroprotective or neurotoxic roles following TBI.

Disclosures: N. Yasin: None. **T. Vu:** None. **Z.R. Lybrand:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.22/D8

Topic: C.10. Brain Injury and Trauma

Support: N000142112044
N000142112855

Title: Investigating Excitotoxic Neuronal Network Disruptions via In Vitro Traumatic Brain Injury Models

Authors: *J. SERGAY[1,2], A. HAI[3], C. FRANCK[4];
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Abstract: Traumatic brain injury (TBI) arises from mechanical trauma to the head, resulting in both immediate cognitive deficits and long-term neurodegeneration. Mild TBI (mTBI) shares similar enduring consequences and represents the majority of TBI incidents worldwide, affecting athletes, soldiers, and civilians alike. Despite the widespread occurrence, the cellular aftermath of mTBI remains disputed. Disturbances to electrical activity have been found to impair executive functions at a lower strain threshold than is required to induce cell death. Excitotoxicity, excessive glutamate secretion, is hypothesized to be responsible for the Ca^{2+} dynamic response to TBI. However, the literature shows inconsistencies in the event timeline, relevant mechanisms, and biochemical and electrophysiological responses. This study employs comprehensive data analysis to delineate network disruption under varying mechanical loads at hyperacute and acute time points. Neural co-cultures on flexible substrates are stretched in a

custom uniaxial tension device for precise applied deformation relating to mTBI values. Combinations of strains (0.1, 0.3, and 0.5) and strain rates (1 s^{-1} and 50 s^{-1}) are tested as well as controls. Spontaneous neural network activity is quantified by optically capturing the Ca^{2+} dynamics of AAV1-syn-GCaMP6f. At 20 DIV, two-minute timelapses are recorded immediately before (0-) and after (0+) applied stretch. Samples are incubated at 37ºC for 24 hours and recorded once more. Cell viability is assessed at 0- and 24 hr using Calcein UltraBlue AM and Thiazole Red Homodimer. Lastly, the role of glutamate is assessed in samples where extracellular glutamate is added in place of stretching at varying concentrations up to 10 μM. Preliminary data shows a global network increase in mean Ca^{2+} intensity immediately after stretch and a network hyperexcitation that lasts up to 20 seconds in all strain conditions at 50 s^{-1}. After this initial period, Ca^{2+} activity decreases compared to the 0− data. This effect is not seen in sham samples. The initial data is consistent with the excitotoxic timeline delineated in literature. This study's glutamate experiments will clarify the involved mechanisms in the hyperacute network behavior after TBI. The ability to collect quantifiable network dynamic measurements from over 500 cells in real-time while simultaneously controlling cell deformation mechanics is unique to this experimental setup. The data will serve as the basis for determining a specific critical strain and strain rate threshold for Ca^{2+} signal dysfunction, which is vital for refining predictive models and devising protective strategies against TBI.

Disclosures: J. Sergay: None. **A. Hai:** None. **C. Franck:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.23/D9

Topic: C.10. Brain Injury and Trauma

Support: The State of Indiana

Title: Characterizing Synaptic Loss Following Blast Trauma In Vitro Using bTBI-On-A-Chip

Authors: *T. B. BEAUCLAIR[1], J. MARTINEZ[1], C. ADAM[2], S. MUFTI[1], N. KRISHNAN[1], R. SHI[3];
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Abstract: Traumatic brain injuries (TBI) caused by explosive blast waves (bTBI) are becoming increasingly common and can result in severe pathologies such as neurodegeneration developing years after injury. Unfortunately, the mechanisms of bTBI progression remain poorly understood. Experiments in animal models have shown irreversible loss of synapses following bTBI and linked this loss to increased risk of developing neurodegeneration. However, the effects of bTBI on individual synapses remain unclear as animal models generally lack the spatial and temporal resolution needed for single cell or single synapse investigation and few *in*

vitro models exist. *In vitro* studies of single cell and single synapse dynamics after blast injury could elucidate the underlying mechanisms of bTBI synapse pathology and thereby help inform research efforts aimed at improving the treatment of bTBI victims. To this end, we used our bTBI on a chip device to study synapse loss after bTBI. BTBI on a chip is an *in vitro* system in which primary murine cortical cultures are grown on microelectrode arrays (MEAs) inside a portable incubation chamber. The chamber is then exposed to clinically relevant blast waves generated by a shock tube prior to electrophysiological or immunocytochemical (ICC) analysis. Previous work using bTBI on a Chip showed that average network spiking activity is significantly reduced following bTBI, and that inflammation and reactive aldehydes are elevated in cultures as early as 24 hours post blast. In our current study we aimed to determine the magnitude of synaptic loss due to the initial physical primary injury, the ensuing biochemical secondary injury, or both. Murine cortical cultures were secured in bTBI on a chip's portable incubation chamber, exposed to a blast wave and incubated for 24 hours, after which ICC staining of synaptophysin (presynaptic) and PSD-95 (postsynaptic) and fluorescent analysis were performed. We discovered a significant loss of synaptic terminals post injury. Cultures were also treated with clinically relevant concentrations of acrolein, a reactive aldehyde and secondary injury product produced in abundance post bTBI. Acrolein alone significantly reduced synaptic terminals in otherwise uninjured cultures, though at a lower magnitude than blast injured cultures. These results suggest that both the primary and secondary injury are responsible for synaptic loss following bTBI. Further, as synaptic loss has been linked to neurodegeneration, this platform provides an opportunity to study up to the moment interactions of synaptic loss and neurodegenerative marker generation, linking synapse loss to the development of neurodegeneration.

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Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.24/D10

Topic: C.10. Brain Injury and Trauma

Support: AR3T Technology Grant
NIH Grant R01NS099596

Title: A 3D TBI-on-Chip Platform to Model Brain Damage and Recovery Responses

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Abstract: *in vitro* models of traumatic brain injury (TBI) provide a valuable means of evaluating temporal neuronal responses to mechanical injury and high-throughput testing of single-agent and combination therapies. However, current *in vitro* 3D models that recapitulate the TBI microenvironment do not provide detailed assessments of population level neuronal network activity and injury progression. In order to address this gap, we designed a microfluidic device made of a flexible polydimethylsiloxane (PDMS) polymer shell that can transfer the impact force of a falling object to underlying 3D hydrogel encapsulated neurons cultured in spatially constrained chambers, and can be used to monitor neuronal network dynamics in real-time. Human prefrontal cortex hPFC neurons were encapsulated in Geltrex^{TM}, and then seeded in volume constrained PDMS reservoirs. Following the establishment of 3D neuronal networks after 2 weeks *in vitro*, we confirmed the maturation of neurons within the device by immunocytochemically stained for mature neuronal marker, B3T and activity marker, synaptophysin. We induced a weight drop injury of the underlying hydrogel encapsulated hPFC neurons and calcium imaging using the cell permeant calcium indicator - Fluo4-AM was performed at 0, 24, 72 hours post injury. Waveform and network analysis of calcium imaging data revealed an acute shift across multiple parameters in response to the injury compared to the control. This was characterized by a significant decrease in mean firing rate (control 0.58±0.07, injury 0.47±0.07, $p<0.0001$), neuronal cluster presence (number of cluster: control 44.6±13.36, injury 32.56±17.27, $p<0.01$; weight modularity: control -0.65±0.14, injury -0.74±0.17, $p<0.05$; cluster coefficient: control 0.018±0.008, injury 0.007±0.004, $p<0.0001$), and network efficiency (global efficiency: control 0.08±0.02, injury 0.04±0.02, $p<0.0001$), and a significant increase in burst activity (mean fall time: control 47.70±10.80, injury 64.15±16.20, $p<0.001$; mean band width: control 79.31±21.83, injury 122.08±29.92, $p<0.0001$) and synchronization (global synchronization index: control 0.19±0.06, injury 0.39±0.11, $p<0.0001$). The acute shift in neuronal activity observed in the injury group returned to the control baseline levels 72 hours post injury. This study establishes the application of a simple and inexpensive 3D neuronal cell culture platform to model neuronal responses to mechanical injury. Results demonstrate the scalability of this platform for inducing a weight-drop injury, obtaining real-time quantification of neuronal network responses, and potentially monitoring therapeutic efficacy post-injury.

Disclosures: R. Tang: None. **N. Gonsalves:** None. **A. Chopra:** None. **M. Sarkar:** None. **H. Wu:** None. **N. Zeltner:** None. **L. Karumbaiah:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.25/D11

Topic: C.10. Brain Injury and Trauma

Title: Characterization, mechanism, and mitigation of neuronal network activity dysfunction in an in vitro model of blast-induced TBI

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Abstract: Traumatic brain injury (TBI) is a major cause of morbidity and mortality worldwide, with no established treatment. Common in war zones, blast-induced TBI (bTBI) presents a significant challenge. The lack of effective treatments is largely due to an incomplete understanding of the mechanisms involved, especially in mild Blast-Induced TBI (mbTBI), the most prevalent form of bTBI. mbTBI often exhibits minimal initial symptoms post-injury, leading to a high rate of underdiagnosis and missed therapeutic opportunities. It is well-established that secondary pathological biochemical cascades in TBI can lead to long-term neurodegeneration and potentially chronic neurodegenerative diseases such as Alzheimer's and Parkinson's. Research using TBI models has identified oxidative stress as a crucial secondary injury mechanism. Specifically, acrolein, a harmful aldehyde and both a product and catalyst of oxidative stress, significantly contributes to post-trauma neuronal damage by destroying crucial cellular organelles in animal models. However, these in vivo models do not offer the adequate spatial or temporal resolution necessary for detailed mechanistic studies. To address this, we utilized an in vitro blast model known as 'bTBI-on-a-chip' to examine changes in neuronal network function following a blast. This model utilized neuronal networks grown on MicroElectrode Arrays (MEAs), permitting monitoring of single-channel and network-level activity in real-time before, during, and after injury. The Plexon neurotechnology system was used for recording and analysis, and a shock tube-based blast device was adapted to produce the blast shockwave. With this model, we observed an immediate decrease in network activity following a mild blast, specifically a reduction in spike rate, burst rate, burst duration, spikes in burst, and burst amplitude, a measure of burst synchronization. Interestingly, the application of hydralazine, a known acrolein scavenger, could mitigate the decrease in network activity following the blast. These data have revealed an immediate network abnormality following a mild blast and a critical pathological role of acrolein in functional loss. Furthermore, this initial finding also suggests anti-acrolein as a potential intervention to mitigate post-blast network function loss. Taken together, this in vitro mbTBI offers a unique platform to examine post-blast neural network activity dysfunction with unprecedented temporal resolution, providing insights into the dynamics and mechanisms of functional deficits, facilitating the investigation to identify effective interventions for blast-induced neurotrauma recovery.

Disclosures: J. Martinez: None. **C. Adam:** None. **T.B. Beauclair:** None. **S. Mufti:** None. **N. Krishnan:** None. **R. Shi:** None.

Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR161.26/D12

Topic: C.10. Brain Injury and Trauma

Title: An in vitro model of acute traumatic brain injury associated neurodegeneration

Authors: *N. WACHTLER[1,2], X. GONG[1], Z. M. KHAN[1], L. KRUCKENHAUSER[1,2], M. MAK[1], B. E. EHRLICH[1], D. MCGUONE[1];
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Abstract: Traumatic brain injury (TBI) is one of the leading causes of death and disability in the world. Despite decades of research, promising drug targets have yet to be identified. Although perturbations of calcium signalling influence early cellular responses to penetrating and blunt head injuries, the role of calcium homeostasis after blast exposure is poorly understood. However, just like repetitive impacts, low-level blasts may be associated with increased risk of subsequent neurodegeneration. In these studies, we aim to understand the interplay of mechanical compression and blast injury with altered calcium signalling and the expression of canonical proteins associated with neurodegeneration in TBI.
Methods: Neuroblastoma (SK-N-AS) cells and human-derived astrocytes were subjected to controlled compressive pressure or changes in hydrostatic pressure through single or repeated low-level blasts. Live cell calcium imaging obtained before and during the injury were correlated with structural changes in cell morphology and protein expression. These changes were correlated with protein expression changes in human thalamus tissue slices obtained from TBI patients and healthy controls.
Results: Our findings in neuroblastoma cells revealed distinct disruptions in calcium homeostasis after compression, which included increased intracellular calcium levels and decreases in cellular calcium oscillations. The cellular response among repetitions heavily depended on the severity of the initial insult. The interplay between calcium and cell morphology was underlined by the changes in cell morphology shown by live cell imaging, such as membrane blebbing and changes in general cell composition. An upregulation in expression of proteins associated with cellular stress and long term neurodegeneration, including APP, TDP-43, and ATF4 was observed through immunostaining and verified by western blot analysis. Correlations with results in human-derived astrocytes following blast, and post-mortem human TBI brain tissues are in progress.
Conclusion: Our in vitro model is able to monitor both immediate and secondary consequences of several types of traumatic injuries to neurons. These in vitro results are being validated using human post-mortem tissue. The long-term aim is to develop methods to manage the initial effects of TBI and attenuate subsequent chronic neurodegenerative disorders.

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Poster

PSTR161: Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Brain Injury and Trauma

Support: Medical Research Council Doctoral Training Programme (MRC DTP)
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NIHR Global Health Research Group on Acquired Brain and Spine Injury

Title: In vitro modelling and therapy development: Investigating the efficacy of a novel neurotherapeutic for secondary injuries following traumatic brain injury

Authors: *C. A. HALL, K. BARANES, M. KOTTER, K. L. H. CARPENTER, P. J. A. HUTCHINSON;
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Abstract: Following traumatic brain injury (TBI), several downstream cascades are initiated, including deranged cerebral metabolism and inflammation, contributing towards secondary injury. This often manifests in patients as a high brain extracellular lactate/pyruvate ratio (LPR), which correlates significantly with unfavourable patient outcome. Initial in-vitro research has shown that succinate, an intermediate of the tricarboxylic acid cycle, can protect against rotenone-induced metabolic dysfunction. It has been shown that complex I of the electron transport chain (ETC) is acutely susceptible to damage following TBI. Using human induced neurons (iNs) and human induced astrocytes (iAs), we replicate this damage mechanism using rotenone, an inhibitor of complex I of the mitochondrial ETC. Subsequently, treating with succinate which interacts with complex II, bypasses the induced injury mechanism to provide protection. Utilising the novel cellular reprogramming technique Optimised Inducible Overexpression, we produced iNs and iAs through transcription factor overexpression. These cultures were treated with varying concentrations of rotenone and disodium succinate. At select time points, cellular metabolism and LPR were assessed using an ISCUSflex analyser and cell viability was determined using selected cellular stains. We also measured extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) in response to treatment. iAs proved to behave more consistently than iNs in the testing regime, and produced results suggesting metabolic rescue, such that LPR was decreased, and cell viability increased following succinate treatment, compared with non-succinate control cells. We also showed that ECAR and OCR were positively affected by succinate and in a dose-dependent manner. iNs responded to treatment of both rotenone and succinate, though not consistently. Co-culturing experiments have been performed to investigate a mechanism whereby the astrocytes may provide a supportive or protective role to the neurons. In addition to succinate rescue models, we have also investigated the link between TBI and Alzheimer's disease (AD). We are also investigating the ability of succinate to not only provide metabolic rescue at the time of injury, but also reduce

likelihood of AD development following injury. We have shown that both iNs and iAs respond to rotenone treatment, confirming our secondary injury model. Furthermore, treatment with succinate has provided evidence of metabolic rescue in iAs cultures and gone a significant way in proving its potential use as a novel neurotherapeutic.

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Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.01/D21

Topic: C.10. Brain Injury and Trauma

Support: DGAPA Fellowship
PAPIIT: IN223417
PAPIIT: IN228320
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Title: The effect of thioredoxin1 in a rat model of traumatic brain injury depending on diurnal variation

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Abstract: Traumatic brain injury (TBI) is a public health concern with limited treatment options because it causes a cascade of side effects that are the leading cause of hospital death. Thioredoxin is an enzyme with neuroprotective properties such as antioxidant, antiapoptotic, immune response modulator, and neurogenic, among others; it has been considered a therapeutic target for treating many disorders. The controlled cortical impact (CCI) model was used to assess the effect of recombinant human thioredoxin 1 (rhTrx1) (1 μg/2 μL, intracortical) on rats subjected to TBI at two different times of the light-dark cycle (01:00 and 13:00 h). We analyzed the food intake, body weight loss, motor coordination and pain perception. Body weight loss, reduced food intake, spontaneous pain and motor impairment are more evident in rats subjected to TBI in the light phase than in the dark phase of the cycle and in groups that did not receive rhTrx1 or minocycline (as positive control). Three days after TBI, there is a recovery in body weight, food intake, motor impairment, and pain, which is more pronounced in the rats subjected to TBI at the dark phase of the cycle and those that received rhTrx1 or minocycline. Knowing the time of day a TBI occurs in connection to the neuroprotective mechanisms of the immune response in diurnal variation and the usage of the Trx1 protein might have a beneficial therapeutic impact in promoting quick recovery after a TBI.

Disclosures: R.I. Noriega: None. **R.J. Martínez-Tapia:** None. **L. Navarro:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.02/D22

Topic: C.10. Brain Injury and Trauma

Support: CBIR 22 PIL022
Dept of Veterans Affairs, Technology Transfer Program, Office of Research and Development, Wash. D.C

Title: Effects of a Neuroprotective Serotonin Receptor Peptide on Behavioral Pattern Separation Following mild Traumatic Brain Injury in the Rat

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Abstract: Accelerated cognitive decline frequently complicates traumatic brain injury (TBI). Human TBI patients and several rat species harbored spontaneously-occurring circulating G-protein coupled receptor agonist IgG autoantibodies whose *in vitro* neurotoxicity was prevented by a novel synthetic peptide fragment of the second extracellular loop of the serotonin 2A receptor (SN..8). The SN..8 peptide was metabolized slowly *in vitro* (t ½ ~ 8 hours) by both human hepatic and renal microsomes consistent with its having a relatively long (~8 hours) half-life in rat plasma following a single intraperitoneal (IP) injection. The SN..8 peptide potently lowered blood pressure in hypertensive rat species and it was safe and well-tolerated. Prior systemic (IP) administration of SN..8 (*vs*. scrambled peptide) strengthened both recall and acquisition of spatial learning after sham injury (but not mild traumatic brain injury, mTBI) in Zucker lean rats. Since behavioral pattern separation is early behavioral marker of cognitive decline, here we tested for differential effects from SN..8 (*vs* scrambled peptide), each at 2mg/kg IP doses given 1-,3- and 5-days after mTBI on behavioral pattern separation. Male Sprague-Dawley (SD) rats were trained (pre-injury) to differentiate between stable and unstable swim platforms (located 1.5, 3.0 or 4.5 feet apart) in a modified Morris water maze protocol, behavioral pattern separation (BPS), to assess deficits in pattern separation at 2- and 5-weeks after mTBI injury. Rats made fewer errors (14.2 *vs* 26.7 *vs* 35.8%) both pre- and post-injury when (stable and unstable) platforms were positioned farther apart: 4.5 *vs* 3.0 *vs* 1.5 feet. At two week's post-injury, the error rates were significantly lower (at 4.5 and 3.0 feet separation) in rats treated with SN..8-(*vs* scrambled peptide) (6.7 *vs* 25.9 %; N=19, P< 0.01; 4.5 feet) and (20.0 *vs* 42.7% (N=19; P= 0.039; 3.0 feet). At five weeks' post injury there was no longer a significant drug effect, and there was no significant drug effect in sham-injured rats. Across all drug and injury groups, the composite error rate was significantly lower at five- *vs*. two- weeks' post-injury (7.75 +/- 4.4 *vs* 17.09 +/9%; P = 0.009) perhaps consistent (in part) with recovery from injury 5 weeks post injury. In summary, systemic SN..8 (2 mg/kg) administered in three successive doses (starting 1 day after mTBI) appeared to have a neuroprotective effect on early

loss of behavioral pattern separation in adult male SD rat. Since dentate gyrus is thought to play a role in BPS, we are investigating effects of SN..8 on neurons in the dentate gyrus and hippocampus.

Disclosures: X.M. Agbolou: None. **C. Yoe:** None. **T. Cominski:** None. **M. Zimering:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.03/D23

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS121706-01A1

Title: In-vivo neuronal mitochondrial mapping of rotenone-induced complex 1 dysfunction and kainic acid induced injury in adult rats

Authors: *D. R. E. CORTES[1], K. SCHWAB[2], N. COULSON[3], D. WEST[3], T. BECKER-SZURSZEWSKI[2], S. HARTWICK[2], Y. WU[4];
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Abstract: Epilepsy affects 45000 children and 300000 adults in the United States. The most common form of epilepsy is Temporal Lobe Epilepsy (TLE). TLE onset is often characterized by an initial brain injury (status epilepticus (SE)) that is followed by physiological remodeling that may result in chronic TLE. The gold standard methods for seizure monitoring and detection such as EEG are not capable of predicting the onset of chronic TLE. Additionally, further research into the role of mitochondrial during epileptogenesis supports mitochondrial dysfunction as an inciting factor for epilepsy due to a traumatic brain injury or genetically associated epilepsy. We hypothesize that longitudinal monitoring of mitochondrial function during acute and chronic phases post an SE-event can predict the development of downstream epileptogenesis. However, there is a current methodological gap: no methods exist to monitor mitochondrial dysfunction non-invasively and longitudinally. Therefore, to address this research gap, we have developed novel 4D Oxy-Wavelet MRI to characterize spatial and temporal mitochondrial function in rat brains, non-invasively. In this study we first show that our methodology is specific to detect complex-1 mitochondrial dysfunction. We use a well-studied model of Rotenone exposure, a known complex-1-inhibitiory drug to show regional and temporal loci of mitochondrial dysfunction in the rat brain. Our second goal is to demonstrate the ability for our 4D-Oxywavelet MRI to characterize the acute and chronic mitochondrial injury profile of rat brains exposed to low doses of TLE-inducing Kainic Acid. Sprague-Drawley rats are provided subcutaneous rotenone daily for 21 days to inhibit their complex-1 function. Rats are imaged at baseline and after treatment. We utilize a well-known rat model of Kainic-Acid induced TLE. Sprague

Dawley rats are subject to multiple low doses of Kainic Acid to elicit a Status Epilepticus event. The Racine scale to evaluate SE event severity with the goal of producing a 3/4/5 level event. 4D OxyWavelet MRI is performed longitudinally at both acute and chronic timepoints of 3, 7, 14, 21 days as well as 1 month and 2-month timepoints. Positive Oxy-wavelet indexes reflecting mitochondrial dysfunction were seen in hippocampus, fimbria, amygdala and piriform cortex, the brain regions known to be affected by KA-induced SE. We show that 4D Oxywavelet fMRI can detect local foci of mitochondrial dysfunction following Kainic Acid insult. We perform longitudinal analysis to show that 4D Oxywavelet fMRI can detect changing loci, as well as monitor whole brain insult via a normalized oxywavelet score of overall mitochondrial damage.

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Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.04/D24

Topic: C.10. Brain Injury and Trauma

Support: 798636

Title: Cognitive & gonadal dysfunction associated with neurodegeneration of the hippocampus

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Abstract: The ovarian activity is regulated by the hypothalamic-pituitary-gonadal axis and direct neural pathways between the central nervous system and the ovaries. The hippocampus is not yet considered part of the reproductive regulation, but several studies have shown that cognitive alterations associated with neurodegeneration of the hippocampus in Huntington's disease and, interestingly, also in reproductive problems. The objective of this study was to analyze the effects of the dorsal or ventral hippocampus lesion in the female rat on memory, learning and density of dendritic spines of hippocampal pyramidal neurons of areas CA1, CA3, and GD. Similarly, the onset of puberty, the ovarian morphology, and the serum levels of steroid and gonadotropic hormones. Aged 21-day old prepubescent female rats of the CII-ZV strain were used. Ventral or dorsal hippocampus lesions were performed by stereotactic surgery as were their

respective controls, a fifth group was used as an absolute control. At 30 days of age all groups underwent novel object recognition tests (NORT). The age of the vaginal opening was recorded, and they sacrificed at first vaginal estrus. The results of the NORT memory test showed a decrease in short-and long-term memory in animals from groups with ventral or dorsal hippocampal lesion compared to their respective controls and the absolute control group. A significant decrease in the density of mushroom type dendritic spines and an increase in thick spines were observed in the three studied areas of the hippocampus. Animals with hippocampal injury showed a delay in the age of the vaginal opening, decrease in the number of ovarian follicles and an increase in follicular atresia. These changes were accompanied by a decrease in serum levels of estradiol, progesterone, gonadotropic hormones, and an increase of testosterone levels. These findings support the idea that the hippocampus participates in a stimulatory manner not only in memory and learning processes, but also in the regulation of the gonadal function (Supported by CONACyT and VIEP).

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Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.05/D25

Topic: C.10. Brain Injury and Trauma

Support: DHA 65642-3.00-310288

Title: Investigating the regional distribution of phosphorylated tau and behavioral changes in P301S tau rats four months after repeated mild rotational acceleration traumatic brain injury

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Abstract: Repeated mild TBI (rmTBI) is associated with an increased risk for dementia. The chronic degenerative process after rmTBI has been attributed to the abnormal accumulation of tau, an essential microtubule-associated protein. 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 assess whether rmTBI may trigger pathological and behavioral alterations in rats that are predisposed to tauopathy. 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. HO rats spontaneously develop tauopathy with age, while HE rats do not, and appear similar to WT rats. We hypothesized that rmTBI in young adulthood would trigger the development of tauopathy in HE rats. Rats were exposed to five rmTBI via Closed-Head Impact Model of Engineered Rotational Acceleration (CHIMERA) or sham procedures at four months of age. At four months post-injury, rats underwent three behavioral tests: open field test (OFT) to assess locomotor activity and anxiety, the novel object recognition test (NOR) to assess episodic learning and memory, and the Y-maze spontaneous alternation task, to assess spatial working memory. On the OFT, 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 rats. We also performed immunohistochemistry to quantify the accumulation of phosphorylated tau (AT8) across various brain regions. In the amygdala and hippocampus, HO rats exhibited higher levels of phosphorylated tau, which were minimal in HE and WT rats. In the paraventricular nucleus of the thalamus, there was an effect of genotype on AT8 levels (HO > HE > WT), yet no injury effect. In the piriform cortex, HO rats exhibited increased levels of AT8 compared to HE Sham and WT rats. Interestingly, injured HE rats displayed greater accumulation of AT8 in the piriform cortex compared to HE Sham and WT rats, suggesting a predisposition to injury-induced tauopathy. These data suggest the piriform cortex may be one of the first vulnerable regions to injury-induced tauopathy in HE tau rats, and this pathological change may precede any noticeable behavioral deficit.

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Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

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Program #/Poster #: PSTR162.06/D26

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 1 R01 AG071228

Title: The effect of mild traumatic brain injury in early adulthood on the neuropathological markers of alzheimer's disease on 3xTg-AD mice

Authors: *C. C. H. BARKER[1], D. A. LINSEMAN[2];
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Abstract: Repetitive traumatic brain injuries (rTBIs) have been shown to increase neuroinflammation in both mouse models and human studies and are predicted to increase risk for neurodegenerative disorders including Alzheimer's disease (AD). Given the number of TBIs reported in the US each year, a large part of the population could be at an increased risk of developing AD, especially if predisposed. By using a combination of behavioral tests and histopathology, we investigated whether brain trauma accelerates cognitive dysfunction, and the brain pathology of neurodegeneration and neuroinflammation in 3xTg-AD mice subjected early in life to repetitive mild TBI (rmTBI). Four groups of mice, 3 control groups and 1 rmTBI treatment group, were aged to 10 months old. These groups were: naïve 3xTg-AD mice, sham 3xTg-AD mice, rmTBI 3xTg-AD mice, and naïve wild type mice. At 3 months old, mice in the rmTBI group were given 5 mTBIs, each separated by 48 hours. At 10-months old, all groups of mice were assessed for cognitive function using the Barnes maze, Y-maze, and Novel Object Recognition (NOR) behavioral tests. Blood and brain tissue were taken immediately after the conclusion of the behavioral tests. Blood samples were centrifuged to separate and collect plasma while brain tissue samples were fixed or frozen following dissection. Hippocampal sections of brain were stained for amyloid-beta (Aβ), and phosphorylated-Tau (p-Tau), proteins that constitute pathological hallmarks of the disease. Immunostaining for GFAP, Iba1, and NeuN was also employed to collect data on the extent of glial reactivity and neuronal death in the hippocampi. Preliminary results from the behavioral tests indicate that there are no significant differences in cognitive function between any of the 3xTgAD mouse groups. However, the wild type mice do perform better across all behavioral tests than any of the 3xTg-AD mouse groups. Our preliminary results do not show a significant difference in the total number of Aβ plaques or p-Tau aggregates between rmTBI and control (naïve or sham) 3xTgAD mouse groups. Our data thus far suggest that receiving rmTBIs early in life may not accelerate progression or enhance the magnitude of disease in mice that are genetically predisposed to developing AD. Future analysis of our histopathology data will show if levels of neuronal death, astroglia reactivity, and microglial reactivity differ significantly between the groups. These data will potentially allow for conclusions to be drawn concerning the amount of long term neuroinflammation caused by rmTBI early in life, as well as the amount of irreparable neuronal death rmTBI may have caused in the 3xTgAD mice.

Disclosures: C.C.H. Barker: None. **D.A. Linseman:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.07/D27

Topic: C.10. Brain Injury and Trauma

Title: A modified mouse blast traumatic brain injury (bTBI) model reveal sex difference in fear response and oxidative stress buildup.

Authors: *Z. ZHANG[1], A. ALFORD[2], R. SHI[3], T. B. BEAUCLAIR[4];
[1]Purdue Univ., West Lafayette, IN; [2]Biomed. Engin., Purdue Univ., Lafayette, IN; [3]Depat. Basic Med. Sci., Purdue Univ., West Lafayette, IN; [4]Purdue Univ., Lafayette, IN

Abstract: Due to the continuous global war conflicts, blast traumatic brain injury (bTBI) remains a prominent health concern in the affected military and civilian population. Furthermore, while there are 15.6% of the US army members are female, little is known about the sex difference in response to bTBI. In this animal study, we used a modified mouse repeated mild bTBI model to investigate the pathological alterations of oxidative stress, blood-brain barrier integrity, and neuroinflammation in both male and female mice in the acute and subacute timepoint post injury. We also examined the fear memory retrieval after bTBI using the foot-shock fear conditioning model. We found significant difference between control and injured mice, as well as between male and female mice in behavior and cellular response after bTBI. Specifically, male mice showed a prolonged retention of fear memory whereas female mice showed an early loss of fear memory. There is also a differential distribution of oxidative stress (e.g. acrolein adducts) and neuroinflammation markers in the male and female mice after bTBI, suggesting distinct brain regions vulnerable to bTBI that is sex dependent. Overall, these results revealed that while bTBI lead to a significant functional and behavioral deficit, it affects male and female differently, a likely important factor in post bTBI pathogenesis. These findings are expected to inspire further studies to provide more knowledge in both the mechanisms of injury, as well as the treatment that is sex dependent for bTBI victims.

Disclosures: Z. Zhang: None. **A. Alford:** None. **R. Shi:** None. **T.B. Beauclair:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.08/D28

Topic: C.10. Brain Injury and Trauma

Support: Department of Veterans Affairs [Merit Review I01-BX005017 (D.K.C.)

Title: A comparative study of lethality thresholds, neurological impairment, and neuropathology following blast injury in genetically diverse strains of mice

Authors: *K. D. BROWNE[1,2], A. GEORGES[3], D. AUGUSTIN[3], A. BELLO[1,2], D. F. MEANEY[4], D. CULLEN[5,2];
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Abstract: Blast-induced traumatic brain injury (bTBI) has been called the “signature injury” of recent wars and is a critical issue facing Veterans and civilians. However, the relationship

between various genetic backgrounds and susceptibility or resilience to blast-induced neuropathological sequelae has not been established. To help address this, we characterized the responses following bTBI of six murine genetic strains (A/J, 129S1/SvlmJ, NOD/LtJ, NZO/HILtJ, C57BL/6J, CAst/EiJ) across a range of blast peak overpressures. For each strain, we measured apnea, dyspnea, and righting time (RT) after exposure. Next, we determined the lethal dose for 50% of the population (LD_{50}) and evaluated neuropathological changes in mice that died as a result. C57BL/6J strain had significantly longer normalized RTs (7.9 ± 5.1 seconds) than other strains. Presence of respiratory symptoms across all strains and blast levels varied, with CAst/EiJs having the highest instance of immediate apnea (47.8%) and the 129S1/SvlmJs having the lowest (14.0%). Thresholds for lethality varied significantly across strains, with the CAst/EiJ and A/J strains being most susceptible to lethality following bTBI, and the NZO/HILtJs and NOD/LtJs being most resilient. The predominant neuropathology present was vascular disruption. C57BL/6Js were the most susceptible to subdural hematomas in fatal cases (71.4%), while the NZO/HILtJ strain frequently showed pulmonary damage (80.0%). This work, coupled with ongoing examinations of behavioral outcomes and gene expression, will help to identify the relative contributions of various underlying genotypes on injury thresholds, behavioral deficits, gene expression patterns, and neuropathology resulting from blast exposure.

Disclosures: K.D. Browne: None. **A. Georges:** None. **D. Augustin:** None. **A. Bello:** None. **D.F. Meaney:** None. **D. Cullen:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.09/D29

Topic: C.10. Brain Injury and Trauma

Support: 1P30DA056410-01A1

Title: Adolescent THC exposure and spatial working memory after acute concussion/mTBI.

Authors: *C. W. HUBBARD[1], G. NAH[2], N. L. PORT[3], H. B. BRADSHAW[4], J. D. CRYSTAL[5];
[1]Indiana Univ. Program in Neurosci., Bloomington, IN; [2]Neurosci., Indiana Univ., Bloomington, IN; [3]Sch. of Optometry, Indiana Univ., Bloomington, IN; [4]Psychological and Brain Sci., Indiana Univ. Bloomington, Saco, ME; [5]Dept Psychological and Brain Sci., Indiana Univ. -- Bloomington, Bloomington, IN

Abstract: Title: Adolescent THC exposure and spatialworkingmemory after acute concussion/mTBI.**Authors:** Cody Hubbard, GabrielNah, NicholasPort, Heather Bradshaw, Jon CrystalMildtraumaticbraininjury(mTBI) isa common injuryinadults of all ages, witha reportedincidentrate inthe UnitedStates of 1.6-3.8 million.Among adolescents, tetrahydrocannabinol(THC) isthe most commonly used illicitsubstance, creatingconcern that

adolescent exposure toTHC could affect mTBI outcomes. The purpose of thisstudy isto model the effect of adolescentTHC exposure on inflammatorysignalingand cognitiveprocessingfollowingadulthood mTBI,as measured by lipidomicsand spatialworkingmemory performance, respectively.Forty LongEvans rats (20 male and 20 female) were randomly selected to receivedailyintraperitonealinjectionsof THC or vehicle(Ethanol) from post-natal day 28 to 49, whichwas followed by aneleven day washout period.Rats then receivedpretrainingon 8-arm radialmaze, followed byfifteensessionsof 8-arm training,and then twenty sessionsof 2-phase testingbeforeintervention.A randomizedblock designwas used to eliminatevariationinradialmazeperformance priorto the intervention.On post-natal day 116, rats were randomly selected toreceiveeitherweightdrop (450g dropped from 1m, skull and skinintact)or sham intervention.Followingthe injuryor sham, the rats were tested on the radialmaze for 15 additionaldays.Blood samples were obtainedat sixtimepoints:three priorto and three followingtheintervention.They were then euthanizedby decapitation,and theirbrainswere rapidlyextractedfor analysisviahighpowered liquidchromatography. Preliminarydata existfor the firstcohortof rats (n=20), and ongoingtestingwillbe completed on the second cohort (n=20). In regards tothe cognitivetestingof the firstcohort, the mTBI rats performed 5% worse (75% mTBI vs 80%sham) duringdays 1-5 post-injury/sham.Duringdays 6-10, the mTBI rats performed similarlytothe sham rats (82% mTBI vs 81% sham), whereas duringdays 11-15, the mTBI rats plateaued incomparisonto sham (80% mTBI vs 84 % sham). When factoringTHC exposure duringthe first5 days post injury/sham,the THC+mTBI group had the worst performance (73%), whereas theTHC+sham, vehicle+mTBI,and vehicle+shamgroup had similarperformances to baseline(82%,77%, 79% respectively).ThisindicatesTHC exposure may be associatedwithcognitiveimpairmentduringthe acute phase of TBI injury.

Disclosures: C.W. Hubbard: None. **G. Nah:** None. **N.L. Port:** None. **H.B. Bradshaw:** None. **J.D. Crystal:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.10/D30

Topic: C.10. Brain Injury and Trauma

Support: R01 NS104573
R01 DE030313

Title: Age-at-injury determines the extent of long-term neuropathology and cognitive outcomes in novel repetitive less-than-mild closed head injury model

Authors: *E. KARAKAYA[1], J. EDWARDS[1], O. ALBAYRAM[2];
[1]Med. Univ. of South Carolina, Charleston, SC; [2]Beth Israel Deaconess Med. Ctr., Med. Univ. of South Carolina Neurosci. Grad. Program, MT PLEASANT, SC

Abstract: Age-at-injury determines the extent of long-term neuropathology and cognitive outcomes in novel repetitive less-than-mild closed head injury model.

E. Karakaya[1], N. Oleinik[2], J. Edwards[1], A. Ergul[1], S. Beyaz[3], B. Ogretmen[2], O. Albayram[1]; [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

None of the authors has a disclosure.

Repetitive mild traumatic brain injury (rmTBI) involves complex pathological processes consisting of primary insults and secondary complications and is a prerequisite for chronic traumatic encephalopathy (CTE). The existing models of rmTBI can replicate histopathological and functional outcomes that are observed in the clinical features of CTE. However, these models don't explain why the development of secondary complications may not necessarily progress every time. The exact prevalence of CTE is still unknown, and despite extensive research, the factors that cause neuropathology have not yet been definitively identified. To investigate this phenomenon, we have developed a new experimental model of repetitive concussive brain injury, known as the repetitive-less-than mild TBI (rlmTBI) paradigm. As part of a proof-of-concept experiment, 2-month-old male wildtype (WT) mice were subjected to seven mild (54-g weight drop from 36″) OR less-than-mild (54-g weight drop from 24″) hits to the dorsal aspect of the skull over 9 days. At 8 months postinjury, the existing rmTBI model induced significant long-term neurological and cognitive deficits accompanied by cortical degeneration of white matter (WM). In contrast, receiving rlmTBI at 2 months of age didn't cause any long-term impairments. The aged brain is particularly vulnerable to secondary disease development after TBI, making it more susceptible to chronic neurodegenerative changes following TBIs. To test if age-at-injury hinders long-term recovery in a new rlmTBI paradigm, young (2-month-old) and old (12-month-old) male WT mice were subjected to 7 less-than-mild hits to the dorsal aspect of the skull in 9 days. The average time elapsed before recovery of the righting reflex was significantly increased in both young and old rlmTBI mice. Old rlmTBI-induced mice developed significant neurobehavioral deficits at 8 months postinjury, analyzed by Barnes maze along with subcortical WM pathology. The present study is the first to introduce a new experimental model, rlmTBI paradigm, to demonstrate how additional confounding factors cause resistance and resilience to the neuropathology of CTE.

Disclosures: E. Karakaya: None. **J. Edwards:** None. **O. Albayram:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.11/D31

Topic: C.10. Brain Injury and Trauma

Support: NSF 2313303
International Brain Research Organization

Title: Olfactory system neurodegeneration and regeneration following brain injury in adult zebrafish

Authors: *W. SNYDER[1,2], M. KUSSMANN[3], E. CALVO-OCHOA[3];
[1]Hope Col., Holland, MI; [2]Biology and Neuroscience, Hope College, Holland, MI; [3]Biol. and Neurosci., Hope Col., Holland, MI

Abstract: Zebrafish are one of the few vertebrates that maintain the ability to generate new neurons throughout their lifespan, contrary to mammals, whose ability to repair and regenerate their nervous system is very limited. Thus, zebrafish is an ideal model for studying mechanisms of neural recovery and regeneration. In particular, the olfactory system, which processes odors and is formed by the olfactory bulb (OB) and epithelium (OE), exhibits extensive neuroplasticity and repair mechanisms in response to damage. It has been shown that following damage to the olfactory system there is a complete recovery of these regions. It has been established that the OE and OB of zebrafish recover after peripheral damage to the OE. However, the repair and recovery mechanisms of the olfactory bulb following direct injury have not been thoroughly studied. In this study, we established a novel model of excitotoxic injury in the zebrafish OB to study mechanisms of structural and functional recovery in the degenerated olfactory system over time. We used adult zebrafish of both sexes and induced damage via a unilateral focal excitotoxic lesion in the right olfactory bulb by injecting 1 μl of 15 mM quinolinic acid (QA). We then assessed markers of cell proliferation and inflammation following recovery using immunohistochemical assays. To test olfactory function, we performed olfactory-mediated behavioral assays to three types of species-relevant odorants. Our results show extensive neurodegeneration across the olfactory system, along with neuroinflammation and an increase in proliferation markers. We also found that the QA lesion causes olfactory loss in the three odorants tested. We observed progressive recovery of the tissue with transient proliferation, neuroinflammation, and neurodegeneration markers throughout recovery. These results contribute to the understanding of the neurological mechanisms and associations between inflammation, neurogenesis, and neuronal recovery following damage.

Disclosures: W. Snyder: None. **M. Kussmann:** None. **E. Calvo-Ochoa:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.12/D32

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R21 NS114771-01A1

Title: Cerebral artery supply territory modification and acute microhemorrhage following single and repeated mild traumatic brain injury in mice

Authors: *D. N. NTHENGE-NGUMBAU[1], N. W. LILLY[2], K. E. SAATMAN[3,1];
[1]Spinal Cord and Brain Injury Res. Ctr. & Dept. of Physiology, Col. of Medicine,, [2]Spinal Cord and Brain Injury Res. Center, Col. of Medicine,, [3]Univ. of Kentucky, Lexington, KY

Abstract: Mild traumatic brain injury (mTBI) is accompanied by headaches, dizziness and other neurological dysfunction, often in the absence of discernable changes in gross brain structure. Changes in cerebral blood flow may contribute to deficits in neuronal function. To better understand the effects of single and repeated concussion-like mTBI on the cerebrovasculature, we examined the major cerebral surface artery territories and labeled brain sections for intraparenchymal microhemorrhage and microglial activation. Adult male mice were subjected to either one (n=7) or two (n=19, 1-day inter-injury interval) mild, midline impact(s) to the closed, exposed skull using a pneumatic impactor or a sham injury (n=3). At 1 day after the final injury, mice were transcardially perfused with ink-containing gelatin for *ex vivo* visualization and quantification of the cortical area of the territory supplied by the anterior, middle, or posterior cerebral artery equivalents, as well as the extent of territory overlap. At one day after a single mTBI, the territory supplied by the posteriorly (PCA) increased by 17%, while that supplied by the middle cerebral artery (MCA) decreased by 10% compared to sham. These changes were amplified after double mTBI, with a 29% increase and 20% decrease, respectively, compared to sham. Mild TBI resulted in sporadic microhemorrhages in multiple brain regions, as detected by Prussian blue staining. Modest CD68-positive microgliosis was observed in the hippocampus, entorhinal cortex and optic tract, with more prominent microgliosis in lateral cerebellar regions. Diffusely distributed microhemorrhages did not appear to be associated with regionally restricted microgliosis.

Disclosures: D.N. Nthenge-Ngumbau: None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.13/D33

Topic: C.10. Brain Injury and Trauma

Support: R01NS108190-01

Title: 3r tau mediates acute neurodegeneration following closed head injury

Authors: *R. MORRONE, E. NIKULINA, R. FURHANG, P. J. BERGOLD;
Physiol. and Pharmacol., SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY

Abstract: White matter is particularly vulnerable to traumatic brain injury (TBI). Acceleration-deceleration of the head during TBI injures and demyelinates axons both proximal and distal to the injury site. This produces cytoskeleton damage that impairs axonal transport. Axonal microtubules are crosslinked and stabilized by tau protein. Alternative splicing of the tau gene results in tau protein isoforms containing either three (3R) or four (4R) microtubule binding

sites. Adult mouse axons express only 4R tau; human axons express both 3R and 4R tau. 3R tau containing-axons are less stable and more flexible than 4R tau containing-axons. TBI produces cognitive deficits that arise, in part, from white matter degradation. I tested if 3R tau expression alters disease course after an experimental TBI. Using a closed head injury TBI model, white matter damage and cognition was compared between C57/Bl6 wildtype mice (WT) with 4R tau containing-axons, and microtubule-associated protein tau knock-in (MAPTKI) mice with 3R and 4R tau containing-axons. Righting reflex, a measure of initial injury, does not differ between WT and MAPTKI mice. At 14 days post injury, spatial memory is evaluated using Active Place Avoidance in WT and MAPTKI mice. Injured MAPTKI mice acquire Active Place Avoidance, whereas injured WT mice are significantly impaired. Following behavioral testing, amyloid precursor protein assesses impaired fast axonal transport; the myelin probe Fluoromyelin-Red assesses myelin content; and NeuN assesses hippocampal neuronal density. The corpus callosum of injured WT mice has significantly greater demyelination and accumulates more amyloid precursor protein than injured MAPTKI mice. Injured WT and MAPTKI mice, however, have similar hippocampal neuronal loss. These data suggest that Active Place Avoidance deficits in injured WT mice arise from increased white matter damage. These data suggest that 3R tau expression mediates acute neurodegeneration following closed head injury.

Disclosures: R. Morrone: None. **E. Nikulina:** None. **R. Furhang:** None. **P.J. Bergold:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.14/D34

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R35-NS116852

Title: Brain extracellular matrix alters local anion concentrations and responses to injury

Authors: *K. P. NORMOYLE[1], V. I. DZHALA[2], K. P. LILLIS[3], K. EGAWA[4], J. GLYKYS[5], K. J. STALEY[6];
[1]Neurol., Massachusetts Gen. Hosp. / Harvard Med. Sch., Boston, MA; [2]Dept. of Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [3]Neurol., Harvard Med. Sch., MGH, Charlestown, MA; [4]Dept. of Pediatrics, Hokkaido Univ. Grad. Sch. of Med., Sapporo, Japan; [5]Pediatrics and Neurol., The Univ. of Iowa, Iowa City, IA; [6]Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: The reversal potential of $GABA_A$ receptors (E_{GABA}) is dependent upon the chloride concentrations on both sides of the neuronal membrane. We recently discovered that the extracellular chloride concentration is non-uniform and half the chloride in bulk cerebrospinal fluid. We also observed that removal of polyanionic glycosaminoglycans (GAGs) from the extracellular space, mimicking injury-induced metalloprotease (MMP) activation, results in a shift toward higher local extracellular chloride concentrations. We used 2-photon Fluorescence

Lifetime Imaging (FLIM) of a custom chloride-sensitive fluorophore constrained to the extracellular space by conjugation with 10 kilodalton dextran in acute and organotypic cultures of hippocampal slices. We used slice injury as well as 2-photon photolysis of single neurons within organotypic slices to model acute brain injuries. Intraneuronal chloride was measured with the ratiometric reporter Super Chlomeleon. Having discovered that the extracellular chloride ($[Cl^-]_o$) between neurons both *in vitro* and *in vivo* is only about half that of bulk CSF chloride, and that digestion of a prominent sulfated GAG in the brain (chondroitin sulfate) leads to release of these anionic moieties and a shift to higher chloride concentrations, we next asked what would happen to $[Cl^-]_o$ when the sulfated moieties of the matrix are freed by endogenous MMPs after brain injury. We found a strong dependence of $[Cl^-]_o$ vs distance from injury, with Cl concentration increasing to the ACSF levels near the injured surface of acute slices or proximity to photolysed neurons in organotypic slices. These changes in $[Cl^-]_o$ should also alter the neuronal intracellular chloride via the activity of the high-velocity equilibrative membrane chloride transporters. We compared $[Cl^-]_o$ and intracellular chloride ($[Cl^-]_i$) in each of these models, and confirmed results *in vivo* using cortical window implantation of adolescent mice. Finally, the release of sulfates and subsequent changes to $[Cl^-]_{o/i}$ should be inhibited by MMP antagonists. We confirmed that broad-spectrum inhibition using the zinc chelator ZX-1 or the more specific MMP-2/9 inhibitor SB3CT also reduced $[Cl^-]_i$ and neuronal volume after injury. These changes were evident at the cut surface of acute brain slices and in proximity to photolysed neurons. In conclusion, $[Cl^-]_o$ is partially displaced by sulfates in the extracellular matrix. Damage to the extracellular matrix following brain injury alters the distribution of chloride in both the extra- and intracellular spaces. These findings have immediate implications for the treatment of cytotoxic edema and seizures after acute brain injury.

Disclosures: K.P. Normoyle: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent on ABP fluorophore. **V.I. Dzhala:** None. **K.P. Lillis:** None. **K. Egawa:** None. **J. Glykys:** None. **K.J. Staley:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent on ABP fluorophore.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.15/D35

Topic: C.10. Brain Injury and Trauma

Support: Indiana State Funding

Title: Sleep spindles alteration after Blast-induced Traumatic Brain Injury: characterization of a perspective thalamus-cortical lesion biomarker using a novel automatic detection algorithm based on Otsu's method.

Authors: *M. DALOLIO[1,2], R. SHI[3,2,4];
[1]Ctr. for Paralysis Res., Purdue Univ., West Lafayette, IN; [2]Biomedical Engeneering, Purdue University, West Lafayette, IN; [3]Basic Med. Sci., Purdue Univ., West Lafayette, IN; [4]Center for Paralysis Research, Purdue University, West Lafayette, IN

Abstract: Blast-induced traumatic brain injury (bTBI) accounts for over a third of all TBI cases in military personnel, often leading to cognitive impairments and memory deficits. Sleep spindles (SSPs), 10-15Hz waves during non-rapid eye movement (NREM) sleep, reflect thalamus-cortical activity and play a role in memory consolidation. Previous research has linked SSPs alterations to posttraumatic epilepsy after contusion TBI. This study examines SSPs following bTBI, alongside sleep patterns and susceptibility to seizures. Video-EEG and EMG were recorded in C57BL/6 male mice for 1 week at 30 days after repetitive (1x3days) 150kPa blast exposure (bTBI group, n=10) or not (sham group, n=10). SSPs were identified using a customized MATLAB algorithm, applying Otsu's method threshold to the root mean squared transformation of 10-15 Hz filtered (bandpass Butterworth) parietal EEG. The algorithm returns a list of all detected events with annotation of duration, amplitude and peak frequency for each of them, as well as the SSPs density in NREM over 24 hours. At last day of recording seizure susceptibility was assessed administering pentylenetetrazol (PTZ, 50mg/kg). The mortality rate post-bTBI was 20% (n=2 bTBI group) and 1 noisy EEG recording was excluded from analysis (sham group). Time spent in wake, NREM, and rapid eye movement (REM) phases, as well as power spectral analysis 1-30Hz band (average spectrum, Welch method), revealed no significant differences between the groups (analysis conducted with 2-way ANOVA). SSPs' mean normalized amplitude and peak frequency were indeed significantly lower in the bTBI group (mean±se 0.328±0.001 vs 0.393±0.001, $p<0.0001$, and 11.718±0.007 vs 11.742±0.006, $p=0.0002$ respectively, Mann-Whitney nonparametric t-test), while both groups shown SSPs' mean density of 8/min and duration of 1.1 s. Neither the occurrence of spontaneous seizures, nor the differences in seizure susceptibility were observed. Based on these discoveries, it appears that bTBI induces a persistent morphological alteration of SSPs, which could suggest a consolidated disruption of thalamocortical circuitry, justifiable by the white matter damage known to follow blast exposure, rather than be related to increased cortex excitability. To our knowledge, this is the first investigation of SSPs following bTBI, marking the initial phase of further research into the potential role of SSPs as indicators of thalamus-cortical damage and memory impairments after bTBI.

Disclosures: M. Dalolio: None. **R. Shi:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.16/D36

Topic: C.10. Brain Injury and Trauma

Support: NIEHS R21ES034191
NINDS R21NS119991
RCMI8G12MD00760
Hispanics in Research Capability (HiREC)
Title V Pilot Project (PiP)

Title: Effects of closed-head injury on pain-like behaviors in rats

Authors: *L. VICENTE-RODRÍGUEZ[1], P. VÁQUEZ MARTÍNEZ[1], D. NAZARIO[1], Y. CARRASQUILLO[2], D. SIERRA-MERCADO[3];
[1]Biol., Univ. of Puerto Rico, Cayey, PR; [2]Natl. Ctr. for Complementary and Integrative Hlth., NIH, Bethesda, MD; [3]Anat. & Neurobio., Univ. of Puerto Rico, Sch. of Med., San Juan, PR

Abstract: One of the most common sequelae of traumatic brain injury (TBI) is the development of chronic pain distant from the site of injury, which occurs in close to 60% of those patients. However, the underlying mechanisms leading to the development of chronic pain post head injury are largely unknown. Functional changes in anatomical pain regions have been correlated with chronic pain in both humans and pre-clinical models. We hypothesize that maladaptive changes in the connectivity between pain regulatory regions contribute to the development of chronic pain after a mild traumatic brain injury (mTBI). To test this hypothesis, we first confirmed a persistent hypersensitivity phenotype in a rat model of mTBI. To do this, we used a well-established closed head injury (CHI) model, namely the weight drop model. No significant changes were observed in waking-time (p=0.5250), righting-time (p=0.7632), or time to ambulate between CHI and sham groups (p=0.6385) immediately following the CHI. These results are consistent with mild TBI, with no loss of consciousness when compared to sham. We then evaluated mechanical sensitivity using von Frey's filaments and thermal sensitivity using the Hargreave's test at various days post the injury (dpi), specifically 3, 5, 7,14, 21, 28 and 35 dpi. The results show that CHI causes significant mechanical hypersensitivity (p=0.0045) across all time points when compared to sham, but not thermal hypersensitivity (p=0.1952). Ongoing experiments are examining markers of neuronal inflammation and neuronal activity in brain regions that are part of the pain axis, such as the anterior cingulate cortex, the locus coeruleus, and the ventrolateral periaqueductal gray. The results of this study will contribute to a comprehensive understanding of the brain structures involved in pain sensitization after a brain injury and will set the ground for future experiments exploring the contribution of specific pain circuits to CHI-induced persistent pain.

Disclosures: L. Vicente-Rodríguez: None. **P. Váquez Martínez:** None. **D. Nazario:** None. **Y. Carrasquillo:** None. **D. Sierra-Mercado:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.17/D37

Topic: C.10. Brain Injury and Trauma

Title: Antibiotic treatment induces microbiome dysbiosis and reduction of neuroinflammation following traumatic brain injury in mice.

Authors: *H. FLINN[1], S. SORIANO[2], S. VILLAPOL[2];
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Abstract: The gut microbiome is linked to brain pathology in cases of traumatic brain injury (TBI), yet the specific bacteria that are implicated in this gut-brain axis link are not well characterized. To address this gap, in this study, we induced traumatic brain injury (TBI) in male C57BL/6J mice using the controlled cortical impact injury (CCI) model. After 35 days, we administered a broad-spectrum antibiotics (ABX) cocktail (ampicillin, gentamicin, metronidazole, vancomycin)through oral gavage for 3 days to diminish existing microbiota. Subsequently, we inflicted a second TBI on the mice and analyzed the neuropathological outcomes five days later. Longitudinal analysis of the microbiome showed significant shifts in the diversity and abundance of bacterial genera during both acute and chronic inflammation. These changes were particularly dramatic following treatment with ABX and after the second TBI. ABX treatment did not affect the production of short-chain fatty acids (SCFA) but did alter intestinal morphology, characterized by reduced villus width and a lower count of goblet cells, suggesting potential negative impacts on intestinal integrity. Nevertheless, diminishing the intestinal microbiome reduced cortical damage, apoptotic cell density, and microglial/macrophage activation in the cortical and thalamic regions of the brain. Our findings suggest that eliminating colonized gut bacteria via broad-spectrum antibiotics reduces neuroinflammation and enhances neurological outcomes in TBI, despite implications to gut health.

Disclosures: H. Flinn: None. **S. Soriano:** None. **S. Villapol:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.18/D38

Topic: C.10. Brain Injury and Trauma

Support: CIHR PJT376309
CIHR PJT156179
CIHR PJT178059

Title: Cerebrovascular Networks Coordination Following Traumatic Brain Injury (TBI)

Authors: *M. ROZAK[1], A. ATTARPOUR[2], A. E. DORR[3], J. R. MESTER[4], M. GOUBRAN[5], B. STEFANOVIC[6];

[1]Univ. of Toronto, York, ON, Canada; [2]Univ. of Toronto, Toronto, ON, Canada; [3]Sunnybrook Hlth. Sci., Toronto, ON, Canada; [4]Physical Sci., Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada; [5]Med. Biophysics, Univ. of Toronto, Toronto, ON, Canada; [6]Imaging Res., Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada

Abstract: Blood flow distribution determines metabolic support afforded to brain tissue via neurovascular coupling, whereby neuronal activation leads to a local increase in blood flow. Brain vasculature is damaged during traumatic brain injury (TBI) and undergoes repair and reorganization. Persistent blood flow alterations are associated with altered behaviour/cognition and adverse outcomes; however, the underlying network-level structural and functional changes in brain vasculature post-trauma are still incompletely understood. Current methods that directly interrogate brain vessels are unable to discern network-level changes to vascular morphology and reactivity. We developed a deep learning-based pipeline using in situ two-photon fluorescence microscopy (2PFM) data to conduct high throughput analysis of cerebrovascular network alterations. We applied this pipeline in a model of moderate TBI, involving three closed-head impacts with a three-day inter-impact interval in Thy1-ChR2-YFP mice (16 TBI (8M/8F), 13 sham (6M/7F)). Two weeks after the final impact, mice were implanted with cranial windows centred over the impact location. The underlying cortex was imaged on 2PFM during baseline periods alternated with focused blue light photostimulation (458 nm, 4.3 mW/mm^2, 239 um diameter, 250 um depth). We used data from 15 mice to train a 3D UNETR for the neuron and vessel segmentation, validated on 4 mice, and tested on 6 mice. To enable detailed morphological analysis, the segmented cerebrovascular networks were rendered as graphs, with vessel segments' morphometrics stored as the edges of the graph. The pipeline also classified vessels as arteries, capillaries, or veins. The model achieved an F1 score of 0.76±0.10 on segmentation of the vessel class. Following optogenetic stimulation, vessel diameter changes in TBI mice, across 2953 responding vessels, were attenuated, by 0.19 ± 1.06 um on average, compared to those seen in SHAM mice (1222 vessels). Furthermore, TBI was found to decrease assortativity of cerebrovascular responses to optogenetic stimulation by 10 ± 43%, indicating a TBI-elicited decrease in coordination between adjacent vessels at the sub-network level.
Our pipeline enables mapping changes in the cerebrovascular structure and function post-TBI. Its application in the subacute phase of moderate TBI revealed spatial patterns of altered cerebrovascular reactivity and coordination in the concussed cortex.

Disclosures: M. Rozak: None. **A. Attarpour:** None. **A.E. Dorr:** None. **J.R. Mester:** None. **M. Goubran:** None. **B. Stefanovic:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.19/D39

Topic: C.10. Brain Injury and Trauma

Support: Combat Casualty Care Research Program at MRDC

Title: Blast overpressure exposure causes tauopathy and axonal degeneration in a ferret model of blast-induced traumatic brain injury

Authors: *P. ARUN;
Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: Blast overpressure exposure causes tauopathy and axonal degeneration in a ferret model of blast-induced traumatic brain injury
Manoj Govindarajulu, Aymen Al-Lami, Jishnu Krishnan, Gaurav Phuyal, Rex Jeya Rajkumar Samdavid Thanapaul, Joseph B. Long and Peethambaran Arun
Blast-Induced Neurotrauma Branch, Center for Military Psychiatry and Neurosciences, Walter Reed Army Institute of Research, Silver Spring, MD 20910
Blast-induced traumatic brain injury (bTBI) causes acute and chronic neurobehavioral abnormalities and is one of the major causes of persistent disabilities in Service Members. Clinical observations of several military blast casualties have revealed tauopathy and development of chronic neurodegenerative disorders post-blast. However, the mechanisms by which bTBI initiates the neurodegenerative process, however, are not completely understood. Hence, we investigated the differential expression of markers of axonal injury such as phosphorylated Tau (pTau) and phosphorylated neurofilament heavy chain (pNFH) in various brain regions following blast exposure. Ferrets were exposed to two tightly coupled blasts (19psi) using an advanced blast simulator. Different regions of the brain (prefrontal cortex, cortex, midbrain, cerebellum and brainstem), plasma and cerebrospinal fluid (CSF) were collected at 24h and 1-month post-blast. Protein levels of pTau and pNFH were quantified by Western blotting. pNFH levels in plasma and CSF were quantified by commercially available ELISA kit. Our results indicate increased phosphorylation of Tau at serine (Ser396 and Ser404) and threonine (Thr205 and Thr231) at 24h and 1-month post-blast exposure with associated activation of protein kinases (GSK3β, CDK5 and MAPK) involved in the phosphorylation of Tau protein in prefrontal cortex, cortex, cerebellum and brainstem. Increased protein expression of pNFH was also evident at both time points in those brain regions. Furthermore, increased pNFH levels were noted at both time points in the CSF. However, in the plasma, statistically significant increase in pNFH levels were noted only at 24h time point. Our results indicate that blast exposure causes acute and persistent tauopathy and axonal degeneration in different brain regions. While compelling mechanistic links have been identified, significant extensive investigation in the field is needed to develop therapies to protect bTBI patients from the increased risk of developing neurodegenerative diseases.

Disclosures: P. Arun: None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.20/D40

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01NS132772-01A1 (YX)

Title: Mesenchymal stem/stromal cell-derived small extracellular vesicles for treatment of traumatic brain injury in female rats

Authors: *Y. ZHANG[1], Y. ZHANG[2], M. CHOPP[5,6], H. PANG[3], L. CHEN[1], Z. ZHANG[4], A. MAHMOOD[7], Y. XIONG[3];
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Abstract: Background: Our previous study has demonstrated that small extracellular vesicles (sEVs) derived from bone marrow mesenchymal stem cells (hMSCs) improve functional recovery in male rats subjected to traumatic brain injury (TBI). The aim of this study was to determine the therapeutic effects of sEVs on functional recovery in female rats subjected to TBI. **Methods:** Anesthetized female age-matched young rats (2-3 months) subjected to severe TBI (sTBI) induced by controlled cortical impact (CCI) over the left parietal cortex were randomly divided into the following treatment groups (n=8/group): 1) phosphate-buffered saline (PBS as Vehicle group); and 2) sEVs administered at 1 day (sEVs group) post TBI. PBS or sEVs at a dose of 100 µg/rat were injected via a tail vein in 0.5 ml PBS for 5 min starting at 1 day after TBI. Sham female animals with surgery without treatment were included as TBI controls (Sham, n=8). We performed the functional tests 1 day and then weekly post-injury including foot-fault, adhesive removal, modified neurological severity score (mNSS) and Morris water maze tests (MWM). Animals were sacrificed at day 35 after TBI for immunostaining analyses of neuroinflammation, neuronal cell loss and lesion volume. **Results:** We found that female rats with sTBI exhibited spontaneous functional recovery specifically for forelimb footfault and adhesive removal tests and these functions were not fully recovered at 35 days post injury. Compared to the vehicle treatment, treatment with sEVs at a dose of 100 µg/rat starting 1 day post TBI significantly reduced foot-fault, adhesive removal functional deficits and mNSS score ($p<0.05$) and significantly improved cognitive function (reduced the latency and increased % time spent in the correct quadrant in the MWM test) ($p<0.05$). Treatment with sEVs significantly reduced lesion volume, neuronal cell loss in the hippocampus measured by NeuN staining and reduced neuroinflammation in the injured brain measured by CD68+ microglia/macrophages and GFAP+ astrocytes ($p<0,05$). **Conclusions**: Our data demonstrate that MSC-derived sEVs treatment initiated at 1 day post-injury at a dose of 100 µg/rat provides a significant therapeutic effect on functional and histological recovery in female rats after sTBI. Our data from previous and current studies indicate that MSC-derived sEVs holds potential treatment for both male and female subjects with TBI.

Disclosures: Y. Zhang: None. **Y. Zhang:** None. **M. Chopp:** None. **H. Pang:** None. **L. Chen:** None. **Z. Zhang:** None. **A. Mahmood:** None. **Y. Xiong:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.21/D41

Topic: C.10. Brain Injury and Trauma

Support: Loyola University - Chicago Burn Shock Trauma Research Institute

Title: Subacute testosterone administration improves repetitive mild traumatic brain injury-induced recognition memory deficits by modulating hippocampal cellular senescence

Authors: *J. E. EXLINE[1,2], M. VOLYANYUK[1,2], K. M. LOTESTO[3,2], S. C. BYRAM[4,5], E. M. FOECKING[6,2,7,8];
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Abstract: Traumatic brain injury (TBI) is a leading cause of death and disability worldwide with 75-90% of all TBI being classified as concussions, or mild TBIs (mTBIs). The acquisition of a single mTBI increases the risk of repetitive mTBI (rmTBI), which can exacerbate the persistent memory deficits experienced by many rmTBI patients. Cellular senescence is a pro-inflammatory cell cycle arrest that is implicated in persistent rmTBI-induced memory deficits. Acute testosterone (T) treatment following rmTBI has been shown to mitigate cognitive deficits and cellular dysfunction that can induce cellular senescence. However, the use of T at subacute-to-chronic time points for the treatment of persistent memory deficits has yet to be well explored. Here, we examine the progression of hippocampal cellular senescence after rmTBI and evaluate the effects of subacute T administration on persistent memory deficits and senescence-associated markers post-injury. Male Long-Evans Hooded Rats (8 weeks) were subjected to five closed-head mTBIs spaced 48 hours apart. A subset of rats received subcutaneous T implants 5 weeks post-injury (WPI). Recognition memory was assessed by novel object recognition (NOR) testing performed at 1, 4, and 6 WPI. NOR performed at 1 and 4 WPI demonstrated persistent recognition memory deficits which subacute T treatment improved by 6 WPI. In a second cohort, the subacute progression of cellular senescence was explored by molecular analysis of whole hippocampal homogenate comparing 5 and 9 WPI. Molecular analysis showed increased p53 protein quantity and $p21^{WAF1/CIP1}$ gene expression indicating a progressive increase in senescence-associated cell cycle arrest markers. Additionally, we found a progressive reduction in the gene expression and protein quantity of SIRT1 and the gene expression of HMGB1 suggesting the derepression of senescence-associated pro-inflammatory gene expression. Subacute T administration reduced p53 protein quantity and $p16^{INK4A}$ gene expression when evaluated at 9 WPI. Interestingly, IL-1β gene expression was reduced between 5 and 9 WPI but increased with subacute T administration by 9 WPI. Together, these findings suggest that rmTBI induces a progression in hippocampal cellular senescence associated with long-term memory deficits that are ameliorated by subacute T treatment. These exciting findings encourage the development of pharmacological agents to target persistent memory deficits at time points distal to rmTBI for the improvement of patient quality of life.

Disclosures: J.E. Exline: None. **M. Volyanyuk:** None. **K.M. Lotesto:** None. **S.C. Byram:** None. **E.M. Foecking:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.22/D42

Topic: C.10. Brain Injury and Trauma

Title: Assessment of Early Neurological Outcomes Post-Mitoquinone Supplementation in a Mouse Model of Repeated Mild Traumatic Brain Injury

Authors: M. RESLAN[1], Z. SHAKKOUR[2], L. NASRALLAH[3], M. HAIDAR[4], ***F. KOBAISSY**[5];
[1]Div. of Neurobio.,, Freie Univ. Berlin, Institute of Biology, Germany; [2]Pathology and Anatom. Sci., Univ. of Missouri, Columbia, Columbia, MO; [3]Dept. of Psychiatry and Neurosci.,, Univ. Laval,, Quebec City, QC, Canada, QC, Canada; [4]Div. of Neurobio.,, Freie Univ. Berlin, Institute of Biology,, Germany; [5]Morehouse Sch. of Med., Atlanta, GA

Abstract: Mild traumatic brain injury (mTBI) or concussion accounts for the bulk of all head injuries and represents a major health concern. Although an mTBI event may not manifest in neurobehavioral impairment, repeated injuries, known as repeated mTBI (rmTBI), can result in a cumulative effect that may progress into long-term cognitive and functional deficits. To date, there is no FDA-approved drug for TBI in general and rmTBI in particular. In previous studies, we have demonstrated the neuroprotective role of mitoquinone (MitoQ), a mitochondrial antioxidant, in an open-head injury model and a model of repeated mild TBI (rmTBI) at a chronic time point (30 days). This current study assesses the trajectory of how MitoQ exerts its neuroprotective effects at acute (3 days) and subacute (7 days) time points post-injury and compares to a chronic time point of 30 days in a controlled cortical impact model of rmTBI. C57BL/6 male mice were injected intraperitoneally with MitoQ (5 mg/kg). Cognitive function was evaluated using the Morris water maze (MWM) while gross and fine motor functions were evaluated by the pole climbing, grip strength, and ladder rung tests. Dihydroethidium (DHE) staining was performed to evaluate oxidative stress while qRT-PCR was used to measure the gene expression of different antioxidant enzymes. Also, immunofluorescence staining was performed on brain tissue to assess the degree of microgliosis and astrocytosis. Our results showed that MitoQ conferred significant protection on days 3 and 7 post-injury against fine motor function impairment induced by rmTBI. Moreover, MitoQ enhanced cognitive function and reduced astrogliosis, microgliosis, and levels of oxidative stress on day 7 post-injury. In light of our results, MitoQ administration may be considered a preventive approach that helps to alleviate the neurological manifestations associated with rmTBI early before symptoms progress to long-term deficits.

Disclosures: M. Reslan: None. **Z. Shakkour:** None. **L. Nasrallah:** None. **M. Haidar:** None. **F. Kobaissy:** None.

Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.23/D43

Topic: C.10. Brain Injury and Trauma

Support: NS111378
NS117148
NS116383

Title: Delayed administration of BDNF mimetic R13 boosts the beneficial effects of exercise on TBI pathogenesis

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[1]UCLA, Los Angeles, CA; [2]Neurosurg., UCLA David Geffen Sch. of Med., Los Angeles, CA; [3]Dept of Integrative Biol. and Physiol., UCLA, Los Angeles, CA

Abstract: Background: Traumatic brain injury (TBI) is a leading cause of neuronal and cognitive dysfunctions. Exercise has shown beneficial effects on increasing brain function and plasticity and reducing the negative effects of TBI. It is still controversial when exercise should be applied for maximal benefits after TBI, and how the action of exercise could be boosted using pharmacological agents. We used resting state functional connectivity (FC) with MRI and protein chemistry to assess the action of the BDNF mimetic R13 on TBI animals exposed to exercise. **Methods:** Sprague Dawley rats received moderate lateral fluid percussion injury (FPI). R13 (7.25 mg/kg, i.p) and vehicle were administered at 7 days post-FPI for 7 consecutive days to rats that either had access to a voluntary running wheel or were sedentary. Memory and anxiety-like behaviors were assessed two weeks (15^{th} day) post-TBI. Magnetic resonance imaging (MRI) was performed on post-TBI days 1 and 30^{th}-day in rats receiving R13 or vehicle intervention with or without a voluntary running wheel. Gene expression and protein levels were measured in the ipsilateral to the injury hippocampus. **Results:** Animals exposed to FPI showed a reduction in spatial memory and anxiety-like behavior at 2 weeks post-TBI which was counteracted by either R13 or exercise. Injured animals showed upregulation in the gene expression of lactate (MCT1 and MCT2) and glucose (GLUT3) transporters as well as inflammatory marker (TNF-a) in the hippocampus at 30th-day post-injury which was neutralized by either R13 or exercise intervention. Following TBI treatment with either R13 or exercise tended to improve FC at 30 days post-injury. FC tended to be improved in several cognitively-relevant brain regions including thalamo-cortical connectivity to the sensory cortex and hippocampal retrosplenial connections **Conclusion:** This study showed that delayed administration of either R13 or

exercise counteracted cognitive deficits post-TBI and increased FC. Overall, these observations support that R13 and exercise have therapeutic potential against TBI.

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Poster

PSTR162: Brain Injury: Animal Models and Therapeutics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR162.24/D44

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01NS104282-01A1

Title: Antagonizing Major Histocompatibility Complex Class II-associated invariant peptide (CLIP) improves neurobehavioral deficits and neuropathological outcomes after fluid percussion injury

Authors: *J. IANNUCCI[1], L. VENKATASAMY[1], M. DAVIS[1], S. BROWN[1], G. YADAV[1], T.-A. NGUYEN[1], A. PEREVERZEV[1], M. N. ROGERS[2], L. A. SHAPIRO[1];
[1]Texas A&M Hlth. Sci. Ctr., Bryan, TX; [2]Med. Physiol., Texas A&M Univ. Col. of Med., Bryan, TX

Abstract: Traumatic Brain Injury (TBI) is a major cause of death and disability worldwide, affecting upwards of 50 million people annually. TBI can cause permanent damage to the brain and highly debilitating post-traumatic syndromes, including depression, cognitive impairment, and increased susceptibility to neurodegenerative diseases. TBI causes an inflammatory response, including initiation of a non-specific innate immune response, and the potential transition to an antigen-specific adaptive immune response. We previously identified adaptive immune B cell expansion after TBI, including expansion of CLIP+ B cells. We developed a CLIP antagonist peptide (CAP) that reduces CLIP+ B cells by competitively antagonizing CLIP binding to the antigen presenting groove of MHCII. We've previously shown that CAP is neuroprotective after TBI, suggesting a detrimental role of CLIP+ B cells. However, the influence of CLIP on TBI-induced sex differences to chronic functional outcomes have not been previously examined. We hypothesized that CAP administration after TBI would improve TBI-induced neurobehavioral deficits and neuropathological outcomes. 10-week-old male and female C57bl/6J mice received either lateral fluid percussion injury (FPI) or Sham surgery, followed 30 minutes later by the administration of CAP or vehicle. Digigait was used to assess acute motor deficits after injury. Starting at 35 days post-FPI, all mice underwent neurobehavioral testing using the, novel object recognition test (NORT), object location test (OLT), and pattern separation test (PST). At the conclusion of behavioral testing sixty days post-FPI, harvested brains were analyzed for Iba1+ microglia, GFAP+ astrocytes, and DCX+ newborn neurons in the hippocampus. In both male and female mice, FPI induced cognitive impairment in NORT, OLT,

and PST that can be differentially improved by CLIP antagonism. Sex differences were also observed for neuroinflammatory outcomes and hippocampal neurogenesis following FPI that was altered by CLIP antagonism. Taken together, these findings support a potential pathological role for CLIP+ B cells after FPI and highlight the importance of evaluating sex as a biological variable (SABV).

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Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR163.01/D45

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CDMRP Grant W81XWH-18-1-0766

Title: Delayed intranasal insulin delivery improves outcome after moderate contusion spinal cord injury

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Abstract: Intranasal insulin (INI) has the ability to enter the central nervous system quickly without systemic effects; our previous studies have shown that INI increases glucose uptake and improves functional and behavioral outcomes after mild and moderate traumatic brain injury. Previously, we demonstrated that INI improved motor function after moderate contusion spinal cord injury (SCI) in adult and aged male Sprague Dawley rats. However, effects of INI administration in a clinically relevant window on a wider range of motor, sensory and autonomic function has not been explored. We therefore evaluated the effects of INI administered 7 days after injury on a panel of motor, sensory and autonomic functions after moderate contusion injury in young adult Sprague Dawley rats. These rats underwent a moderate spinal cord contusion injury at the T9 and received 7 daily intranasal administrations of saline or insulin (6IU) starting at 7 days post injury. Initial data shows that delaying INI to 7 days in young adult rats demonstrates a similar motor function improvement observed with acute (4 hours post-injury) treatment, as measured by the BBB score. In addition, improvement in penile dorsiflexion reflex toward baseline levels, suggesting an improvement in autonomic sexual function, was observed with INI administration. Finally, slight improvement in sensory function, with a small reduction in latency to tail flick response was noted in the INI group in comparison to the saline treated group. These data demonstrate that INI improves a wide range of motor, sensory and

autonomic function outcomes within a therapeutic window up to 7 days after SCI, and suggests that INI holds promise for translation in SCI therapy.

Disclosures: S. Nagrabski: None. **B. Smith:** None. **K. Byrnes:** None.

Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR163.02/D46

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH-NINDS (R21NS130241)
IND DEPT HLTH (55051, 74247, 74244)
US ARMY (HT94252310700; SC220152)

Title: Sexual dimorphism in the therapeutic effects of FDA approved sedative dexmedetomidine in treating rodent contusive spinal cord injury

Authors: *L. DENG[1], **A. KHABBAZ**[2], S. ZHANG[3], X. DU[4], S. CHAKRABORTY[5], K. COHEN[6], F. YUAN[7], Y. ZHANG[8], X. GAO[7];
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Abstract: Spinal cord injury (SCI) is a devastating health problem in the United States that has a catastrophic impact on tens of thousands of patients and their families, with no effective treatment available to date. The FDA-approved sedative Dexmedetomidine (Dex) has shown great neuroprotective potential in various traumatic CNS injuries, including SCI. However, considering sex as an important biological variable, whether the efficacy of Dex treatment differs between males and females in SCI needs to be verified. Young adult (8-10 weeks old) age-matched male and female C57 BL6 mice received a 60-kdyn impact using the Infinite Horizon system at T9 to generate moderate contusive SCI. One dose of Dex (100 μg/kg) was administered intraperitoneally for neuroprotection. Open-field Basso Mouse Scale (BMS), Rotarod, and Grid Walk behavioral tests were performed. Histological analysis was conducted at the endpoint of 6 weeks post-injury to measure male and female differences in motor function recovery and lesion size. SCI drastically compromised motor function in both sexes of mice. Female mice showed subtle but advantageous spontaneous functional recovery compared to the male mice. Dex treatment exerted significant neuroprotection in both genders; however, it displayed sex-based differences in improvement. In the BMS and grid walk tests, despite the male mice treated with Dex showing a higher rate of improvement compared with the untreated control, the treated female mice ended up with a higher score. Interestingly, some male mice with Dex treatment failed in the Rotarod test even though their BMS scores were over 5, while

many more female mice with Dex treatment completed the Rotarod test. Histological analysis also showed more preserved white and grey matter in the epicenter of female-treated mice than in male-treated mice. Our data recapitulated the sexual dimorphism in response to pharmacological therapy in SCI mice and highlight the importance of sex as a biological variable in experimental SCI studies.

Disclosures: L. Deng: None. **A. Khabbaz:** None. **S. Zhang:** None. **X. Du:** None. **S. Chakraborty:** None. **K. cohen:** None. **F. Yuan:** None. **Y. Zhang:** None. **X. gao:** None.

Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR163.03/D47

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant 1R01NS122371
Government of Canada's New Frontiers in Research Fund, Mend the Gap, No. NFRFT-2020-00238
National Natural Science Foundation of China No. 82201533

Title: Collagen I is a critical organizer of scarring and CNS regeneration failure

Authors: *Y. BI, W. DUAN, J. SILVER;
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Abstract: Although axotomized neurons retain the ability to initiate the formation of growth cones and attempt to regenerate after spinal cord injury, the scar area formed as a result of the lesion in most adult mammals contains a variety of reactive cells that elaborate multiple extracellular matrix and enzyme components that are not suitable for regrowth. Newly migrating axons in the vicinity of the scar utilize upregulated LAR family receptor protein tyrosine phosphatases, such as PTPσ, to associate with extracellular chondroitin sulphate proteoglycans (CSPGs), which have been discovered to tightly entrap the regrowing axon tip and transform it into a dystrophic non-growing endball. The scar is comprised of two compartments, one in the lesion penumbra, the glial scar, composed of reactive microglia, astrocytes and OPCs; and the other in the lesion epicenter, the fibrotic scar, which is made up of fibroblasts, pericytes, endothelial cells and inflammatory cells. While the fibrotic scar is known to be strongly inhibitory, even more so than the glial scar, the molecular determinants that curtail axon elongation through the injury core are largely uncharacterized. Here, we show that one sole member of the entire family of collagens (collagen I) creates an especially potent inducer of endball formation and regeneration failure. The inhibitory signaling is mediated by mechanosensitive ion channels and RhoA activation. Staggered systemic administration of two blood-brain barrier permeable-FDA approved drugs, aspirin and pirfenidone, reduced fibroblast incursion into the complete lesion and dramatically decreased collagen I, as well as CSPG

deposition which were accompanied by axonal growth and functional recovery. The anatomical substrate for robust axonal regeneration was provided by laminin producing $GFAP^+$ and $NG2^+$ bridging cells that spanned the wound. Our results reveal a collagen I-mechanotransduction axis that regulates axonal regrowth in spinal cord injury and raise a promising strategy for rapid clinical application.

Disclosures: Y. Bi: None. **W. Duan:** None. **J. Silver:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); J.S. is an advisor with NervGen Pharma who has licensed ISP from CWRU and are now undergoing a phase 2 clinical trial of a humanized version of ISP, NVG291 (ClinicalTrials.gov Identifier: NCT05965700).

Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR163.04/D48

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Merit Review Award # B3986-R/1 I01 RX003986-01A1, from the United States Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D)
Spinal Cord Injury Research Program (SCIRP) Investigator-Initiated Research Award # SC210266 from the United States Department of Defense (DoD)

Title: Experimental iron chelator therapy reduced signature spinal cord injury (SCI) motor disabilities in a rodent model: neurobiology of improvements

Authors: *P. BOSE[1,2,3], J. HOU[1,2], S. TSUDA[1,2], D. PLANT[1], G. DOOLEY[4], S. LULU[1], G. CHENG[1], G. A. VARGAS[4], J. BREINER[1], G. FABER[1], R. CARRASCOSA[1], K. S. KLIPPEL[4], N. M. WESTON[1], G. HWANG[4], R. J. BERGERON[5], F. J. THOMPSON[1,6];
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Abstract: Cervical spinal cord injury (C-SCI) has been a major component of injury in battlefields, sports, and accidents with lingering quality of life eroding disabilities such as spasticity and gait disability that produce substantial health care burdens. There are rapidly growing concerns that contusion injury-induced hemorrhagic “free iron” is a significant risk factor for lingering oxidative stress/inflammation and chronic SCI-disabilities. Acceleration/deceleration and contusion SCI cause micro-vessel shear injury, blood spinal cord

barrier (BSCB) dysfunction, and hemorrhage. Iron deposited by diffuse micro-hemorrhage fuels oxidative stress and inflammation through reactive oxygen species (ROS), which may further induce progressive disabilities. There is an urgent need to address both specific disabilities and risk factors for long-term progressive disabilities and to develop effective therapies that have excellent potential for translation. The current set of studies was performed to test the preclinical evaluation of the safety and efficacy of a new iron chelator, SP420, in a rodent model of contusion C-SCI. SP420 was administered SQ (66 mg/kg; represents the human phase II dose) in both acute and chronic time points and tested against saline placebo controls. Treatment was initiated 30 minutes after SCI and post-injury week 4 for two weeks, each using a separate cohort of animals. Quantitative physiological measures of spasticity, gait, and the integrity of axonal conduction of descending locomotor pathways functions were the primary outcomes, along with clinically relevant T1/T2W, SWI/QSM, and DTI MRIs. A comprehensive list of safety outcomes was applied during the treatment. A cause-effect relationship between iron deposition, tissue damage, and treatment effects of iron chelator was studied using a combination of histological and immunohistochemical assays to evaluate bleed iron, oxidative stress, inflammation, markers for BSCB integrity, and neural and vascular protective factors. Our data indicate that free bleed iron fuels oxidative stress and neuroinflammation through ROS, which, in part, drives the progression of neurological damage and motor disabilities. Our data also indicate that the SP-420 therapy reverses the iron-mediated neurological damage and delayed neurological sequelae in spasticity and gait disabilities in both acute and chronic SCIs. These data may provide innovative, non-invasive, and patient-centered technologies and treatments that will significantly facilitate the treatment of patients with SCI. Translation of this new, user-friendly therapeutic can significantly benefit veterans and civilians.

Disclosures: P. Bose: None. **J. Hou:** None. **S. Tsuda:** None. **D. Plant:** None. **G. Dooley:** None. **S. Lulu:** None. **G. Cheng:** None. **G.A. Vargas:** None. **J. Breiner:** None. **G. Faber:** None. **R. Carrascosa:** None. **K.S. Klippel:** None. **N.M. Weston:** None. **G. Hwang:** None. **R.J. Bergeron:** None. **F.J. Thompson:** None.

Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR163.05/D49

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS/NIH grant 5R01 NS111037-01

Title: Effect of Rolipram-loaded PgP Nanoparticles on Motor Function and Secondary Injury in Male and Female Rat Contusion SCI Model

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Abstract: Spinal cord injury (SCI) damages neurons and axons, leading to permanent motor, sensory, and autonomic dysfunction. Cyclic adenosine monophosphate (cAMP) is critical in all neural cell types and its sharp decline after SCI contributes to secondary injury through neuroinflammation, while limiting remyelination and neuronal survival / growth potential. In our lab, we developed an amphiphilic polymeric nanocarrier, poly (lactide-co-glycolide)-graft-polyethylenimine (PgP) for delivery of the phosphodiesterase inhibitor, rolipram (Rm). The objective of this study was to evaluate the effect of Rm-PgP on neuroprotection, motor function recovery and neuropathic pain after SCI in male and female rats. A moderate T9 contusion SCI model was generated and intrathecal catheters inserted at lumbar level (L4-5). Rats were divided into 4 groups: 1) sham, 2) untreated SCI (saline, 40 µl), 3) Rm-PgP-S : Rm-PgP (20 µg Rm, 40 µl) single injection immediately post-injury (time 0), 4) Rm-PgP-R :Rm-PgP (20 µg Rm, 40 µl) repeated injection at 0, 2 and 4 days post-injury (DPI) for male and 0 and 7 DPI for female. Rm-PgP or saline was injected using microinjection pump with Hamilton syringe (28G) via intrathecal catheter. Motor function and neuropathic pain were evaluated using BBB scoring system and von Frey test, respectively. At 6 weeks post-injury (WPI), rats were sacrificed via cardiac perfusion and the spinal cords retrieved for histological analysis. In separate studies, animals were sacrificed at 1WPI (male) and 2 WPI (female), fresh spinal cords harvested, and cAMP levels measured by ELISA. cAMP levels in Rm-PgP treatment groups were significantly higher than that in untreated SCI group in both male and female rats. Rm-PgP single treatment group showed significantly higher BBB scores compared to untreated SCI group in both male and female rats. For neuropathic pain, Rm-PgP treatment groups showed significantly lower pain levels than that in untreated SCI group in both male and female rats. Interestingly, Rm-PgP-S treatment group showed slightly higher motor function and lower neuropathic pain compared to Rm-PgP-R treatment group even though it was not significantly different in both male and female rats. We also observed that Rm-PgP treatment groups showed significantly increased spared myelin area, increased number of $NeuN^+$ cells and reduced fluorescence intensity from $GFAP^+$ activated astrocytes compared to untreated SCI group in both male and female rats. In conclusion, Rm-PgP reduced secondary injury and improved motor functional recovery in both male and female animals after contusion SCI.

Disclosures: Z. Liao: None. **C.E. Jones:** None. **B. Elliott:** None. **M.R. Detloff:** None. **K. Webb:** None. **J. Lee:** None.

Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR163.06/D50

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH 5R21NS115094-02

Title: Endogenous Acrolein Scavenging as a Novel Neuroprotective Strategy After Spinal Cord Injury

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Abstract: Spinal cord injuries (SCI) result in a slew of secondary biochemical reactions exacerbate the degree and scope of the initial mechanical trauma. Acrolein, a highly toxic aldehyde generated from the oxidative stress-associated lipid peroxidation has emerged as a key mediator in the secondary injury that contributing significantly to the neurological deficits. A key endogenous oxidoreductase, mitochondrial aldehyde dehydrogenase-2 (ALDH2), is known to detoxify acrolein, and therefore play a critical role in acrolein-mediated pathology. Using a novel transgenic (TG) ALDH2*2 deficiency mouse model that recapitulates a clinical genetic condition in 600 million people worldwide, and a recently discovered ALDH2 activator, we planned to assess the acrolein-clearing and neuroprotective role of ALDH2 after SCI through different pathological aspects including inflammation infiltration, tissue preservation and axon degeneration.Following a thoracic contusion SCI, the ALDH2*2 TG mice showed an exaggerated ascension of the acrolein level when compared to the wildtype (WT) mice at 2 to 28 days post-injury. In addition, the level of proinflammatory cytokines and macrophage/microglia activation are also significantly higher in the ALDH2 deficiency mice group compared to WT at 1 week post injury. The application of ALDH2 activator rescued the enzymatic activity in the spinal cord, mitigated the post-SCI acrolein elevation in the spinal cord. Accordingly, the pro-inflammatory response, lesion area, neuron loss and demyelination in the spinal cord in the group received ALDH2 activator was significantly decreased compared to the ones without treatment at 7 and 28 days. Behavior tests assessing both locomotor and sensory function revealed a significant better recovery at 4 weeks following SCI in both TG and WT groups that received ALDH2 activator compared to untreated groups. This study has further demonstrated acrolein as a critical player in the pathogenesis of SCI, as well as an effective therapeutic target to enhance recovery in SCI. Furthermore, our data also supports the role of ALDH2 as a potential target for potentiating endogenous anti-acrolein capability through pharmaceutical intervention in SCI and other neurotrauma and degenerative diseases where acrolein is known to play a role.

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Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR163.07/

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Cox2 expression plays a role in spinal cord injury induced neuropathic pain

Authors: *M. TOI[1,6], T. TACHIBANA[2], H. YAMANAKA[3], K. KISHIMA[4], M. OKUBO[7], Y. T. DAI[8], K. NOGUCHI[5];

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Abstract: Although it has been reported that cyclooxygenase 2 (COX2) is involved in neuropathic pain (NeP) in rat models of peripheral nerve injury and that COX2 inhibitors can alleviate NeP, these mechanisms after spinal cord injury (SCI) have not been fully investigated.Purpose:The purpose is to investigate whether the thoracic SCI affects the expression of mRNAs for COX1 and COX2 in the lumbar spinal cord, and the effect of COX2 inhibitor on its behavior.Study Design:Male Sprague-Dawley (SD) rats underwent thoracic (T10) spinal cord contusion injury using an Infinite Horizon (IH) impactor device. SCI rats received COX2 inhibitors (50 μg/day) on days 5 and 6 after SCI.Methods:Male SD rats underwent T10 laminectomy under mixed anesthesia, and IH impactors were applied to the same site to create a rat SCI model. Rats that underwent only laminectomy were designated as sham. Lumbar spinal cord at the L4-5 level was harvested at 3, 5, 7, 14, and 28 days after SCI, and COX2 and COX1 were quantified by reverse-transcription PCR (RT-PCR). COX2 expression, expression site, and expression time were determined by immunohistochemistry (IHC) and *in situ* hybridization histochemistry (ISH) at the same time points. The expression site and time of COX2 expression were also examined at the same time point by ISH. On 5th and 6th day after SCI, saline and COX2 inhibitor (50 μg/day) were administered into the subarachnoid space as a single dose, and the two groups were compared in terms of mechanical withdrawal latency using the dynamic plantar esthesiometer, which is an automated von Frey-type system.Results:COX2 was significantly increased at 5 and 7 days after SCI, but no significant difference in COX1 was observed after SCI. ISH targeting COX2 showed clear expression of COX2 in spinal cord vascular endothelial cells at 5 and 7 days after SCI. COX2 expression was almost abolished at days 14 and 28. Behavioral experiments showed that pain was significantly improved from day 2 after COX2 inhibitor administration compared to the saline group, with improvement up to day 14 after SCI, but no significant difference was observed after day 21.Conclusions:The present findings suggest that thoracic SCI increased COX2 in vascular endothelial cells in the lumbar spinal cord and that the administration of COX2 inhibitor significantly alleviated mechanical hypersensitivity of the hind-paw following the thoracic SCI. Therefore, endothelial cell derived COX2 in the lumbar spinal cord may be involved in the induction of neuropathic pain in the SCI model rats.

Disclosures: M. Toi: None. **T. Tachibana:** None. **H. Yamanaka:** None. **K. Kishima:** None. **M. Okubo:** None. **Y.T. Dai:** None. **K. Noguchi:** None.

Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR163.08/D51

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant R01 NS120877
Mayo Clinic Center for Regenerative Biotherapeutics
Michael S. and Mary Sue Shannon Foundation
Mayo Clinic Center for Biomedical Discovery

Title: Delayed Statin Treatment Enhances Recovery from Spinal Cord Injury

Authors: *S. BUCHL[1], H. KIM[5], B. HUR[2], W. SIMON[3], M. R. LANGLEY[4], J. SUNG[2], I. A. SCARISBRICK[4];
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Abstract: Background:Spinal cord injury (SCI) significantly disrupts spinal cord gene expression, potentially impeding recovery. This study evaluated the impact of a common cholesterol-lowering statin on gene expression and functional recovery in a chronic SCI mouse model.
Methods:Female C57BL/6 mice (8 weeks old) underwent moderate (0.25 mm lateral compression) SCI. Starting two weeks post-injury, mice received daily intraperitoneal injections of either a statin dissolved in DMSO or DMSO alone for four weeks. Recovery was assessed using the Basso Mouse Scale (BMS), BMS Sub-score, and Inclined Plane Test. Spinal cord tissues were analyzed via bulk RNA-seq, with differential expression analyzed using DESeq2 and pathway enrichment via Ingenuity Pathway Analysis and PANTHER Gene Ontology.
Results:Statin treatment significantly improved outcomes in the BMS and Inclined Plane tests (ANOVA $p < 0.001$). RNA-seq revealed 3509 genes consistently altered by SCI ($\Delta > 25\%$ vs. uninjured controls, FDR < 0.05), with 13 genes significantly affected by statin treatment compared to control ($p < 0.05$). Pathway analysis highlighted crucial roles of statin-modulated genes in fatty acid transport and axon guidance ($p < 0.05$), suggesting mechanisms for neuroregeneration.
Conclusion:Statin administration two weeks post-SCI enhances sensorimotor recovery and activates neuroregenerative gene programs, supporting further research into statins as a potential therapy for chronic SCI in humans.

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Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR163.09/D52

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation, grant # 890901, to EG

Title: The effect of regional application of 17-B estradiol prior to thoracic Spinal Cord Injury (SCI) in ovariectomized rats

Authors: *K. N. COLPITTS[1], T. JOHNSTON[3], R. S. ELEJALDE[2], S. PATIL[2], A. AWALT[2], K. SANCHEZ[4], C. GIBSON[4], E. GILES[4], J. W. GRAU[2];
[1]Inst. for Neurosci., [2]Texas A&M Univ., College Station, TX; [3]McGovern Med. Sch. at UTHealth Houston, Houston, TX; [4]Univ. of Michigan, Ann Arbor, MI

Abstract: Previous work has shown that nociceptive stimulation after injury promotes a breakdown of the blood spinal cord barrier (BSCB), increases hemorrhage, and undermines locomotor performance in male rats (Grau et al., 2017, J Neurotrauma, 34, 1873). It has been shown that female animals demonstrate a protective effect after SCI in comparison to male animals. Baine et al. (2022, Neurotrauma Rep, 3.1) showed that estrous cycle may be implicated; long-term locomotor recovery was worse when female animals were injured during the estrous stage compared to other stages. To elucidate the role of hormones in recovery after SCI we have tested systemic application of estradiol and progesterone prior to injury and/or prior to pain in ovariectomized rats. Animals were given a minimum of 3 weeks to recover from ovariectomy procedures and then were randomized to receive drug (estradiol or progesterone) or vehicle intraperitoneal injections prior to a moderate T12 contusion injury or prior to pain. One day after injury animals received 6 minutes of noxious, variable intermittent tail shock or the control procedure. We found that estradiol, but not progesterone, given prior to injury attenuated the shock induced locomotor deficit in the acute phase. Treatment prior to pain input did not attenuate the effect. In the present study we used the same design to test the local application of estradiol via intrathecal injection prior to injury or pain. The result suggests that regional application of estradiol prior to SCI does not attenuate the shock induced locomotor deficit. Ongoing work is exploring whether a clinically applicable injection regime, wherein estradiol is given an hour after injury, has a protective effect. For all experiments, we have also collected tissue from the site of the injury to assess the extent of hemorrhage in these animals via western blotting.

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Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR163.10/D53

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H Neilsen Foundation Grant 1000927

Title: Protecting the Injury Spinal Cord: Application of a Local Anesthetic (Bupivacaine) Reduces Secondary Injury

Authors: *N. KOBAYASHI[1], C. T. PHINNEY[1], J. DAVIS[2], J. W. GRAU[1];
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Abstract: Prior work has shown that engaging pain fibers a day after a thoracic spinal cord injury (SCI) impairs long-term recovery in rats. These effects have been related to increased hemorrhage and tissue loss at the site of injury, which has been linked to the breakdown of the blood spinal cord barrier (BSCB). Placing animals in a state akin to a medically-induced coma using the general anesthetic pentobarbital reduced hemorrhage after injury (Davis, 2023, J Neurotrauma, 40). Prior research has also shown that local application of an anesthetic (lidocaine) at the site of injury blocks the adverse effect of nociceptive stimulation (Turtle, 2017, J Neurotrauma, 34). The present experiment examined whether local (regional) anesthesia protects the injured spinal cord when applied soon after injury, in the absence of additional pain. Male rats received a moderate thoracic (T10-11) contusion injury and were fitted with an intrathecal catheter for drug infusion. The anesthetic bupivacaine was selected for the drug of interest because it is widely used in the clinic and has a longer duration of action than lidocaine. Bupivacaine can also dampen pain signals without loss of consciousness, providing a clinical benefit over general anesthesia. Given that secondary processes can begin within minutes following the primary injury, injections were administered as soon as possible after the contusion injury. Within five min after injury, animals received 30 µl of 0.75% bupivacaine or it's saline vehicle, followed by 10 µl of saline to flush the catheter. Separate groups then received 0, 1, 3, or 7 additional injections at 90 min intervals. This yielded four treatment conditions: 1.5, 3, 6, or 12 hrs of local anesthesia. A day after injury, hindleg locomotor performance was assessed using the BBB scale and a 1 cm region of the spinal cord encompassing the area of injury was taken and assayed for hemorrhage using spectrophotometry and the Drabkin's assay. Western blots were then run to measure hemoglobin levels in each sample by combining the relative amount of monomeric, dimeric, and trimeric forms of α-hemoglobin. Animals that received bupivacaine for 3-12 hrs exhibited improved locomotor performance and had less hemorrhage at the site of injury. Further work is being conducted to determine the effect of bupivacaine treatment on indices of inflammation and cell death. We are also examining whether bupivacaine has a beneficial effect when treatment is initiated 0.5-3 hrs after injury.

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Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR163.11/D54

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS R01NS110385
NINDS R01NS079702

Title: Pten inhibition promotes robust growth of bulbospinal respiratory axons and partial recovery of diaphragm function in a chronic model of cervical contusion spinal cord injury

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Abstract: High spinal cord injury (SCI) leads to persistent and debilitating compromise in respiratory function. Cervical SCI not only causes the death of phrenic motor neurons (PhMNs) that innervate the diaphragm, but also damages descending respiratory pathways originating in the rostral ventral respiratory group (rVRG) located in the brainstem, resulting in denervation and consequent silencing of spared PhMNs located caudal to injury. It is imperative to determine whether interventions targeting rVRG axon growth and respiratory neural circuit reconnection are efficacious in chronic cervical contusion SCI, given that the vast majority of individuals are chronically-injured and most cases of SCI involve contusion-type damage to the cervical region. We therefore employed a clinically-relevant rat model of chronic cervical hemicontusion to test therapeutic manipulations aimed at reconstructing damaged rVRG-PhMN-diaphragm circuitry to achieve recovery of respiratory function. At a chronic time point post-injury, we systemically administered: an antagonist peptide directed against phosphatase and tensin homolog (PTEN), a central inhibitor of neuron-intrinsic axon growth potential; an antagonist peptide directed against receptor-type protein tyrosine phosphatase sigma (PTPσ), another important negative regulator of axon growth capacity; or a combination of these two peptides. PTEN antagonist peptide (PAP4) promoted partial recovery of diaphragm motor activity out to nine months post-injury, while PTPσ peptide did not impact diaphragm function after cervical SCI. Furthermore, PAP4 promoted robust growth of descending bulbospinal rVRG axons caudal to the injury within the denervated portion of the PhMN pool, while PTPσ peptide did not affect rVRG axon growth at this location that is critical to control of diaphragmatic respiratory function. In conclusion, we find that, when PTEN inhibition is targeted at a chronic time point following cervical contusion that is most relevant to the SCI clinical population, our non-invasive PAP4 strategy can successfully promote significant regrowth of damaged respiratory neural circuitry and also partial recovery of diaphragm motor function.

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Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

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Program #/Poster #: PSTR163.12/D55

Topic: C.11. Spinal Cord Injury and Plasticity

Support: R01EY024575
R01NS105961-01
R01NS079432

Title: Developing selective peptides against non-muscle myosin II for regenerating injured optic nerve axons in adult mammals

Authors: *S. WANG[1,2], S. LI[3,1];
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Abstract: Currently, there is no cure for patients with CNS axon injury and it is essential to develop highly effective therapeutic strategies for regenerating lesioned CNS axons, including the retinal ganglion cell axons. Recent transgenic studies demonstrate that non-muscle myosin II A/B, the cytoskeletal proteins presented in the axons, significantly suppress the elongation of injured optic nerve axons in adult mice. Transgenic deletion of these proteins in conditional knockout mice dramatically stimulates axon regeneration of crushed optic nerves. The major goal of this project is to develop post-injury deliverable therapeutic reagents for promoting robust axon regeneration following CNS injuries, including optic nerve crush. We designed six antagonist peptides for non-muscle myosin II A/B (NM IIA/B) by targeting the critical activity domains of these proteins selectively. Pre- or post-injury intravitreal treatments with our selective peptides stimulated robust axon regeneration in wildtype mice with optic nerve crush. Impressively, two of our peptides almost mimicked the effects of transgenic deletion of NM IIA/B or PTEN. PTEN suppression has been frequently used to promote CNS regeneration. Also, our peptide treatments significantly increased the survival of retinal ganglion cells after optic nerve injury. Furthermore, NM IIA/B suppression combined with the inhibition of Let7 signaling by AAV vectors showed synergistic actions in promoting the regeneration of axotomized optic nerve axons in adult mice. Therefore, this project may facilitate the development of effective regenerative treatments for lesions to neural visual pathways and other neurological disorders.

Disclosures: S. Wang: None. **S. Li:** None.

Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR163.13/D56

Topic: C.11. Spinal Cord Injury and Plasticity

Support: GR023063

Title: Inhibition of the fibrotic scar to promote axon regeneration following SCI

Authors: *E. MCGINN[1], K. JEFFRIS[2], S. M. WHEELER[3], O. SEIRA[4], F. ROSSI[2], W. TETZLAFF[5];
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Abstract: Introduction: The fibrotic scar appears to be part of the barrier to axon regeneration following spinal cord injury (SCI). Cytokine release from immune cells triggers proliferation and migration of fibroblasts to the lesion, where they express extracellular matrix proteins and CSPGs that may be inhibitory to axonal regeneration. Primary signaling pathways responsible for the activation of fibroblasts include TGF-b, FGF, and PDGF. These pathways can be inhibited by drugs that are FDA approved to treat fibrotic conditions in humans. Some of these drugs inhibit the TGF-b pathway while others inhibit the PDGF and FGF pathways. Given that these activation pathways are conserved throughout the body, we aimed to assess the effectiveness of these drugs to reduce fibrotic scarring in the spinal cord after injury. **Methods:** A T9/10 complete spinal cord crush injury was administered to C57Bl/6 mice and 24 hours after injury, treatment with antifibrotic drugs alone or in combination was started. Control animals in this experiment received vehicle solutions used for these drugs in equivalent volumes. After 14 days of treatment, the animals were euthanized, and histology is currently underway to evaluate fibrotic scarring and axon regeneration. Our analysis will include immunofluorescence for PDGFRβ, CSPGs and ECM proteins as well as GFAP and various axonal populations.**Significance:** Our studies will contribute to a further assessment of fibrotic scarring as a barrier to axon regeneration. By using FDA approved drugs such treatment could be readily translated to the clinic and possibly combined with other SCI repair strategies.

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Poster

PSTR163: Spinal Cord Injury: Therapeutic Strategies: Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR163.14/D57

Topic: C.11. Spinal Cord Injury and Plasticity

Support: FAPESP # 2021/02754-9
FAPESP # 2018/05006-0

Title: Rescue of avulsed spinal motoneurons with N, N-dimethyltryptamine (DMT) and fibroblast growth factor 2 (FGF-2) following ventral root repair

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Abstract: Brachial plexus injuries result in extensive retrograde degeneration of spinal motoneurons (MN), significantly reducing the prospects for functional recovery. In turn, the combination of root repair and neuroprotective pharmacological strategies needs to be developed. Thus, we investigated the effects of combining N, N-dimethyltryptamine (DMT) and Fibroblast Growth Factor 2 (FGF-2, thermostable, Core Biogenesis FGF-2 STAB®) therapy following ventral root avulsion (VRA) and reimplantation with a heterologous fibrin biopolymer (VRR). Adult female Lewis rats were subjected to unilateral L4-L6 rhizotomy and were divided into five groups (N=5/group): VRA and VRR alone, VRR+DMT (1mg/kg, i.p., daily for 2 weeks), VRR+FGF-2 (2 μg/ml, locally at the site of repair), and VRR+DMT+FGF-2. Lumbar spinal cords were collected two (acute phase) and twelve weeks (chronic phase) after surgery for MN survival (Nissl staining) and immunofluorescence analysis (synaptophysin, GFAP, and IBA-1). Behavioral (walking track test - CatWalk system) and electroneuromyography (ENMG) tests were performed throughout the chronic phase experiment. All procedures were approved by the Institutional Committee for Ethics in Animal Use (CEUA/IB/UNICAMP/Brazil, protocol number 5921-1/2021). The two-week analysis of MN survival following VRA revealed that 89% of MNs had died, accompanied by a markedly increased reactivity of glia and synaptic detachment. It is noteworthy that both the DMT and the FGF-2 demonstrated a significant increase in the survival of the MN, from 22.97 ± 0.2% (VRA group) to 77.11 ± 0.62% ($p=0.0001$) and 73.47 ± 0.14% ($p=0.0001$), respectively. Moreover, when the DMT and the FGF-2 were employed in conjunction, 76.94 ± 0.03% of the MNs survived. The rescue of axotomized MNs was accompanied by a significant downregulation of the glial reaction markers, which may have positively influenced the preservation of presynaptic inputs found in the spinal cord ventral horn (lamina IX of Rexed). Of note, the long-term functional recovery demonstrated a significant improvement both in gait parameters as well as nerve conduction amplitude, latency, and duration. Overall, our findings indicate that both DMT and FGF-2 are promising therapeutic approaches to treat CNS/PNS interface injuries.

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Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.01/D58

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation Grant 647897
National Institute of Health R01 Grant NS119475
National Institute of Health T32 Grant NS121768

Title: The Effects of Transcutaneous Spinal Cord Stimulation on Spasticity and Inhibitory Circuitry in Rats when Initiated in the Acute vs Chronic Phase of Spinal Cord Injury

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Abstract: 75% of individuals with spinal cord injury (SCI) develop spasticity. However, current treatment options are limited and often have a deleterious effect on motor function. Transcutaneous spinal cord stimulation (tSCS) has emerged as a promising treatment for spasticity in recent clinical studies, using motor activation rather than depression to treat spasticity. We have previously demonstrated the efficacy of repeated tSCS sessions initiated in the acute phase of SCI in preventing the development of spasticity in rats, but how repeated tSCS may affect spasticity once it has been established after SCI is unclear. This study aims to determine the efficacy of tSCS in preventing and improving spasticity following SCI and identify potential mechanisms through which tSCS may be acting to preserve/restore spinal inhibition and prevent/improve spasticity.
Following a severe (250 kDyn) T9 contusion injury, adult female Sprague-Dawley rats (N=41) were randomly assigned to an experimental group (acute SCI + tSCS or chronic SCI + tSCS) or control group (acute SCI + sham stimulation or chronic SCI + sham stimulation). Intact rats (N=8) were also used for comparison. Stimulation sessions began either before spasticity had developed fully (5 days post-injury/acute phase of SCI) or after spasticity had developed (4 weeks post-injury/chronic phase of SCI). Sessions lasted 18 min/day, 5 days/week for 6 weeks. Frequency-dependent depression and presynaptic inhibition of the H-reflex were assessed terminally to evaluate hyperreflexia and restoration of spinal inhibition. Furthermore, tissue from the lumbar enlargement was collected and immunohistochemistry was performed to assess the rearrangement of $GAD65^+$ on $VGlut1^+$ afferents which could underlie the improvement of presynaptic inhibition. Membrane expression of KCC2, a protein that plays a vital role in maintaining chloride homeostasis for efficient inhibitory signaling, was also quantified. Our data indicates that repeated tSCS initiated in the acute or chronic phase of SCI increases frequency-dependent depression and motoneuronal membrane expression of KCC2 toward intact levels. Furthermore, tSCS initiated in the chronic but not the acute phase of SCI can restore primary afferent depolarization-mediated presynaptic inhibition of the H-reflex to intact levels and increase the number of contacts between $GAD65^+$ and $VGlut1^+$ terminals. These findings suggest that tSCS initiated in either the acute or chronic phase of injury can improve spasticity and spinal inhibition, however circuit-related plasticity due to tSCS may have an intervention timepoint dependent effect.

Disclosures: N. Yakas: None. **D.C. Malloy:** None. **C. Buhalo:** None. **M. Côté:** None.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

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Program #/Poster #: PSTR164.02/D59

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant NS119475

Title: How to combine transcutaneous stimulation and step-training to maximize the recovery of spasticity and locomotor function after SCI

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Abstract: Following spinal cord injury (SCI), about 75% of patients develop spasticity. Spasticity is a condition involving pathological motor behaviors including heightened muscle tension, involuntary movements, and hyperactive reflexes. Activity-based therapies, including step-training, are commonly used in the clinic to restore function. However, hyperactive reflexes and involuntary movements due to spasticity impede rehabilitation. Current pharmacological treatments effectively treat spasticity but cause side-effects that further impede residual motor function such as reductions in muscle activity and muscle weakness. Transcutaneous spinal cord stimulation (tSCS) is a non-invasive treatment that can diminish spasticity after SCI and contribute to functional recovery through neuroplastic changes without depressing motor function. tSCS decreases spasticity and hyperreflexia acutely up to 2 hours post-treatment and contributes to long-term spinal plasticity when repeated over multiple sessions. By decreasing spasticity with tSCS immediately before step-training, we hypothesize that step-training will further improve locomotor recovery. Adult female Sprague-Dawley rats received a severe T9 contusion injury (250 kdyn) and were randomly assigned to a treatment group: tSCS, sham stimulation, tSCS followed by step-training, or sham stimulation followed by step-training. For 6 weeks starting 5d post-SCI, rats receive 18 minutes of tSCS or sham stimulation, with or without a subsequent 10 minutes of step-training. A terminal experiment revealed that whether stimulation and step-training were delivered in isolation or in combination, frequency-dependent depression and presynaptic inhibition were restored. We further performed kinematic recordings during treadmill walking for analysis of locomotor function. Preceding the step-training session with tSCS further improved locomotor recovery as compared to tSCS or step-training alone. Together, our results suggest that tSCS and step-training aid in reducing symptoms of spasticity, and tSCS treatment delivered immediately before step-training improves functional recovery better than step-training alone.

Disclosures: T. Gray: None. **M. Côté:** None.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.03/D60

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Department of Defense SC210210

Title: Attenuation of SCI-induced chronic pain by peripheral blood derived recombinant hiPSCs chromaffin cells releasing serine-histogranin and endomorphin grafted with silicone-based matrix support

Authors: *B. RAHIMI[1], S. JERGOVA[1], J. LEE[2], A. EESWARA[1], D. A. ABDENNOUR[1], H. CUKIER[1], J. SAGEN[1];
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Abstract: Spinal cord injury (SCI) pain reduces quality of life and hampers rehabilitation participation, necessitating new pain management approaches. Highly secretory chromaffin cells synthesizing analgesic substances are potential candidates for new pain interventions. The induced pluripotent stem cell (iPSC) technology enables derivation of chromaffin cells (hCC) from recipients themselves, eliminating the need for immunosuppression. Using gene engineering, we have previously generated recombinant hiPSCs derived chromaffin cells releasing NMDA antagonist serine-histogranin (SHG) or a combination of SHG with opioid peptide endomorphin 1 (EM1) and demonstrated their analgesic potency in SCI pain model. This study aims to analyze the effect of different SHG/EM gene combinations in recombinant cells and to define the most promising for clinical transition. In addition, to enhance survival of the cells, limit their diffusion from grafted sites, and possibly to reduce immune response, a silicone-based organic polymer polydimethylsiloxane (PDMS) was used to engineer 3D-printed matrix and tested to serve as a cell-infused implant. Cell lines from different donors were engineered as 1SHG, 6SHG, 1SHG/EM and 6SHG/EM recombinant cells and the phenotype was confirmed by FLISA and immunostaining. 3D matrix was engineered in layers with specific design of channels for cell seeding. Cells were seeded with differently designed patterns of the matrix and their survival and release of catecholamines were evaluated in vitro. Analgesic effects of selected naïve and recombinant cell lines were evaluated in Sprague Dawley rats with the clip compression SCI model. Cells were grafted 3-4weeks post injury and hypersensitivity to tactile, cold, and heat stimuli was assessed weekly. The recombinant peptides were detected in culture media from all recombinant cells, with differences in the amount of SHG related to 1SHG/6SHG format of the gene. Cells seeded with PDMS patterns displayed relatively stable survival rate and catecholamine production with no major differences between patterns. Some differences were observed related to donor age groups. Animals receiving intrathecal grafts of both non-recombinant and recombinant cells displayed a gradual reduction in SCI pain hypersensitivity, with effects partially mitigated by the injection of specific antibodies to SHG or EM, suggesting the involvement of the peptides in pain alleviation. These results suggest that peripheral blood-derived hCCs are a promising source for generating chromaffin cells to alleviate SCI pain, with the potential for further enhancement through the release of additional analgesic peptides.

Disclosures: B. Rahimi: None. **S. Jergova:** Other; The University of Miami and J.S. and S.J. hold rights to intellectual property used in the study and may financially benefit from the commercialization of the intellectual property. **J. Lee:** None. **A. Eeswara:** None. **D.A. Abdennour:** None. **H. Cukier:** None. **J. Sagen:** Other; The University of Miami and J.S. and S.J. hold rights to intellectual property used in the study and may financially benefit from the commercialization of the intellectual property.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.04/E1

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CIHR

Title: Characterizing Inducible Oligodendrogenically-Biased Neural Progenitor Cells in Spinal Cord Injury

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Abstract: Spinal cord injury (SCI) is associated with the loss of oligodendrocytes, which play key roles in myelination and in modulating interactions between glia and neurons. Neural progenitor cells (NPCs) are a promising source of cells for SCI treatment, due to their ability to replace the lost oligodendrocytes, neurons and astrocytes. However, the differentiation of NPCs into oligodendrocytes is often inefficient, whereby the majority of the cells differentiate into astrocytes following transplantation. Therefore, we aimed to enhance oligodendrocyte differentiation by generating an inducible oligodendrogenic NPCs (ioNPCs) in which the extent of oligodendrocyte differentiation could be carefully regulated. Human ioNPCs were prepared by engineering NPCs to express Olig2 under the control of the conditional doxycycline-inducible tet-ON promoter, in which doxycycline administration regulates Olig2 expression. The cells were characterized in vitro using a combination of qRT-PCR analysis, immunostaining, and bulk RNA sequencing, which confirmed that the ioNPCs had higher expression of oligodendroglial lineage genes, including OLIG1, OLIG2 and PDGFRA, and differentiated into a greater proportion of O1+ oligodendrocytes (39.44 ± 16.5%) compared to NPCs (24.73 ± 6.5%). To assess the cells in vivo, athymic Rowett nude rats were subjected to a 23 g cervical clip-compression injury and half were transplanted with 2 x 10^6 ioNPCs one week after the injury. After eight weeks the rats were sacrificed, RNA was isolated from the spinal cord and bulk RNA sequencing was performed. Preliminary RNA sequencing analyses suggest that a total of 181 genes were differentially expressed following ioNPC transplantation, including several genes related to extracellular matrix proteins, cell substrate adhesion and detoxification. In conclusion, our study aims to assess the therapeutic potential of ioNPCs in cervical SCI.

Disclosures: K. Pieczonka: None. **M.G. Fehlings:** None.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

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Program #/Poster #: PSTR164.05/E2

Topic: C.11. Spinal Cord Injury and Plasticity

Support: EU FLAG-ERA JTC 2021 (RESCUEGRAPH)
H2020 GRAPHENE FLAGSHIP CORE 2
TÜBİTAK 221N399

Title: Effects of transcutaneous electrical nerve stimulation on rats with spinal cord injury

Authors: B. Y. KARAHARMAN[1], P. KURU BEKTASOGLU[2], P. INCE[1], M. KAHRAMAN[3], Z. OZDEMIR KUMRAL[3], B. YEGEN[3], ***B. GUCLU**[1];
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Abstract: Several alternative strategies of neuromodulation (NM) therapy have been reported in the literature to help with some of the sensorimotor losses in spinal cord injury (SCI). In this preliminary study, we tested the bilateral electrical stimulation of the peripheral tibial nerve transcutaneously (pulse width: 0.3 ms, pulse frequency: 2 Hz, amplitude: 2 × motor threshold, duration: 30 min. at 3 days/week) and studied its effects during recovery of rats with SCI. The study consisted of sham (n=9), SCI (n=13), NM-sham (n=6), and NM-SCI (n=14) groups with rats terminated at different endpoints (D1, D7, D14, M1, M1.5, M2) post-op (T8-T9 laminectomy). Contusion-type SCI was induced with a computer-controlled custom-made impactor device (contactor diameter: 2.3 mm, indentation peak force: 0.9-1 N, peak displacement: 1.5-1.75 mm, duration: 0.3-0.5 s) to produce moderate injury as observed with the Basso, Beattie and Bresnahan (BBB) locomotor rating scale. High-speed (120 fps) video recordings of rats walking on a platform were obtained pre-op and during recovery for BBB scoring and gait analyses. Hind and fore paws were tracked by using DeepLabCut software and gait parameters (stance/swing/stride durations, limb duty factor, stride length, footprint area) were extracted in Matlab after detrending for speed effects. SCI caused a significant decrease in the BBB scores (0-7 at D1) as expected, and the SCI group improved to much higher scores (10-20) after 1 month. NM-SCI group, however, resulted in significantly lower BBB scores than the SCI group during recovery ($p<0.001$) and reached to a score range of 1-21 after 1 month. On the other hand, stride length ($p=0.004$) and stride duration ($p=0.011$) developed better in the NM-SCI group compared to the SCI group for the hind limbs. This shows the positive effect of NM once the steps are established. Overall, our preliminary results suggest that non-specific peripheral NM may not be beneficial during the recovery of SCI if the stimulation is not synchronized with locomotor activity. As such, functional electrical stimulation strategies combined with movement therapy may be better in that respect.

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Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.06/E3

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation SCIRTS 890307

Title: Skin-derived recombinant hiPSC chromaffin cells releasing MVIIA-serine-histogranin construct grafted with enhanced PEG biomatrix attenuates chronic SCI pain

Authors: *J. SAGEN[1], B. RAHIMI[1], B. MARIN[2], I. COZZONE[2], A. EESWARA[1], D. A. ABDENNOUR[1], C. DUMONT[2], S. JERGOVA[1];
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Abstract: Pain management post spinal cord injury (SCI) presents a significant clinical challenge, necessitating the exploration of novel therapeutic approaches. The transplantation of chromaffin cells into the lumbar spinal subarachnoid space has demonstrated substantial alleviation of chronic pain in various preclinical rat models. Enhancing their pain-alleviating efficacy, these cells could be engineered to produce additional analgesic neuroactive substances. Our previous findings indicated the efficacy of the N-type Ca2+ channel blocker omega conopeptide MVIIA (known as Prialt) and NMDA antagonist serine-histogranin (SHG) in various pain models. The aim of this study is to assess and refine parameters to produce recombinant human chromaffin cells (hCC) from human fibroblast-derived iPSCs and to access their analgesic potential in SCI pain model. To achieve more targeted effects of the graft within subarachnoid space, we explored gel biomatrices based on FDA-approved polyethylene glycol (PEG) to increase transplant cell stability, vascularization, and local adherence at desired spinal levels. Maleimide functionalized PEG microspheres were developed and tested as supportive biomatrix for hiPSC chromaffin cells in vitro and as an anchoring biomatrix for grafting in an SCI model. Sprague Dawley rats were subjected to SCI clip compression injury. Four weeks post-injury, age-matched naïve and recombinant cell lines suspended either in media or PEG matrix were intrathecally injected into the animals. Weekly assessments were conducted to evaluate tactile, cold, and heat hypersensitivity. All cell lines were successfully engineered into recombinant cells and the phenotype and the presence of recombinant peptides SHG and MVIIA in cells and media was detected by immunostaining and FLISA. Selected cell lines were evaluated in vitro with PEG biomatrix to establish the composition of the material most supportive for cell growth. The same composition was prepared and used for intrathecal injection for naive and recombinant cells with PEG matrix in the SCI model. Behavioral tests showed attenuation of tactile, cold and heat hypersensitivity in animals treated with cells in PEG compared to PEG only treatment with stronger effects observed for recombinant cells, especially in MVIIA and MVIIA-SHG groups. The presence of grafted cells and recombinant peptides was confirmed in the spinal tissue. These findings showed the promise of using PEG as a biomatrix for intrathecal cell grafting and further indicated that recombinant hiPSC-derived chromaffin cells can enhance effectiveness of hCC transplantation in chronic pain management.

Disclosures: J. Sagen: Other; The University of Miami and J.S. and S.J. hold rights to intellectual property used in the study and may financially benefit from the commercialization of the intellectual property. **B. Rahimi:** None. **B. Marin:** None. **I. Cozzone:** None. **A. Eeswara:** None. **D.A. Abdennour:** None. **C. Dumont:** None. **S. Jergova:** Other; The University of Miami

and J.S. and S.J. hold rights to intellectual property used in the study and may financially benefit from the commercialization of the intellectual property.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.07/E4

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Promoting neuroprotection through genetic engineering: therapeutic implications of ALDH2 upregulation

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Abstract: The primary damage from spinal cord injury (SCI) is significantly worsened by a series of biochemical cascades, known as secondary injury, that can last for months to years following initial physical trauma. Despite tremendous medical advancements, there is no established treatment for SCI and, consequently, recovery can be limited. Methods aimed at mitigating the progression of the secondary injury may provide neuroprotection and improve recovery prognosis. As a key component of secondary injury, oxidative stress-induced lipid peroxidation (LPO) resulting in the formation of reactive aldehyde byproducts, such as acrolein, is extremely destructive and self-propagating. Acrolein has been shown to induce mitochondrial dysfunction, myelin damage, and cell death even in the absence of trauma. Due to its multi-faceted role in SCI degeneration and its relatively long half-life, acrolein has emerged as a promising target for therapeutic intervention. Indeed acrolein scavengers such as hydralazine have yielded promising results in preclinical studies but pose a risk of side effects due to its vasodilating properties. We sought to promote acrolein scavenging through a novel approach by upregulating aldehyde dehydrogenase 2 (ALDH2) using genetic engineering. ALDH2 is an endogenous oxidoreductase with powerful aldehyde detoxification capabilities. We hypothesized that targeted upregulation of ALDH2 in the nervous system could provide neuroprotection following SCI by decreasing acrolein levels thus suppressing other pathologies. Using an adeno associated virus (AAV) to upregulate ALDH2 and a contusion model of SCI, we found that 1) acrolein levels were lower in mice receiving AAV-ALDH2 compared to the control group (AAV-GreenLantern) and 2) mice exhibited superior locomotor recovery starting 4 days post-SCI. It is expected that this effort may help establish new methods to promote neuroprotection and thus enhance the effectiveness of SCI treatment and recovery.

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Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: RS-2023-00208315
RS-2023-00254156
RS-2024-00419269
2019R1A6A1A11034536

Title: Enhanced Axonal Regeneration with Multichannel Scaffolds for Peripheral Nerve and Spinal Cord Injuries

Authors: *J. HYUN, **J. JEON**;
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Abstract: Peripheral nerve injuries often result in significant functional deficits due to limited spontaneous regenerative capacity, particularly in injuries involving extensive nerve gaps. Traditional treatments using autologous nerve grafts or allografts often fail to achieve full functional recovery. Similarly, spinal cord injuries (SCI) present profound challenges due to permanent sensory and motor deficits, as well as severe complications such as neuropathic pain and autonomic dysfunction. To address these limitations, we have developed innovative artificial scaffolds that incorporate finely aligned microchannels to provide physical guidance for regenerating axons, coupled with nanoporous structures to facilitate ion exchange and waste removal. These scaffolds, constructed from FDA-approved biomaterials, are ready for clinical application.In our experimental models, these scaffolds were implanted into 16 mm and 30 mm gap lesions in the sciatic nerves of rats and minipigs, respectively. Comparative analyses revealed that the re-innervated muscle cross-sectional area in scaffold-implanted groups significantly exceeded that of groups receiving autologous nerve grafts. Functional assessments showed superior recovery in motor functions, as measured by the Sciatic Functional Index (SFI) and Sciatic Static Index (SSI), and sensory functions, assessed via von Frey hair and thermal tests in scaffold-treated subjects compared to controls.In addition, spinal cord-sized scaffolds were implanted into full-thickness transections at the thoracic level in SCI models. Subsequent evaluations indicated encouraging axonal outgrowth across the scaffold and notable improvements in locomotor function in both rats and minipigs. Currently, these multichannel scaffolds are being produced in various sizes at a GMP-certified facility to prepare for imminent clinical trials. The promising results from animal models support the potential of these unique nerve conduits to significantly improve outcomes for patients with severe peripheral nerve and spinal cord injuries.

Disclosures: J. Hyun: None. **J. Jeon:** None.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

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Program #/Poster #: PSTR164.09/E6

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DH-2022-00922

Title: Three-dimensional motor mapping of the lumbar spinal cord with dorsal, ventral, and lateral epidural stimulation

Authors: *O. EDDAOUI[1], J. HARNIE[2], O. L. BERNARD[3], C. NADEAU[4], S. YASSINE[5], R. AL ARAB[5], S. TONLEU DONGMO[6], S. MARI[7], P. JEHANNIN[8], J. AUDET[4], A. FRIGON[5], C. IORIO-MORIN[9];
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Abstract: CONTEXT: Spinal cord stimulation (SCS) holds potential to restore locomotor movements following severe SCI by reactivating motor circuits below the injury. However, precision in muscle activation is lacking, hindering functional recovery. The most common approach involves dorsal epidural SCS, which recruits spinal motoneurons via reflex pathways. Yet, this approach struggles to produce functional movements because muscle activations are often diffuse. The location of the electrodes is the main factor influencing evoked motor responses. We hypothesize that SCS near ventral roots enables greater selective activation of leg muscles. The project's goal was to map and compare hindlimb muscle motor responses with dorsal, ventral, and lateral epidural SCS of the lumbar cord.
METHODS: Experiments were performed under general anesthesia in 4 intact cats (2F, 2M). Multiple electrode grids, totaling around 180 contacts, were implanted on the dorsal, ventral, and lateral epidural surface, covering L2 to L6. Cats were installed in a stereotaxic frame allowing free hindlimb movements. We recorded electromyography (EMG) from 32 hindlimb muscles. Bipolar stimulations were delivered, increasing the current from motor threshold to max EMG response. High-resolution 3D lumbar cord MRI images and postoperative CT scans of the electrodes were obtained for each cat.
RESULTS: We applied a sigmoid fit to each recruitment curve and compared various parameters between ventral, lateral, and dorsal SCS, including motor thresholds, max response, half response, and max sigmoid slope. We show that, at anticipated motoneuron pool levels, ventral SCS evoked stronger motor responses at lower thresholds than dorsal SCS. Preliminary 3D maps showed stronger correlations between computed electric fields and motor responses at expected motoneuron levels, side of the spinal cord, and in ventral structures, consistent with greater selective activation using ventral SCS.

CONCLUSION: Our work supports further exploration of ventral SCS to induce more specific motor contractions. Our mapping will guide future electrode development and placement aimed at restoring natural movements following SCI.

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Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

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Program #/Poster #: PSTR164.10/E7

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Department of Florida of Health COPBC - University of Miami

Title: Analgesic potential of naïve and recombinant GABAergic hiPSCs and their exosomes in a model of spinal cord injury induced pain

Authors: *S. JERGOVA, B. RAHIMI, Y. PRESSMAN, L. TIERNEY, S. BURKS, J. SAGEN; Univ. of Miami, Miami, FL

Abstract: Spinal cord injury (SCI)-induced chronic pain presents a therapeutic challenge. Recombinant cell-based therapy can provide sustained and targeted delivery of analgesic compounds, while cells can functionally integrate with the host tissue. Dysfunctional GABAergic signaling and calcium-dependent release of pain neurotransmitters are among major pathologies underlying chronic pain. Our previous studies showed that spinal transplantation of rat GABAergic progenitors releasing an FDA approved calcium channel blocker conotoxin MVIIA can attenuate injury-induced hypersensitivity in rats. Human induced pluripotent stem cells (hiPSCs) enable autologous cell derivation from readily accessible sources like blood or skin. hiPSCs also produce modulatory factors released in the form of exosomes, which have shown promising outcomes in attenuation of chronic pain. The goal of the proposed study is to explore the analgesic and translational potential of hiPSCs derived recombinant GABAergic cells and to explore an early intervention with hiPSCs-derived exosomes for attenuation or prevention of chronic pain development. Fibroblast and blood-derived hiPSCs were used to generate GABAergic neuronal cells using established protocols. Cells were transduced by AAV2/8 MVIIA construct developed by our lab to generate recombinant cells. Both naïve and recombinant cells were grown in sufficient quantities to allow isolation of exosome fractions using ultracentrifugation. The analgesic potency of naïve and recombinant GABAergic hiPSCs and their exosomal fractions were evaluated in clip compression model of SCI pain. Sprague Dawley rats were used in the experiment. Cells were grafted intraspinally at 4 weeks post SCI and rats were evaluated weekly for mechanical and thermal hypersensitivity. In a separated group, exosomal fractions were injected intravenously at 24hours post SCI with a booster

injection at 1 week post SCI. Our results showed GABAergic phenotype and the presence of MVIIA in all transduced cell lines. Exosomal fractions were isolated from selected cell lines and characterized by Nanosight. Attenuation of hypersensitivity was detected in animals grafted with GABAergic hiPSCs with stronger effect observed in the recombinant group. The effect was mitigated by anti MVIIA or bicuculine injection. An early injection of exosomal fractions followed up by booster partially attenuated development of tactile hypersensitivity compared to a single injection. Our data suggests the beneficial effect of recombinant GABAergic hiPSCs in the management of chronic pain and possible attenuation of chronic pain development using exosomal infusion.

Disclosures: S. Jergova: Other; The University of Miami and J.S. and S.J. hold rights to intellectual property used in the study and may financially benefit from the commercialization of the intellectual property. **B. Rahimi:** None. **Y. Pressman:** None. **L. Tierney:** None. **S. Burks:** None. **J. Sagen:** Other; The University of Miami and J.S. and S.J. hold rights to intellectual property used in the study and may financially benefit from the commercialization of the intellectual property.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.11/E8

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Engraftment of Neural Networks via Pre-Formed Ribbons Transplant for Restoring Circuitry after Spinal Cord Injury

Authors: *C. STIGLIANO[1], P. J. HORNER[1], J. L. PALUH[2];
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Abstract: Stem cell technologies represent a forefront strategy to promote tissue repair after spinal cord injury (SCI). While the primary focus is the functional restoration through the repair of specific circuitry, the formation of functional connections between the transplanted neural stem cells (NSCs) and the host needs to be promoted and further investigated. However, stochastic differentiation and tumorigenic risk still represent a challenge associated to the use of NSC. Here, we transplanted differentiated regionally matched cervical spinal motor neurons (SpMNs) and interneurons (SpINs), plus oligodendrocyte precursor cells (OPCs), to promote the engraftment of cells phenotypically appropriate for the location of SCI. To address the need of efficient cell delivery and survival, we encapsulated predefined neuronal networks in alginate hydrogel, here called neural ribbons, that we implanted directly at the injury site of a clinically relevant model of cervical C4 hemicontusion in rat. Following the transplant of the spinal neural glial network, we allowed cell integration for 10 weeks and finally harvested the cord for histological evaluation. Control animals received empty ribbons without cells (CTRL-Ribbon).

The tissue analysis demonstrated viability and retention of the neural ribbon in close proximity of the injury cavity; the shape of the graft resembled the transplanted ribbon with 1-2 mm of maximum length. Cell type characterization showed that the grafted cells are positive to the mature neuron marker NeuN. Moreover, graft-derived neurons were characterized for specific neuronal markers, and the quantification showed that almost 70-80% of the cells are positive to the excitatory interneuron V2a marker Chx10, and around 10% of the cells are ChAT positive, a marker for motoneuron. Transplanted OPCs survived in the host expressing GST-pi marker for mature oligodendrocytes. Next, we evaluated the synaptic marker Synapsin-1 and post-synaptic marker Homer-1 at the graft neurite and the analysis demonstrated the juxtaposition of the synaptic proteins with the host neurons in the grey matter. To assess functional recovery, the Limb Use Asymmetry Test (LUAT) has been performed and ultimately highlighted an improvement of the forelimb use after neural ribbon transplantation compared to the control. In conclusion, the transplant of organized and pre-differentiated neural ribbons successfully integrated new networks at the injury site and advanced the goal of functional recovery after cervical SCI.

Disclosures: C. Stigliano: None. **P.J. Horner:** None. **J.L. Paluh:** None.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.12/Web Only

Topic: C.11. Spinal Cord Injury and Plasticity

Support: SUMS Grant no. 1397-04-11-15704, 2018

Title: Enhanced Tissue Repair and Functional Recovery Through Combined Use of Neural Stem Cells and Ibrutinib in a Mouse Model of Spinal Cord Injury

Authors: *S. TORABI[1], H. AZARI[2], H. ALIGHOLI[3];
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Abstract: Introduction: The treatment of spinal cord injury (SCI) presents challenges due to its complexity and complications, compounded by inflammation that worsens tissue damage. Our recent study highlighted Ibrutinib's potential in alleviating post-SCI inflammation. Neural stem cells (NSCs) also hold promise for tissue regeneration, yet hurdles remain in their survival, differentiation, and integration. To enhance NSC therapy, we investigated Ibrutinib's impact on transplanted NSCs and its synergistic effect with NSC transplantation on motor function recovery and tissue repair post-SCI in mice. Methods: Mice with a contusion SCI model received intraparenchymal injections of green fluorescent protein (GFP)-expressing NSCs or PBS (as a control). Subsequently, they received a single intravenous dose of Ibrutinib (3.125 mg/kg in PBS) or PBS alone (as a control). Over four weeks, we conducted behavioral assessments,

including the Basso Mouse Scale (BMS), inclined plane, and beam balance tests. Tissue analysis evaluated lesion volume, cell viability, and fate post-transplantation.
Results: Our investigation revealed significantly improved hind limb motor function recovery with combined Ibrutinib and NSCs transplantation. Moreover, groups treated by ibrutinib alone, NSCs, and their combination showed a statistically significant decrease in lesion volume, indicating effective reduction of secondary injury mechanisms such as inflammation exacerbating tissue damage post-SCI. Additionally, Ibrutinib led to a 2.9-fold increase in transplanted NSCs' viability, significantly enhancing their expression of oligodendrocyte and neuroblast markers compared to NSCs transplanted without Ibrutinib. Simultaneously, Ibrutinib significantly reduced astrocyte marker expression in transplanted NSCs, potentially mitigating scar formation and fostering an environment conducive to axonal growth and tissue restoration. Furthermore, Ibrutinib therapy resulted in the migration of more NSCs over a longer distance (7 mm) compared to NSCs transplanted groups treated with PBS (5 mm).
Conclusion: The synergy between Ibrutinib and NSCs transplantation holds promise for enhancing tissue repair, facilitating functional recovery, and influencing transplanted NSCs fate post-SCI. This combined approach offers potential for improving NSCs survival and promoting neuronal and oligodendroglial differentiation, thereby addressing critical aspects of SCI pathology.

Disclosures: S. Torabi: None. **H. Azari:** None. **H. Aligholi:** None.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.13/E9

Topic: C.11. Spinal Cord Injury and Plasticity

Support: AMED Grant JP24bk0104175h0001

Title: Lotus gene transfer by adeno-associated virus is efficacious in improving function after spinal cord injury

Authors: *J. MATSUBAYASHI, Y. KAWAGUCHI, K. KOBAYASHI, K. TAKEI; Yokohama City Univ. Sch. of Med., Yokohama, Japan

Abstract: The CNS regeneration is extremely limited after spinal cord injury (SCI). After SCI, several axonal growth inhibitors derived from myelin debris and glial scars bind to Nogo receptor-1 (NgR1) or Paired immunoglobulin-like receptor B (PirB), resulting in CNS regenerative failure. It is required to promote axon regrowth and synapse formation beyond the injured area while suppressing these axonal growth-inhibiting receptors to facilitate functional restoration. Lateral olfactory tract usher substance (LOTUS) contributes to axonal tract formation in the developing brain and axon regrowth synaptic formation in the adult brain as an endogenous NgR1 and PirB antagonist. Previous studies reported that neuronally LOTUS-

overexpressing transgenic mice showed axonal elongation and functional recovery after SCI (Hirokawa et al., Sci Rep. 2017; Ito et al., eNeuro. 2018). Therefore, LOTUS is expected to be useful for future SCI therapy by inhibiting NgR1 and PirB functions. However, the expression level of LOTUS drastically decreases after SCI. The decrease in LOTUS expression can be considered one of the major causes of delayed functional restoration following SCI. In addition, to apply the beneficial effects of LOTUS to clinical treatment, establishing a non-invasive and adaptive therapeutic strategy is desirable. The adeno-associated virus (AAV) vector is an available system to express target genes in the long term and deliver gene safety. Herein, we evaluated the therapeutic effects of AAV transduction of the LOTUS gene in contusive SCI model mice. In this study, we used AAV.GTX vector penetrates blood-brain barrier (BBB) and transduces target genes extensively, specifically in the CNS. We performed intrathecally induction of AAV.GTX-LOTUS in the acute phase or subacute phase of SCI. As a result, AAV.GTX-LOTUS-transduced mice expressed higher levels of LOTUS in the injured spinal cord compared to AAV control. Histological analysis revealed that raphespinal serotonergic fibers are beyond the epicenter in AAV.GTX-LOTUS-transduced mice were increased, suggesting transduced LOTUS may promote axonal regrowth of the rapfespinal tract. Moreover, overexpression of LOTUS via AAV significantly enhanced weight gain and restored functional motor activity, as determined by the Basso Mouse Scale (BMS) locomotion score and Grid-walking test 28 days after SCI. These findings suggest that overexpression of the LOTUS gene using an AAV.GTX vector compensates for LOTUS expression and inhibits NgR1 and PirB functions, promoting neuronal regeneration after SCI. Thus, AAV.GTX-LOTUS could be a promising therapeutic strategy for treating humans with acute SCI.

Disclosures: J. Matsubayashi: None. **Y. Kawaguchi:** None. **K. kobayashi:** None. **K. Takei:** None.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.14/E10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Government of Canada NFRFT-2020-00238

Title: Development of an injectable anisotropic hydrogel to promote aligned axon regeneration in a rat model of spinal cord injury

Authors: *K. G. JEFFRIS[1], A. LOONG[2], M. LU[1], A. BURDEN[1], E. MCGINN[3], P. PIETRYSZEK[4], H. BRIGGS[1], E. RAFFAELE[2], L. DE LAPORTE[4], W. TETZLAFF[5]; [1]ICORD, [2]Univ. of British Columbia, Vancouver, BC, Canada; [3]Univ. of British Columbia, Coquitlam, BC, ; [4]DWI - Leibniz Inst. for Interactive Materials, Aachen, Germany; [5]ICORD, Univ. of British Columbia - ICORD, Vancouver, BC, Canada

Abstract: After a spinal cord injury (SCI), the damaged tissue fails to regenerate, often leading to formation of a cavity at the lesion site. This cavity lacks a substrate that is permissive to axon outgrowth. Previous studies indicate that physical cues such as longitudinally-oriented microchannel scaffolds can guide aligned axon growth across an SCI lesion. Currently, these scaffolds must be pre-formed and implanted into the lesion, posing risk of further tissue damage. We are investigating an anisotropic hydrogel that can be injected minimally-invasively to direct aligned axon regrowth. It utilizes a surrounding polyethylene glycol (PEG)-based hydrogel to deliver 2.5 um by 25 um rod-shaped microgels (developed in the De Laporte lab) that serve as physical guidance cues for axons. These microgels contain magneto-responsive nanoparticles, enabling their rostro-caudal alignment using a low strength magnetic field (<100mT). The microgels and the surrounding hydrogel comprise an "Anisogel", which has been shown by the De Laporte lab to promote aligned neurite outgrowth of chick embryo dorsal root ganglia (DRGs) in vitro. Here, we hypothesize that the Anisogel platform can induce aligned axon outgrowth in a rat SCI model. We have functionalized the surrounding hydrogel with various cell-adhesive peptides such as IKVAV or RGD, known integrin receptor-binding sequences derived from extracellular matrix molecules laminin and fibronectin, respectively, to promote axon outgrowth. Both peptide sequences promoted improved 3D neurite outgrowth of cultured rat pup cortical explants compared to peptide-free control hydrogels and therefore represent promising candidates for spinal cord repair. These two hydrogel modifications are currently being tested in rat SCI models; both a dorsal column resection model as well as in a severe midline cervical contusion model. The inflammatory response as well as axonal growth into the hydrogels will be evaluated. We are further optimizing the hydrogel formulation for in vivo delivery and alignment of the microgels to ultimately evaluate the ability of the platform to facilitate aligned axon regrowth after SCI.

Disclosures: K.G. Jeffris: None. **M. Lu:** None. **A. Burden:** None. **H. Briggs:** None. **E. Raffaele:** None. **W. Tetzlaff:** None.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.15/E11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant 1R01DK133195-01A1

Title: Lower urinary tract response to spinal cord epidural stimulation with peripheral neurectomy in spinally injured rats.

Authors: *K. BEASLEY[1], D. MEDINA AGUINAGA[2], B. PEREZ DE CORCHO VAZQUEZ[3], N. WILKINS[2], C. HUBSCHER[2,4];
[1]Univ. of Louisville Anatom. Sci. & Neurobio., Louisville, KY; [2]Anatom. Sci. and Neurobio.,

[3]Anatom. Sci. & Neurobio., Univ. of Louisville Sch. of Med., Louisville, KY; [4]Kentucky Spinal Cord Injury Res. Ctr., Louisville, KY

Abstract: Spinal cord injury (SCI) often leads to severe impairment of multiple body systems, greatly impacting quality of life. While the urinary bladder is initially areflexic during spinal shock following SCI, reflexive voiding usually develops within 2-12 weeks in humans and 1-2 weeks in the rat. However, voiding post-SCI is often disordered and may display detrusor-sphincter dyssynergia (DSD), which is characterized by uncoordinated bladder and external urethral sphincter (EUS) contractions, causing inefficient emptying and smooth muscle hypertrophy. Likewise, the frequency of bladder contractions may increase and contribute to dangerously high intravesical pressures and storage dysfunction in a condition known as neurogenic detrusor overactivity (NDO). Spinal cord epidural stimulation (scES) is a novel therapy that has been shown to improve lower urinary tract (LUT) function in both humans and pre-clinical experimental models post-SCI. It is hypothesized that the improvements in LUT function seen with scES result from modulation of the neural networks which project to the bladder or EUS that are located within these sites of stimulation. To gain insight into the neural mechanisms behind scES-induced effects on the LUT, the Hubscher laboratory has developed a model combining thoracolumbar or lumbosacral scES with a neurectomy of either the pelvic, hypogastric, or pudendal nerves in female rats with moderate-severe SCI (215 kdyn) during urethane-anesthetized cystometry-electromyography at 7-, 14-, or 28-days post-injury. Early data indicate a reduction in scES-induced LUT improvements following peripheral neurectomy, implicating their role as a functional target of neuromodulation.

Disclosures: K. Beasley: None. **D. Medina Aguinaga:** None. **B. Perez de Corcho Vazquez:** None. **N. Wilkins:** None. **C. Hubscher:** None.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.16/E12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Li Ka Shing Grant

Title: Poly-l-ornithine coated plant scaffolds support motor recovery in rats after traumatic spinal cord injury

Authors: *L. COUVRETTE[1], A. M. LALIBERTE[2], T. V. BUI[2];
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Abstract: Spinal cord injury (SCI) is a debilitating neurological condition with far-reaching consequences for patients, including loss of motor function and significant limitations to quality of life. Implantable biomaterials have emerged as a therapeutic strategy to modulate the SCI microenvironment and facilitate regeneration of axons. In this study, plant-derived cellulose

scaffolds coated with poly-L-ornithine are shown to support locomotor recovery and neural tissue repair in a rat model of spinal cord injury. Upon complete transection of the spinal cord at T8, animals were implanted with a plant-derived scaffold coated in poly-L-ornithine, a positively charged amino acid chain that is known to promote neural stem cell differentiation into neurons and enhance myelin regeneration. Recovery of motor function is evaluated by the BBB locomotor scale as well as the Karolinska Institutet Swim Assessment Tool. Retrograde tracing of ascending sensory tracts reveals enhanced regeneration in animals that received the PLO-coated scaffold. Immunostaining for β-III tubulin and NF200 shows axonal sprouting within the cellulose biomaterial and LFB staining highlights myelination around the PLO-coated scaffold.

Disclosures: L. couvrette: None. **A.M. Laliberte:** None. **T.V. Bui:** None.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.17/E13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: WFL-US-15/21

Title: Two-tiered combinatorial transplantation strategy for repair of the contused spinal cord

Authors: *G. SCESA[1], W. BOGUE[2], M. OUDEGA[3];
[1]Shirley Ryan Ability Lab., Chicago, IL; [2]Biol., Northeastern Illinois Univ., Chicago, IL;
[3]Biologics Lab., Shirley Ryan Abilitylab, Chicago, IL

Abstract: A typical outcome of spinal cord contusion is an immediate nervous tissue damage associated with function impairments. Repair of the damaged nervous tissue may result in recovery of function. Transplantation of Schwann cells (SC) into the damaged area has been deemed a candidate strategy to repair nervous tissue and, consequently, restore function after spinal cord injury (SCI). Nervous tissue repair by transplanted SC is chiefly due to paracrine stimulation of repair events. However, preclinical and clinical research has demonstrated that the effectiveness of a SC transplant in the injury site to repair nervous tissue and elicit functional recovery is limited. Following contusive SCI, the damaged area remains chronically inflamed and poorly vascularized, which support a continuous degeneration of nervous tissue, and therefore a persistent cytotoxic microenvironment. We hypothesized that the cytotoxic injury site jeopardizes the repair ability of transplanted SC, thereby limiting their therapeutic potential for SCI. We argued that a SC transplant's repair ability in the damaged spinal cord can be affected either by poor survival or by changes in the SC's secretome. We have addressed two questions. 1) Does the contusion microenvironment change the composition of the SC secretome such that less repair is achieved? 2) Can the contusion environment be modified prior to transplantation in order for to SC retain their repair-supporting potential? We show how the contusion milieu changes the secretome of the SC, and thereby their overall efficacy to repair damaged nervous

tissue. The injury-mediated changes in SC secretome are evident in numerous mechanisms that would otherwise strongly support SC-mediated tissue repair, and are dependent on the post-injury time of transplantation. To modify the contusion environment prior to SC injection, we have investigated the potential of an injectable nanofiber hydrogel composite (NHC), which was shown to enhance the formation of a highly vascularized tissue, and to induce a reparative inflammation profile. Overall, our studies support the idea that a contusion environment affects the repair potential of transplanted SC by modifying their secretome. Future SC therapies will need to consider combinatory approaches to prevent/limit such changes in transplanted SC to maximize repair and functional recovery.

Disclosures: G. Scesa: None. **W. Bogue:** None. **M. Oudega:** None.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.18/E14

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Selective knockdown of potassium channel Kv1.2 in spinal-projecting axons as a novel approach to restore functions in chronic spinal cord injury without systemic side effects.

Authors: *J. SALAZAR[1], V. K. SLONE[2], F. SHAH[2], C. BOSSE-JOSEPH[2], A. N. STEWART[2];
[2]Physiology/SCoBIRC, [1]Univ. of Kentucky, Lexington, KY

Abstract: Restoring motor and sensory functions in chronic stages after spinal cord injury (SCI) remains a formidable challenge due to a persistent growth inhibitory environment at the lesion. Prior research has identified that action potentials descending from supraspinal motor neurons dissipate from spared and intact axons as they approach the lesion, and further, that pharmacologic inhibition of potassium channels can improve signal propagation through the lesion and restore motor and sensory functions. Indeed, delivery of the potassium channel blocker 4-aminopyradine (4-AP) has demonstrated clinical efficacy to improve motor and sensory functions in humans with chronic SCI. Unfortunately, the dose of 4-AP which confers functional benefits in most patients with SCI exceeds a safe threshold for inducing seizures and other systemic side effects. Targeting potassium channels, therefore, is an actionable target with demonstrated efficacy to restore functions after SCI, but the systemic complications using pharmaceutical approaches remains a barrier to translation. We hypothesize that selectively targeting only spared and damaged axons that are implicated in the SCI pathology will confer therapeutic improvements in chronic stages after injury that are free from systemic side effects. Our lab has optimized a delivery mechanism using retrogradely transported adeno-associated viruses (AAVrg) which will knockdown the axon-specific potassium channel Kv1.2 specifically in spared and damaged spinal-projecting neurons. We present work on our in vitro validation of Kv1.2 knockdown as well as outcomes after knockdown in mice with chronic SCI. Collectively,

our work supports an emerging understanding that the suppression of neuronal excitability at the lesion remains an unresolved barrier that limits motor and sensory functions through spared and intact pathways chronically after SCI. Further, our work presents a novel approach to overcome complications with systemic delivery approaches aimed at increasing neuronal excitability.

Disclosures: J. Salazar: None. **V.K. Slone:** None. **F. Shah:** None. **C. Bosse-Joseph:** None. **A.N. Stewart:** None.

Poster

PSTR164: Spinal Cord Injury: Therapeutic Strategies: Non-Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR164.19/E15

Topic: C.11. Spinal Cord Injury and Plasticity

Support: 1K99NS133388-01A1
1R01HL139708-01A1
1 R01 HL153140-01
Parker B Francis Foundation Fellowship

Title: Non-invasive transcutaneous spinal direct current stimulation enhances diaphragm activity following high-cervical spinal cord injury

Authors: *S. RANA[1], E. BENEVIDES[2], I. GONZALEZ[1], J. H. MARTIN[3], D. D. FULLER[1]; [1]Physical Therapy, [2]Dept. of Physical Therapy, Univ. of Florida, Gainesville, FL; [3]CUNY Sch. of Med., New York, NY.

Abstract: Respiratory complications stemming from cervical spinal cord injuries (SCIs) are the foremost cause of both mortality and morbidity within this patient demographic. Thus, there is an urgent need to develop therapeutic interventions aimed at reinstating the complete spectrum of breathing functionality following SCI (e.g., eupnea, hyperpnea, coughing, sighing). Given that most of SCIs are incomplete, an extant substrate exists for using neuromodulatory therapies to activate sub-threshold spinal networks and motor neurons. Transcutaneous spinal direct current stimulation (tsDCS) is a non-invasive method in which a constant, low-intensity current is delivered via surface (skin) electrodes. tsDCS enables excitatory neurotransmitter release onto spinal motor neurons and increases overall motor neuron excitability. tsDCS can increase overall ventilation and amplify diaphragm motor evoked potentials in spinal intact humans. However, tsDCS has not been used to improve breathing ability after SCI. The goal of the current work was to evaluate the effect of cathodal tsDCS (2mA, 20 min) on diaphragm muscle activation following cervical SCI. Adult rats were implanted with indwelling diaphragm EMG electrodes and received a C2 hemisection injury. Subsequently, the immediate effects of tsDCS on diaphragm output were measured at a sub-acute (7 days) and chronic time point (8 weeks). At 7 days post-injury, DCS delivered to the ipsilateral hindlimb (i.e., non-spinal “control” stimulation) did not affect diaphragm EMG activity ($p=0.53$). In contrast, tsDCS at the mid-

cervical region increased the peak of the diaphragm inspiratory EMG burst recorded ipsilateral to C2 hemisection by 28 ± 7% ($p<0.001$). Similarly, at 8 weeks post-injury, the non-specific hindlimb stimulation had no effect on diaphragm EMG activation ($p=0.27$), but mid-cervical tsDCS increased ipsilateral EMG activity by 32% ± 14% ($p=0.001$). We conclude that non-invasive cathodal tsDCS delivered at the mid-cervical region can increase diaphragm muscle output after cervical SCI. tsDCS may therefore be useful in the context of respiratory rehabilitation after SCI.

Disclosures: S. Rana: None. **E. Benevides:** None. **I. Gonzalez:** None. **J.H. Martin:** None. **D.D. Fuller:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.01/E16

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH R01NS089787
Martina Stern Memorial Fund
American Cancer Society 122229-IRG-97-153-10-IRG
NSF CMMI 1761432

Title: Shear force activates TrpA1 to drive mechanical nociception in Drosophila

Authors: J. GONG[1], J. CHEN[2], P. GU[1], Y. SHANG[1], F. WANG[1], Q. WEN[2], ***Y. XIANG**[3]; [1]Univ. of Massachusetts Chan Med. Sch., Worcester, MA; [2]Worcester Polytechnic Inst., Worcester, MA; [3]Univ. of Massachusetts Chan Med. Sch. Grad. Program in Neurosci., Worcester, MA

Abstract: Mechanical nociception is essential for animal survival and abnormal mechanical pain is a hallmark of many debilitating diseases including neuropathic pain, cancer pain, and diabetic pain. However, the types of mechanical forces involved in nociceptor activation and the underlying mechanotransduction mechanisms remain elusive. As such, elucidating the molecular and cellular mechanisms of mechanical pain is considered the ‘frontier’ of sensory biology. Here, we address these problems by investigating mechanical nociception in Drosophila larvae, which display robust nocifensive behavior and share conserved sensory transduction mechanisms with mammals. We show that strong poking stimulates nociceptors with a mixture of mechanical forces including shear stress and membrane stretch. Unexpectedly, nociceptors are selectively activated by shear stress, but not stretch. Both the shear stress responses of nociceptors and nocifensive behavior require transient receptor potential A1 (TrpA1), which is specifically expressed in larval nociceptors. We further demonstrate that mammalian or Drosophila TrpA1 channels specifically respond to shear stress but not stretch. Mechanistically, shear stress activates TrpA1 in a membrane-delimited manner, through modulation of membrane fluidity.

Together, our study reveals TrpA1 as a mechanotransduction ion channel that is activated by select force types (i.e., shear stress but not membrane stretch), and unravel a previously unknown role of shear stress in mechanical nociception. Given that shear force sensing is a conserved property of TrpA1 across species and TrpA1 is expressed in mammalian nociceptors, our study also highlights importance of investigating TrpA1 shear stress mechanosensing in other species.

Disclosures: J. gong: None. **J. Chen:** None. **P. Gu:** None. **Y. Shang:** None. **F. Wang:** None. **Q. Wen:** None. **Y. Xiang:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.02/E17

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant UC2AR082195
T32 COSTAR training grant

Title: Identification of Neuronal Subtypes in the Temporomandibular Joint

Authors: *J. ALFARO;
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Abstract: Temporomandibular joint disorders (TMJD) are functionally heterogenous conditions of the mastication system affecting the jaw joint, masticatory muscles, and ligaments. Despite approximately 5-12% of the population suffering from some type of TMJD, treatment remains ineffective. To gain an improved understanding of the pathophysiology of TMJD, the subtypes of sensory neurons that innervate the temporomandibular joint (TMJ) and connected lateral pterygoid muscle (LPM) were identified using immunohistochemistry (IHC) and various established markers. TMJ neurons were primarily C fibers (NFH-), while the LPM has about equal amounts of A fibers (NFH+) and C fibers. Approximately 1/5 of TMJ C fibers were labeled as peptidergic nociceptors and 2/5 in LPM C fibers. Of the A fibers in TMJ, most were Htr3a expressing. The A fibers in the LPM had almost equal amounts of Htr3a+ and CGRP+ expressing fibers. There was little to no MrgprD expression in both TMJ and LPM as well as minimal amounts of PV in TMJ. Our findings convey that the sensory neurons innervating the TMJ and LPM are distinct from each other, and subsequent studies with additional markers will further categorize the subtypes of sensory neurons present. Following, we will use patch clamp electrophysiology to obtain the electrophysiological characteristics of the sensory neurons innervating the TMJ. The goal of the present study is moving towards uncovering the sensory neurons responsible for TMJD pain to develop more specific and long-lasting treatment for TMJD inflicted chronic pain.

Disclosures: J. Alfaro: None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.03/E18

Topic: D.01. Somatosensation – Pain and Itch

Support: Grant ANR-11-LABX-0015-01
Grant ANR-22-CE14-0048-01
Certified team FRM 2020

Title: Exploring the role of THIK potassium channels in the nociceptive pathway

Authors: *N. GILBERT, D. BICHET;
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Abstract: Title : Exploring the role of THIK potassium channels in the nociceptive pathway
Nicolas Gilbert, Delphine Bichet
From the Laboratory of Excellence Ion Channel Science and Therapeutics, Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, and Université Côte d'Azur, 660 Route des Lucioles, 06560 Valbonne, France
Potassium channels play a crucial role in the nervous system, as they can affect resting membrane potential and modulate action potentials making them important targets for the search for new neuronal modulators. The K2P group of potassium channels are involved in various physiological functions mostly cardiac and neuronal. Recently, several K2P channels have been linked to the regulation of pain and mutation in K2P channels are associated with migraine and neurodevelopmental disorders. K2P channels are known to finely regulate neuronal excitability by hyperpolarizing their membrane.Members of the Tandem pore-domain Halothane-Inhibited K+ channels subfamily (THIK1 and THIK2) are highly expressed in the Central and Peripheral Nervous System (CNS and PNS), but their role in the control of pain sensation has not been studied yet. Using in-situ hybridization technique (RNAscope), we have recently shown that THIK channels are co-expressed by non-peptidergic nociceptive neurons that express the Purinergic Receptor 2X3 (P2RX3) in PNS that are unmyelinated nociceptive neurons. These are known to be involved in the transmission of slow nociceptive messages in Dorsal Root Ganglia (DRG) such as inflammatory and chronic ones. Moreover, RNAseq data shows that THIK1 and THIK2 are the most highly expressed K2P channels in microglial cells of the CNS, in which THIK1 has been linked to inflammasome activation. This suggests that these channels might play a role in inflammatory pain.We are now investigating their role in transmitting sensory and nociceptive messages and whether these channels function as homomers or heteromers. Initial studies have shown that THIK2 knockout mice exhibit allodynia and inflammatory hyperalgesia. We aim to further explore the functions of THIK1 and THIK2 in nociception and differentiate the roles of homomeric and heteromeric forms of the THIK channels. This distinction is crucial for the development of specific pharmacology and targeted therapy.

Disclosures: N. Gilbert: None. **D. Bichet:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.04/E19

Topic: D.01. Somatosensation – Pain and Itch

Support: NRF-2020R1A2C1008084

Title: Glp-1 and its derived peptides mediate pain relief through direct trpv1 inhibition without affecting thermoregulation

Authors: *E. GO[1], G. CHUNG[2], T. BERTA[3], Y. KIM[4], C. PARK[5];
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Abstract: Hormonal regulation during food ingestion and its association with pain prompted the investigation of the impact of glucagon-like peptide-1 (GLP-1) on the transient receptor potential vanilloid 1 (TRPV1). Both endogenous and synthetic GLP-1 and an antagonist of GLP-1, exendin 9-39, reduced heat sensitivity in naïve mice. GLP-1-derived peptides (liraglutide, exendin-4, and exendin 9-39) effectively inhibited capsaicin (CAP)-induced currents and calcium responses in cultured sensory neurons and TRPV1-expressing cell lines. Notably, the exendin 9-39 alleviated CAP-induced acute pain, as well as chronic pain induced by complete Freund's adjuvant (CFA) and spared nerve injury (SNI) in mice, without causing hyperthermia associated with other TRPV1 inhibitors. Electrophysiological analyses revealed that exendin 9-39 binds to the extracellular side of TRPV1, functioning as a noncompetitive inhibitor of CAP. Exendin 9-39 did not affect proton-induced TRPV1 activation, suggesting its selective antagonism. Among exendin 9-39 fragments, exendin 20-29 specifically binds to TRPV1, alleviating pain in both acute and chronic pain models without interfering with GLP-1R function. Our study revealed a novel role for GLP-1 and its derivatives in pain relief, proposing exendin 20-29 as a promising therapeutic candidate.

Disclosures: E. Go: None. **G. Chung:** None. **T. Berta:** None. **Y. Kim:** None. **C. Park:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.05/E20

Topic: D.01. Somatosensation – Pain and Itch

Support: JSPS/22K06001

Title: Analgesic effects of linalyl acetate, a component of clary sage and lavender essential oils, involve nociceptive TRPA1 inhibition

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Abstract: Essential oils derived from clary sage and lavender are utilized in perfumery, aromatherapy, and skincare. Linalyl acetate (LA), a primary component of these essential oils, possesses sedative, anxiolytic, and analgesic properties. However, the mechanism of the analgesic action of LA is not clearly understood. Transient receptor potential ankyrin 1 (TRPA1) channel, a non-selective cation channel, is mainly expressed in peripheral sensory neurons and serves as a sensor of various irritants. In this study, we investigated the effects of LA on TRPA1 channel using heterologous expression system and isolated sensory neurons. To detect channel activity, we employed Ca^{2+} imaging and the whole-cell patch-clamp technique. The analgesic action of LA was measured in a pain-related behavioral mouse model. In cells that heterologously expressed TRPA1, LA diminished $[Ca^{2+}]_i$ and current responses to AITC and carvacrol, which are exogenous TRPA1 agonists. The inhibitory effects of LA were more pronounced for the former than for the latter. Moreover, LA suppressed $[Ca^{2+}]_i$ and current responses to PGJ_2, an endogenous TRPA1 agonist. Similar inhibitory actions were observed in native TRPA1 channels that were expressed in mouse sensory neurons. Furthermore, LA diminished PGJ_2-induced TRPA1-mediating nociceptive behaviors in mice. These findings suggest that analgesic effects of LA exert through inhibition of nociceptive TRPA1, making it a potential candidate for novel analgesic development.

Disclosures: M. Hashimoto: None. **K. Takahashi:** None. **T. Ohta:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.06/E21

Topic: D.01. Somatosensation – Pain and Itch

Support: JSPS Grant 22K06001

Title: Nociceptive TRPV1 and TRPA1 channels are involved in the adverse effects of antifungal drugs.

Authors: S. OKABE[1], K. TAKAHASHI[1,2], M. HASHIMOTO[2], ***T. OHTA**[1,2];
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Abstract: Topically-applied antifungal agents can induce adverse effects, such as pain and irritation. The transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1), mainly expressed in sensory neurons, act as nociceptors and sensors for detecting irritants. This study aims to evaluate the involvement of these channels in topical antifungal-induced pain. We tested nine topical antifungals belonging five classes: isoconazole, econazole, miconazole, clotrimazole, and ketoconazole as imidazoles; liranaftate as a thiocarbamate; terbinafine as an allylamine; amorolfine as a morpholine; and butenafine as a benzylamine. Intracellular calcium concentrations ($[Ca^{2+}]_i$) and membrane currents in response to antifungals were measured to estimate channel activity using heterologously expressing cells and isolated mouse sensory neurons. In mouse TRPA1-expressing cells, all the tested drugs induced an increase in $[Ca^{2+}]_i$, which was abrogated or reduced by a TRPA1 blocker. Although many drugs evoked the TRPA1-nonspecific $[Ca^{2+}]_i$ response at high concentrations, responses to clotrimazole, ketoconazole, and liranaftate were TRPA1 specific and elicited current responses in TRPA1-expressing cells. In mouse TRPV1-expressing cells, clotrimazole and ketoconazole elicited $[Ca^{2+}]_i$ and current responses. In mouse sensory neurons, liranaftate-induced increase in $[Ca^{2+}]_i$ was abrogated by a TRPA1 blocker and *Trpa1* deletion. Responses to ketoconazole were inhibited by TRPA1 and TRPV1 blockers, and by the genetic deletion of either channel. These results suggest that topical antifungal-induced pain and irritation are attributable to the activation of nociceptive TRPA1 and/or TRPV1 channels. Consequently, caution should be exercised in the use of topical antifungals with symptoms of pain. Moreover, the simultaneous use of TRPA1 and TRPV1 inhibitors is an effective approach to mitigate the adverse effects of topical antifungals.

Disclosures: S. Okabe: None. **K. Takahashi:** None. **M. Hashimoto:** None. **T. Ohta:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.07/E22

Topic: D.01. Somatosensation – Pain and Itch

Support: R01NS114018
R35GM146862

Title: Eef2k regulates pain through translational control of bdnf

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Abstract: *De novo* translation of mRNA is integral to pain yet the key regulatory factors and their target mRNAs are unclear. Here, we uncover a mechanism that bridges noxious insults to multiple phases of translational control in murine sensory neurons. We find that a painful cue triggers repression of peptide chain elongation through activation of elongation factor 2 kinase (eEF2K). Attenuated elongation is sensed by a ribosome-coupled mechanism that triggers the integrated stress response (ISR). Both eEF2K and the ISR are required for pain-associated behaviors *in vivo*. While eEF2K attenuates global mRNA translation, the ISR induces biosynthesis of brain derived neurotrophic factor (BDNF). Selective blockade of *Bdnf* translation with an oligonucleotide has analgesic effects *in vivo*. Our data suggest that that precise spatiotemporal regulation of *Bdnf* translation is critical for appropriate behavioral responses to painful stimuli. Overall, our results demonstrate that eEF2K resides at the nexus of an intricate regulatory network that links painful cues to multiple layers of translational control.

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Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.08/E23

Topic: D.01. Somatosensation – Pain and Itch

Support: DoD W81XWH-20-1-0509

Title: Vgf-derived peptide TLQP-62 modulates primary afferent neuron activity

Authors: *A. FRIE[1], A. HUNTEMER-SILVEIRA[1], M. S. RIEDL[1], A. PARR[2], L. VULCHANOVA[1];
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Abstract: VGF is a neurosecretory protein upregulated by primary afferent neurons (PANs) after peripheral nerve damage. TLQP-62 is a VGF-derived C-terminal peptide that has been associated with hypersensitivity in rodent models of neuropathic pain. We have previously shown that application of this peptide induces potentiation of glutamatergic responses. In addition, TLQP-62 promotes phosphorylation of TrkB in the hippocampus, suggesting a possible BDNF dependent mechanism. We hypothesized TLQP-62 is also involved in spinal neuroplasticity via modulation of calcium signaling in PANs and that this signaling may be mediated by BDNF/TRKB. To explore this, primary mouse DRGs (PIRT-Cre x Ai96, TRPV1-cre x Ai96) and human stem-cell derived TRPV1 expressing PANs were cultured and studied for peptide modulated transient activity. High resolution two photon time lapse videos of GCaMP fluorescence in PAN cultures following application of TLQP-62 were recorded. TLQP-62 induced calcium transients were observed in both mouse and hiPSC-derived PAN cultures. A

subset of these terminals was also capsaicin responsive. Furthermore, analysis of secreted media from hiPSC-derived PAN revealed the presence of TLQP-62, highlighting the possibility of constitutive release in these cultures. This suggests a functional role of TLQP-62 induced modulation, such that changes in calcium transient activity may contribute to alterations in PAN neurotransmitter release and subsequent pain signaling. Ongoing work is exploring whether TLQP-62 activity affects BDNF release by PANs and may be relevant for transitions from acute to chronic pain.

Disclosures: A. Frie: None. **A. Huntemer-Silveira:** None. **M.S. Riedl:** None. **A. Parr:** None. **L. Vulchanova:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

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Program #/Poster #: PSTR165.09/E24

Topic: D.01. Somatosensation – Pain and Itch

Support: GR012124
Levinson Emerging Scholars Award

Title: Cannabidiol is anti- and pro-nociceptive in larval zebrafish

Authors: *G. SHEN[1], B. LECAMP[3], K. ESANCY[2], A. K. DHAKA[4];
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Abstract: The promiscuous ligand cannabidiol (CBD) shows promise as an analgesic, but its complex pharmacological profile has made it difficult to identify its mechanism(s) of action. Numerous CBD receptors including cannabinoid receptors CB1 and CB2 and noxious heat nociceptor transient receptor potential vanilloid 1 (TRPV1) have been proposed to underpin CBD mediated analgesia. Larval zebrafish have a number of attributes that lend themselves to inquiries into the biology of nociception. The neural circuits underlying nociception in zebrafish larvae are highly analogous to those found in higher vertebrates, such as rodents and humans. Notably, the small size and optical clarity of zebrafish enable holistic evaluation of analgesic function utilizing high-throughput behavioral and imaging platforms. Here we report in larval zebrafish (6dpf) that CBD serves both anti- and pro-nociceptive functions, comparable to mammalian studies. Utilizing place aversion assays ($n \geq 20$ larvae/assay) as a proxy for nociception, we found that CBD inhibits aversion to the noxious transient receptor potential ankyrin 1 (TRPA1) agonist allyl isothiocyanate (AITC) as well as place aversion elicited by the optogenetic activation of TRPA1 expressing nociceptors. Counterintuitively, we found that CBD potentiated thermal aversion. When larvae were given a choice between two temperatures 28.5 ℃ and 31.5 ℃, CBD strongly increased aversion to 31.5 ℃. As CBD has been shown to be a

ligand for mammalian TRPA1 and TRPV1, and these two receptors are known to contribute to thermal hyperalgesia, we investigated whether these receptors contributed to CBD-evoked thermal sensitization. We used ratiometric calcium imaging in human embryonic kidney 293T cells ($n \geq 200$ cells/condition) expressing the zebrafish orthologs of these channels (zTRPA1 or zTRPV1). Our preliminary data suggests that CBD is an agonist for zTRPV1 but not zTRPA1. These data suggest that CBD may be acting on TRPV1 to evoke thermal sensitization. Interestingly, inactivation of zTRPA1 inhibited CBD-mediated thermal sensitization, suggesting that CBD may indirectly activate this channel *in vivo*. Taken together these studies provide a framework to investigate the genetic and neural substrates of CBD mediated analgesia and nociception.

Disclosures: G. Shen: None. **B. Lecamp:** None. **K. Esancy:** None. **A.K. Dhaka:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.10/E25

Topic: D.01. Somatosensation – Pain and Itch

Title: Inhibition of fatty acid binding protein 5 (FABP5) to reduce chronic neuropathic pain following spinal cord injury

Authors: *J. K. GUPTA[1], K. GUNARATNA[2], E. H. SIPPLE[3], H. LIU[4], M. KACZOCHA[2], M. PUOPOLO[2];
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Abstract: Chronic neuropathic pain is a severe complication following spinal cord injury (SCI), affecting up to 60-70% of patients. SCI-induced neuropathic pain (SCI-NP) is often lifelong and therapeutically intractable, resulting in a severe decline in quality of life. Data from our laboratory showed that the increased activity of T-type calcium channels induced by SCI contributes to drive nociceptors' hyperexcitability and the development/maintenance of SCI-NP. Fatty acid binding protein 5 (FABP5) is an intracellular carrier for endocannabinoids and related lipids that mediates anandamide (AEA), palmitoylethanolamide (PEA), and oleoylethanolamide (OEA) transport to fatty acid amide hydrolase (FAAH) for inactivation. AEA has been shown to directly inhibit T-type channels independently of CB receptors. This suggests that inhibition of FABP5 (with subsequent increase in AEA levels) may provide a strategy to reduce nociceptors' hyperexcitability and SCI-NP. Methods. SCI was performed in mice by a midline spinal cord contusion at T10 (50-60 kilodynes). The mechanical allodynia was measured with the von Frey filaments; 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 mice to measure the T-type calcium current during

the interspike interval. Results. In wild type mice, the 50% mechanical threshold dropped from 1.62±0.12 g (pre-injury) to 1.01±0.14 g (post-SCI). TTA-P2 (10 mg/kg i.p.) increased the 50% mechanical threshold to 1.56±0.10 g (n=12) at 1-hour post-injection. In a different cohort of wild-type mice, the 50% mechanical threshold dropped from 1.71±0.19 g (pre-injury) to 1.12±0.18 g (post-SCI), and SBFI103 (40 mg/kg, i.p., a selective inhibitor of FABP5) increased the 50% mechanical threshold to 1.70±0.20 g (n=9) at 1-hour post-injection. When tested with the CPP paradigm to measure spontaneous pain, wild type mice showed an increase in the TTA-P2 paired chamber (10 mg/kg, i.p.) of 40±12 sec and a decrease in the vehicle-paired chamber of -54±16 sec (n=12). When compared to TTA-P2, wild type mice showed an increase in the SBFI103-paied chamber (40 mg/kg, i.p.) of 56±21 sec and a decrease in the vehicle-paired chamber of -49±22 sec (n=8). In voltage clamp experiments, the size of the interspike T-type calcium current measured at -50 mV was reduced from 0.28±0.06 pA/pF in control to 0.11±0.05 in the presence of 5 μM SBFI103, consistent with reduced nociceptors' excitability. Taken together, our data suggest that inhibition of FABP5 reduces nociceptors' hyperexcitability in vitro and SCI-NP in vivo.

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Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.11/E26

Topic: D.01. Somatosensation – Pain and Itch

Support: R01NS116759

Title: Asct2-mediated glutamine metabolism drives pain resolution

Authors: M. HAQUE[1], P. KUPPUSAMY[1], *O. K. MELEMEDJIAN[2];
[1]Univ. of Maryalnd, Baltimore, MD; [2]Univ. of Maryland Dent. Sch., Baltimore, MD

Abstract: Chronic pain is a debilitating condition with limited effective treatments. Understanding the mechanisms of pain resolution is crucial for developing novel therapies. This study uncovered a compensatory mechanism involving enhanced glutamine oxidation and upregulation of the glutamine transporter ASCT2 in the resolution of nerve growth factor (NGF)-mediated allodynia. Using the hyperalgesic priming model, we demonstrated that disruption of mitochondrial pyruvate oxidation persisted even after pain sensitivity returned to baseline. However, pain resolution was associated with increased glutamine utilization and ASCT2 expression in dorsal root ganglia (DRGs). Knockdown of ASCT2 prevented the resolution of NGF-induced allodynia and precipitated the transition to a chronic pain state. The glutamine catabolite dimethyl α-ketoglutarate (DKG) attenuated glycolytic flux and alleviated allodynia in

both acute and chronic phases of the hyperalgesic priming model. Furthermore, ASCT2 knockdown prevented the resolution of allodynia in the plantar incision model. These findings highlight the critical role of adaptive metabolic responses in pain resolution and identify ASCT2-mediated glutamine metabolism as a potential therapeutic target for chronic pain. Understanding the endogenous mechanisms that promote pain resolution can guide the development of novel interventions to prevent the transition from acute to chronic pain.

Disclosures: M. Haque: None. **P. Kuppusamy:** None. **O.K. Melemedjian:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

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Program #/Poster #: PSTR165.12/E27

Topic: D.01. Somatosensation – Pain and Itch

Support: CA263777
CA241627

Title: Resolvin D1 reduces tumor-evoked hyperalgesia by reducing sensitization of DRG neurons

Authors: *V. VIATCHENKO-KARPINSKI, A. HEROLD, M. JOHNS, I. KHASABOVA, D. A. SIMONE, S. G. KHASABOV;
Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Resolvin D1 reduces tumor-evoked hyperalgesia by reducing sensitization of DRG neuronsViacheslav Viatchenko-Karpinski, Annabelle Herold, Malcolm Johns, Iryna A. Khasabova, Donald A. Simone and Sergey G. KhasabovKeywords: Pain, Bone cancer, DRG neurons, Resolvin D1 (RvD1) Pain is one of the most debilitating symptoms accompanying primary and metastatic bone cancer. About 80% of patients with end-stage bone cancer complain of severe persistent pain and hyperalgesia, and in 40% of patients pain is poorly controlled. NSAIDs and opioids remain the primary pharmacotherapies to treat bone cancer pain, despite their complex side effects. New and safe approaches to manage cancer pain are needed. Here we show that Resolvin D1 (RvD1), one of the specialized lipid pro-resolving mediators, is a promising candidate. Tumors were generated in mice by injection of mouse NCTC clone 2472 fibrosarcoma cells unilaterally into and around the calcaneus bone. Unilateral mechanical hyperalgesia developed equally in both male and female mice. Hyperalgesia was defined as an increase in the frequency of paw withdrawal in response to a von Frey monofilament with a bending force of 0.4 g applied to the plantar surface of the paw. Unlike the vehicle, a single intravenous injection of RvD1 (0.001 – 10 μg/kg) decreased hyperalgesia in mice of both sexes with similar potency (ED50=0.0015 μg/kg) and efficacy. RvD1 had no effects in naive mice. Tumor-evoked hyperalgesia was associated with sensitization of primary afferent neurons isolated from the L3-L5 dorsal root ganglia (DRG). Retrograde labeling of neurons innervating

the tumor-affected area of the paw was performed by injection of the lipophilic dye, DiI, into the proximal half of the plantar surface 10 days before tumor implantation. Only DiI-positive neurons were studied. Nociceptive neurons were identified by the shape of their action potential, membrane capacitance (Cm<40 pF), and size (cell area<500 μm2). Sensitization was characterized by a decrease in rheobase and an increase in inward currents. Direct application of RvD1 (5 nM) reduced hyperexcitability of DRG neurons isolated from tumor-bearing mice by suppressing the inward currents and by recovery of the rheobase. Taken together, these data suggest a therapeutic potential for resolvin-based approaches for the treatment of bone cancer pain.Acknowledgments: Supported by NIH grants CA263777 and CA241627.

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Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

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Topic: D.01. Somatosensation – Pain and Itch

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Title: Oral cancer associated functional expression of transient receptor potential cation channel subfamily v member 4 in trigeminal ganglion neurons

Authors: *G. E. HARDEN, Y. MULPURI, K. INOUE, N. HUU-TU, S. NICHOLSON, D. ALBERTSON, B. L. SCHMIDT;
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Abstract: Oral squamous cell carcinoma (SCC) is considered one of the most painful cancers, yet the mechanisms involved are not well understood. Oral cancer patients report high levels of pain associated with mechanical movements and stretch. Transient receptor potential cation channel subfamily V member 4 (TRPV4) is a stretch-induced ion channel, suggesting a role for TRPV4 in mediating stretch induced oral cancer pain. In oral tongue cancer mouse models, expression of TRPV4 is detected in cancer cells, Schwann cells, immune cells, fibroblasts and vascular endothelial cells as demonstrated by immunohistochemistry and/or single cell RNA sequencing. To begin to elucidate the role of TRPV4 in oral cancer pain, we are measuring functional expression of TRPV4 on different cell types in the tumor microenvironment using

calcium imaging. We have demonstrated functional expression of TRPV4 on cancer cells, Schwann cells and oral keratinocytes. This study aims to elucidate the role of TRPV4 in trigeminal ganglion (TG) neurons isolated from naïve and oral tongue cancer bearing mice. TG neurons dissociated from wild type mice were treated with 1μM Fura2-AM and calcium imaging was performed in the presence of a selective TRPV4 agonist (GSK1016790A), Ionomycin (1μM) and a TRPV1 agonist, capsaicin (1μM). Of ionomycin responsive cells tested from TG cultures we find that 27% responded to both ionomycin and GSK1016790A (300nM), whereas 36% of cells responded to both ionomycin and capsaicin. No cells responded to both capsaicin and GSK1016790A. These results align with the results obtained from patch clamp recordings of nociceptive neurons (≤ 25 μm) isolated from the mandibular division of mouse TG where ≥ 40% of neurons responded to capsaicin (1 μM). None of the small-diameter neurons (14/14 recorded) responded to the TRPV4 agonist at 300 nM and 1 μM drug concentrations. Since TG cultures contain endoneural fibroblasts, Schwann cells and neurons, the tissue of origin of GSK1016790A responsive TG cells is being determined in TG cultures from genetically engineered mouse models in which the different cell types are distinguished by fluorescent labeling. It is unclear from published studies whether functionally active TRPV4 is expressed on TG neurons. Our studies will help to define the functional expression of TRPV4 on TG neurons to understand the role of neuronal TRPV4 in oral cancer pain.

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Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

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Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant R01NS121533
NIH COBRE P20GM103642

Title: Rna-binding protein celf4 modulates nerve growth factor signaling

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Abstract: Gene expression is controlled by interacting networks of regulatory pathways before, during and after both transcription and translation. Post-transcriptional regulation involves interactions between mRNAs and the proteins that bind them, controlling their stability,

localization and translation. These RNA binding proteins (RBPs) selectively bind to small RNA motifs, which can be found on multiple sets of mRNAs, forming gene expression regulons coordinated by RBP-transcript interactions. Post-transcriptional regulation of transcripts is especially critical for peripheral neurons, which are large cells with termini remote from the cell body. Required transcripts are transported along axons to the termini where they are translated as needed. Changes in mRNA transport, stability and translation modulate the excitability of neurons, and the understanding of these processes is critical for discovering mechanisms of neuroplasticity and diseases of the peripheral nervous system such as maladaptive pain and neuropathies. PC12 cells are a common cell line used to study neuronal physiology and intracellular signal transduction. When cultured with nerve growth factor (NGF), these cells differentiate in a dose-dependent manner, undergoing physiological and morphological changes and assuming a neuron-like phenotype. Using RNA-sequencing, we profiled NGF-dependent changes in gene expression, revealing hundreds of differentially regulated RBPs. Among these genes is CUGBP Elav-Like Family Member 4 (CELF4), an RBP that has been previously implicated in the regulation of neuronal excitability. This protein is downregulated by NGF withdrawal and upregulated by NGF stimulation. Computational analyses predicted that this protein preferentially binds to NGF-regulated transcripts, including the high-affinity NGF receptor TRKA (gene name *Ntrk1*). This interaction was confirmed by RBP-immunoprecipitation using validated CELF4 antibody. To investigate these interactions, an inducible CELF4-overexpression model was generated in PC12 cells. This upregulation's effect on cellular function is analyzed through morphological and physiological assays, and direct mechanistic analysis of singular protein-RNA interactions. Preliminary findings suggest that CELF4 works to modulate neuronal physiology by post-transcriptional regulation of target transcripts in PC12 cells , including TRKA, and that this function may translate to *in-vivo* models.

Disclosures: P. Neufeld: None. **M. Mueth:** None. **L.A. Fitzsimons:** None. **E. Grlickova-Duzevik:** None. **B.J. Harrison:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.15/E30

Topic: D.01. Somatosensation – Pain and Itch

Support: R01NS121533
P20GM103642

Title: Uncovering mechanisms of post-transcriptional regulation in persistent pain

Authors: *M. MUETH[1], P. NEUFELD[2], L. A. FITZSIMONS[2], E. GRLICKOVA-DUZEVIK[3], B. J. HARRISON[1];

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Abstract: Despite the availability of a wide variety of therapeutic strategies, chronic pain management remains challenging due to limitations including side effects and risks associated with pharmacological interventions, variable response to treatment among patients, and limited long-term effectiveness, leaving patients battling untreated and/or recurrent pain. Due to the limitations associated with current therapeutics, there is a significant need for the development of alternative strategies to manage chronic pain. Persistent inflammatory pain is dependent on de novo protein synthesis in sensory neurons. After injury, release of inflammatory factors such as nerve growth factor (NGF) enhances pain transduction through modulation of nociceptive ion channels, receptors, and neurotransmitters through local and somatic signaling events within sensory neurons. Identifying post-transcriptional regulatory mechanisms that control the translation of these nociceptive mRNAs may allow for successful modulation of sensory neuron sensitivity in persistent pain. Through histological and in silico analyses, we identified that the RNA-binding protein CUGBP Elav-like family member 4 (CELF4) is expressed in TRPV1-expressing sensory neurons in the dorsal root ganglia (DRG) and that CELF4 preferentially associates with many transcripts of pronociceptive genes. Therefore, we generated conditional knockout (KO) mice with Celf4 deleted from adult DRG neurons to investigate its role in pain signaling. This revealed that Celf4 KO causes mouse sensory neurons to become extremely hyperexcitable compared to wild-type controls and these mice display robust mechanical and thermal behavioral hypersensitivities. Additionally, Celf4 KO induces an exaggerated response to low dose intraplantar NGF. To assess the role of CELF4 in regulating the translation of nociceptive mRNAs, we used RNA Immunoprecipitation sequencing and Translating Ribosome Affinity Purification sequencing to confirm CELF4 binding with nociceptive targets and assess changes in translational efficiencies of these targets under conditions of Celf4 KO. Additionally, histology and western blot were used to assess changes in the expression of these nociceptive targets within Celf4 KO sensory neurons. These studies revealed that CELF4 is a powerful negative regulator of sensory neuron excitability and behavioral sensitivities in mice. These findings support CELF4 as a promising candidate regulatory mechanism within sensory neurons that may be leveraged to reduce sensory neuron sensitivity in persistent inflammatory pain conditions.

Disclosures: M. Mueth: None. **P. Neufeld:** None. **L.A. Fitzsimons:** None. **E. Grlickova-Duzevik:** None. **B.J. Harrison:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.16/E31

Topic: D.01. Somatosensation – Pain and Itch

Support: R01NS121533
P20GM103642

Title: Regulation of DRG neuron excitability, glomerular structure, and renal function by the RNA-binding protein CELF4

Authors: L. A. FITZSIMONS[1], M. MUETH[2], ***B. HARRISON**[3];
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Abstract: Over 35.5 million Americans are living with chronic kidney disease (CKD), many of whom develop uncontrolled hypertension, increasing the likelihood of requiring kidney transplantation. One factor influencing onset/progression of CKD is the sustained, pathogenic coactivation of renal sympathetic and sensory reflexes, termed renorenal hyperreflexia (RRH). Activation of RRH via inflammatory factors, such as nerve growth factor (NGF), can lead to structural to the glomerulus, the functional filtration unit of the kidney. Preliminary data from our lab demonstrates that pathogenic activation of sensory nerve fibers is augmented by CELF4 RNA binding protein. We investigated the role of CELF4 as a tonic regulator of renal dorsal root ganglia DRG neuron (RDN) excitability, RRH and kidney function using a tamoxifen-inducible, Calcacre mouse to generate a conditional Celf4-knockout (KO) from adult sensory neurons. Behavioral analyses of Celf4-KO (N=6 male, N=6 female) revealed robust hind-paw hypersensitivity to mechanical and thermal stimuli, that was further exacerbated with NGF injection. RDN and whole kidneys from Celf4-KO mice were processed for histopathological analyses (N=6 male; N=6 female). Patch-clamp recordings confirmed that acutely-dissociated, capsaicin-sensitive (TRPV1+) DRG neurons become hyperexcitable following Celf4-KO. Immunofluorescence analyses show coexpression of CGRP, TRPV1 and CELF4 in RDN. Histological analyses using H&E and PAS-silver stained kidney sections from Celf4-KO revealed a novel inflammatory kidney injury pattern, characterized by pronounced mesangial expansion, extracellular matrix deposition, hypercellularity and incidence of glomerular sclerosis consistent with membranoproliferative glomerulonephritis. Whole-kidney gross examination revealed that Celf4-KO kidneys displayed hydronephrosis and increased pre-fixation weight when compared to vehicle controls in both male and female mice. Taken together, we have shown that CELF4 is co-expressed with TRPV1 in CGRP+ DRG neurons, and that loss of CELF4 in CGRP+ nerve fibers upregulates neural excitability/sensitivity of DRG neurons to NGF, leading to development of MPGN inflammatory kidney disease. We present preliminary findings from an ongoing study investigating CELF4 as a tonic, negative regulator of the renorenal reflex, RDN excitability and inflammation-induced RRH responsible for impairing renal/glomerular function in hypertension and CKD. Our results support the feasibility of targeting this pathway as a potential treatment/management strategy for inflammatory chronic kidney diseases and hypertension.

Disclosures: L.A. Fitzsimons: None. **M. Mueth:** None. **B. Harrison:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.17/E32

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant 7R21NS125484-02
NIH Grant 1P20GM152330-01 8074

Title: Stress Due to Loss-of-Enrichment Induces Mechanical Hypersensitivity and Increased Activity in Satellite Glial Cells

Authors: *L. T. HOWLAND[1,2], A. R. FRENCH[3], L. F. QUEME[3,2];
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Abstract: Chronic pain is a significant problem with approximately 20% of US adults experiencing it at any moment in time. Chronic exposure to stress has been associated with higher risk for developing chronic pain. Although many studies have linked chronic stress with the development of painful conditions, in both animal models as well as human population studies, the underlying mechanisms of how stress can influence the transition from acute to chronic pain are still unclear. Satellite Glial Cells (SGCs) are support cells that envelop sensory neurons located in the Dorsal Root Ganglia (DRGs). SGCs have been shown to present increased activity that is linked with increased expression of Glial Fibrillary Acidic Protein (GFAP) and has been frequently associated with exacerbated pain behaviors in various rodent models of injury. Increased GFAP expression in SGCs has been also associated to mechanical hypersensitivity in mice exposed to IgG from patients with Fibromyalgia, a condition characterized by diffuse musculoskeletal pain that has a higher incidence in patient suffering chronic stress. Our lab has recently developed a modified stress inducing protocol for mice, based on loss of environmental enrichment (LOE) and paired it with a model of hindlimb muscle ischemia with reperfusion injury (I/R). We have previously shown that LOE is capable by itself of inducing mechanical hypersensitivity and changes in stress-related behavior. Recent studies have shown that SGCs can also become activated in rodent models of stress. Thus, we hypothesized that LOE would induce mechanical hypersensitivity and that this would correlate with increased expression of GFAP in the SGCs surrounding the sensory neurons innervating the muscles. To test this, we exposed animals to our LOE stress paradigm that consisted of enriched, group housing environment for 3 week and later to 1 week of solo housing without any environmental enrichment. Mice then received an ischemia with reperfusion injury of the right hindlimb. Pain-related behaviors were assessed at baseline, after completion of the LOE protocol, and 1, 3, 5, and 7 days after I/R. We also collected muscle and DRG samples at the same time points for immunohistochemical analysis. We observed significant mechanical hypersensitivity after LOE that got further exacerbated after I/R that recovered after 5d in the control group but not in the animals exposed to LOE. We also detected increased GFAP signal in the DRG from mice exposed to LOE in comparison with conventional housing controls. These findings suggest that the hypersensitivity observed after LOE may be linked to increased activity from the SGCs.

Disclosures: L.T. Howland: None. **A.R. French:** None. **L.F. Queme:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.18/E33

Topic: D.01. Somatosensation – Pain and Itch

Support: Canadian Institutes of Health Research (FDN167276)

Title: Subversive compensation during chronic sodium channel blockade reduces the efficacy of subtype-selective sodium channel inhibitors

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Abstract: Voltage-gated sodium (Nav) channels play an important role in neuronal excitability. Nav subtypes 1.3, 1.7 and 1.8 are particularly important for the excitability nociceptive sensory neurons, yet drugs that selectively block Nav1.7 or Nav1.8 have struggled in clinical trials. We investigated whether their poor efficacy is because chronic blockade of one type of Nav channel triggers compensatory upregulation of other Nav channels. Such compensation is possible since excitability is degenerate, meaning different channel combinations can produce similar excitability. Cultured nociceptors normally rely on Nav1.7 and Nav1.3 for spike generation, as evident from acutely blocking either channel pharmacologically. But nociceptor excitability was unchanged after blocking either channel chronically (for 24 hours or for 4-7 days). This is due to compensatory upregulation of Nav1.3 when Nav1.7 is blocked, or vice versa. When both channels were blocked with TTX, nociceptor excitability became more reliant on the TTX-resistant channel Nav1.8. Changes in channel expression were verified with immunocytochemistry. In vivo, tactile and thermal hypersensitivity caused by chronic inflammation were reversed by the first dose of a Nav1.7-selective inhibitor, but this effect was absent by the second day of testing after twice daily dosing of the drug. This loss of efficacy is consistent with the time course of compensatory changes observed in vitro. Notably, preclinical pain testing traditionally involves a single drug dose despite patients needing to take drugs chronically. Our results show that subversive compensation can reduce drug efficacy during chronic treatment.

Disclosures: S. Ratte: None. **Y. Xie:** None. **S.A. Prescott:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.19/E34

Topic: D.01. Somatosensation – Pain and Itch

Support: CAPES
FAPEMIG APQ-03767/23
CNPq 310968/2023-2
Faculdade de Saúde Santa Casa BH

Title: Structural characterization nerve terminal actions and motor side effects of PEP2: a synthetic-shortened peptide derived from a Phoneutria nigriventer venom toxin

Authors: *C. CASTRO JUNIOR[1], D. ASTONI[1], B. GOMES[1], C. COUTO ALVARENGA[1], L. ASSIS[2], T. PEREIRA[1], N. RODRIGUES[3];
[1]Pós Graduação em Ciências da Saúde Santa Casa BH, Faculdade de Saúde Santa Casa BH, Belo Horizonte, Brazil; [2]Psychological & Brain Sci., Indiana Univ., Bloomington, IN; [3]Iguaçu Univ. - Itaperuna Campus, Itaperuna, Brazil

Abstract: Introduction: The venom of the Brazilian "armed" spider *Phoneutria nigriventer* is a rich source of bioactive molecules. Some peptidic fractions of this venom are neurotoxic and one of these fractions, Phα1β toxin, is a 55 aminoacids peptide that acts as a blocker of neuronal voltage-gated calcium channels and TRPA1 receptors. Also, Phα1β has a remarkable analgesic activity in pre-clinical studies using rodent models of pain. The difficulty of obtaining Phα1β directly from the venom, the complexity and unresolved tertiary structure of this toxin, and other pharmacokinetic issues represent obstacles to the development of a formulation containing Phα1β directly from the venom. We recently confirmed the hypothesis that synthetic-shortened fragments of Phα1β retain the antinociceptive action of Phα1β. Following computational analysis of Phα1β sequence, we proposed and synthesized PEP2. This is a 10 amino acid peptide with antinociceptive activity in acute and inflammatory pain models in mice. **Aims:** to perform structural studies to elucidate the tertiary structure of the peptide. Also, evaluate motor side effects, in mice, after intrathecal administration of PEP2 and, evaluate the mechanism of action of PEP2 over intracellular calcium dynamics and exocytosis on isolated nerve terminals (synaptosomes). **Methods:** Structural analysis was conducted by mass spectrometry, cellular dichroism, and nuclear magnetic resonance. In vivo motor effects were performed using the open-field test in swiss mice. In vitro mechanisms were evaluated by fluorimetry procedures using mice cortical-brain synaptosomes stimulated with KCl. **Results**: Our data confirmed the molecular weight, and amino acid sequence of the peptide, and demonstrated that a disulfide bond exists in the molecule. Dichroism analysis shows that PEP2 exhibit random coil conformation. The nuclear magnetic resonance demonstrates that PEP2 presents a secondary coiled conformation suggestive of a β-hairpin tertiary structure. Open field tests showed no significant motor alterations after injection of PEP2 at antinociceptive doses. PEP2 dose-dependently reduced the synaptosomal KCl-induced $[Ca2+]_i$ increase with IC50 value = 0.02 nm. At 1 μM, PEP2 reduced the KCl-induced $[Ca^{2+}]$ increase by 43.5%. PEP2 also reduced (up to 58.5%) the KCl-induced exocytosis, suggesting PEP2 is an inhibitor of cortical

neurotransmission. **Conclusions**: PEP2 is a neuroactive molecule with a defined tertiary structure and without acute motor effects. Data from this project provided valuable insights into the structure and mechanisms of a new molecule potentially useful for the treatment of pain conditions.

Disclosures: C. Castro Junior: None. **D. Astoni:** None. **B. Gomes:** None. **C. Couto Alvarenga:** None. **L. Assis:** None. **T. Pereira:** None. **N. Rodrigues:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.20/E35

Topic: D.01. Somatosensation – Pain and Itch

Support: Hospital São José do Avaí
Universidade Iguaçu - UNIG
Marjan Farma

Title: Obtaining a peptide with antinociceptive activity from the in silico analysis of Phoneutria nigriventer venom toxin

Authors: *N. RODRIGUES[1], A. RODRIGUES[1], C. CASTRO JUNIOR[2], D. ASTONI[3], L. ASSIS[4];
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Abstract: *Phoneutria nigriventer* is a spider that has been responsible for several accidents involving poisoning in Brazil, and of which have caused fatalities. The venom of the spider is formed from a variety of molecules, and it is able to interfere with biological mechanisms. Some of these molecules, and especially the toxins, are capable of causing an antinociceptive effect. The research into the formulation of drugs that are capable of becoming future analgesic agents has advanced. This advancement has also come about with the help of bioinformatics that have been used in regards for developing new compounds that are based on molecular modelling. The objective was to develop a synthetic peptide that had an analgesic action similar to Phα1β (PnTx3-6) with a smaller amino acid sequence. Its elaboration was based on a bioinformatic analysis of the Phα1β (PnTx3-6) structure as well as an analysis of all the other toxins that are part of the peptide portion. After the analysis of the similarities among these toxins, and their ability to form epitopes, it was proposed that three peptides (PEP1, PEP2 and PEP 3) be submitted for structural analysis in silico by programs that would be capable of simulating the peptide structures, and of analyzing their functions based on the existing database of experimentally resolved proteins. Once the *in silico* analysis was completed, these peptides were, then, chemically synthesized and subsequently injected intrathecally into Wistar rats in order to assess the ability of the peptides to block nociceptive stimulus in the hot plate test. PEP2 was the

peptide that presented antinociceptive effect at the dose of 1000pmol/site, being purified and resynthesized, starting with the intraplantar capsaicin injection test. Its antinociceptive activity was maintained in the capsaicin test when compared with native and recombinant Phα1β (PnTx3-6) toxin. Some PEP2 action targets have been proposed based on the analysis of their chemical similarity to other experimentally resolved compounds, and the most likely biological targets of PEP2 have been found to be: type 2 FF neuropeptide receptor; delta-type opioid receptor; mu-type opioid receptor; and kappa-type opioid receptor. This result showed that a peptide that was generated by chemical synthesis, which was based on the analysis of the similarity between the primary molecular structure and that of the epitopes belonging to the *Phoneutria nigriventer* toxins, is capable of producing analgesia in pain models, and can pave the way for future analyses of the primary protein structure and epitopes, with the aim of building simpler bioactive compounds that can act as targets for the treatment of various conditions.

Disclosures: N. Rodrigues: None. **A. Rodrigues:** None. **C. Castro Junior:** None. **D. Astoni:** None. **L. Assis:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.21/E36

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant R01NS113965
NIH Grant R01NS105715
NIH Grant F31NS135995

Title: Prolactin receptor modulates hypersensitivity in female mice following repeated ischemia with reperfusion injury

Authors: *M. QUIJAS[1], L. F. QUEME[2], M. C. HOFMANN[3], M. P. JANKOWSKI[4]; [1]Univ. of Cincinnati, Cincinnati, OH; [2]Biomed. Sci., Univ. of New England, Biddeford, ME; [3]Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [4]Dept Anesthesia, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: Myalgia is a common cause of disability, exercise intolerance, and emotional distress. One common cause of myalgia is ischemic injury, which occurs when there is a decrease in blood flow leading to impaired oxygen supply to the affected tissues. This occurs in disorders like fibromyalgia and complex regional pain syndrome which is shown to differentially affect males and females. In our model of prolonged ischemic myalgia, where animals experience repeated I/R injury, we identified sensitization in females to be associated with interleukin 1 receptor type 1 (IL1r1) and transient receptor potential cation channel (TRPV1) in the dorsal root ganglion (DRGs). Additionally, affected muscle from females exhibited higher amounts of

interleukin 1 beta (IL1β). Sex specific alterations in DRG neurons appeared to be due to differential phosphorylation of the RNA-binding protein, AU-rich element RNA binding protein (AUF1). We therefore wanted to determine what might be regulating pAUF1 in females specifically. Hormones are known to modulate preclinical and clinical models of pain, especially prolactin (PRL), which plays a prominent role in inflammatory pain in females. Using a Prlr antagonist in this model, we found inhibited TRPV1 and pAUF1 upregulation in the DRGs and partially blunted mechanical hypersensitivity behaviors. To determine the cell types involved in the observed effects, we developed a TRPV1cre;$Prlr^{f/f}$ mouse model to create a knockout of Prlr in TRPV1+ neurons and a LysMCre;$Prlr^{f/f}$ mouse to delete Prlr in infiltrating macrophages (the putative source of IL1β). This study will be important in potentially identifying factors regulating chronic ischemic myalgia in females.

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Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR165.22/E37

Topic: D.01. Somatosensation – Pain and Itch

Title: Sensory neuron expressed TRPC3 mediates gout pain via the regulation of Cav1.2 channels

Authors: *L. QU[1,3], W. LIU[1], Y. LIU[3], L. YANG[2,1];
[1]Univ. of South China, Hengyang, China; [2]Univ. of South China, Heng Yang, China; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Gouty arthritis is a common inflammatory arthritis, resulting from monosodium urate (MSU) crystal deposition in joints. Joint pain is a major symptom in gouty arthritis, severely affecting the quality of life. However, the underlying mechanisms remain poorly understood. Transient receptor potential canonical 3 (TRPC3) is expressed in a subset of dorsal root ganglion (DRG) neurons. Moreover, TRPC3 was shown to be functionally coupled to the receptors for multiple inflammatory mediators in DRG neurons that triggered peripheral sensitization. Yet, the full extent to which TRPC3 contributes to the sensitization of joint nociceptors and gout pain remains entirely unknown. Using RNAscope in situ hybridization, we found that TRPC3 was expressed in a subset of mouse joint nociceptors and human DRG. Intra-articular injection of TRPC3 agonist evoked calcium responses in joint innervating DRG neurons in vivo and behavioral signs of acute join pain hypersensitivity without obvious concurrent joint inflammation. These effects were significantly diminished in global and sensory neuron specific TRPC3 knockout mice. In a murine model of gouty arthritis, genetic deletion or pharmacological blockade of TRPC3 remarkably attenuated hyperactivity of joint nociceptors in vivo and gout pain without measurably altering joint inflammation. Fura-2 calcium imaging assay revealed that

MSU directly activated recombinant TRPC3 overexpressed in HEK-293 cell lines. MSU evoked calcium responses in a subset of dissociated DRG neurons of wildtype mice. The proportion of MSU responding DRG neurons was significantly reduced in TPRC3 knockout mice, indicating that MSU may serve as an endogenous TRPC3 agonist and directly activates primary sensory neurons through TRPC3. In addition, TPRC3 was coexpressed with Cav1.2 channels on mouse and human DRG neurons. The basal expression levels of Cav1.2 mRNA were lower in the DRG of TRPC3 knockout mice compared to wildtype controls. Genetic knockdown or knockout of Cav1.2 channels reversed TRPC3 agonist induced acute articular hypernociception and gout pain. These findings suggest that neuronal TRPC3 contributes to gout pain via the regulation of Cav1.2 channels and thus define neuronal TRPC3 as a new potential therapeutic target for treating gout pain.

Disclosures: L. Qu: None. **W. Liu:** None. **Y. Liu:** None. **L. Yang:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

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Program #/Poster #: PSTR165.23/E38

Topic: D.01. Somatosensation – Pain and Itch

Support: NSFC grant

Title: A short peptide derived from the nature venom, acts as a non-opioid analgesic to attenuate mechanical hyperalgesia

Authors: J. WANG[1], Y. MEI[1], W. XIAOHUI[2], Z. JUNHAN[1], Y. SHUANGSHUANG[1], F. QINGKE[1], Y. XU[1], M. TANG[3], Z. ZHANG[1], ***Q. TANG**[1];
[1]Jiangsu Province Key Lab. of Anesthesiol., Xuzhou, China; [2]Jiangsu Province Key Lab. of Anesthesiol., Xu Zhou, China; [3]The Affiliated Hosp. of Southwest Med. Univ., Luzhou, China

Abstract: Polypeptide toxins play a central role in understanding the physiological and pathophysiological functions in ion channel studies. They led to important advances in basic research and even to clinical applications in the field of pain. The venom GsMTx4, a selective mechanosensitive (MS) channel inhibitor, was reported to reduce mechanical and neuropathic pain. However, the underlying mechanism as well as the target remain unknown. In this study, by using rodent models of pain, we identified a 17-residue peptide based on the nature sequence of GsMTx4, and showed that this peptide was able to reduce mechanical hyperalgesia and neuropathic pain. This peptide, which we call P10581, is not toxic in mice/rats but shows a potent analgesic effect on mechanical hyperalgesia upon peripheral and central injections that can be as strong as morphine. The anti-hyperalgesic effect of peptide is, however, resistant to naloxone (an μ-opioid receptor antagonist), it causes non-tolerance; no motor impairment, or conditioned place preference (CPP). Pharmacological inhibition of TRPV4 by P10581 in a heterogeneous expression system, combined with the use of TRPV4 knockout mice indicates that

TRPV4 channels may act as the potential target for the analgesic effect of the peptide. This study identified a new potential therapeutic target for mechanical and neuropathic pain and introduced a natural toxin-based short peptide that produces potent analgesia via the blockade of the TRPV4 channel.

Disclosures: J. Wang: None. **Y. mei:** None. **W. xiaohui:** None. **Z. junhan:** None. **Y. shuangshuang:** None. **F. Qingke:** None. **Y. xu:** None. **M. Tang:** None. **Z. Zhang:** None. **Q. Tang:** None.

Poster

PSTR165: Peripheral Mechanisms of Pain and Analgesia

Location: MCP Hall A

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Program #/Poster #: PSTR165.24/E39

Topic: D.01. Somatosensation – Pain and Itch

Support: Craig H Neilsen Foundation 882060
Indiana Spinal Cord & Brain Injury Research Fund from the Indiana State Department of Health (2020) (YX)

Title: Molecular Determinants of Resurgent Sodium Currents Mediated by Navβ4 Peptide and A-type FHFs

Authors: *Y. XIAO[1], Y. PAN[2], T. CUMMINS[2];
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Abstract: Resurgent current (I_{NaR}) generated by voltage-gated sodium channels (VGSCs) plays an essential role in maintaining high-frequency firing of many neurons and contributes to disease pathophysiology such as epilepsy and painful disorders. Targeting I_{NaR}may present a highly promising strategy in the treatment of these diseases. Navβ4 and A-type FHFs have been identified as two classes of important I_{NaR} mediators; however, their receptor sites in VGSCs remain unknown, which has hindered the development of novel agents to effectively target I_{NaR}. Navβ4 and FHF4A mediate I_{NaR} generation through the amino acid segment located at C-terminus and N-terminus, respectively. Here, we show that the receptor of Navβ4 peptide involves four residues N395, N945, F1737 and Y1744 within Nav1.7 DI-S6, DII-S6, and DIV-S6. We show that A-type FHFs mediate generation of Nav1.8 I_{NaR} depends on the segment located at the very beginning, not at the end, of its N-terminus domain. We show that the receptor site of A-type FHFs also resides in VGSC inner pore region, but only partially overlaps with that of Navβ4 peptide. Cryo-EM structures reveal that several of the side chains of these critical residues project into the VGSC channel pore, and therefore our findings not only evidence that Navβ4peptide and A-type FHFs function as open-channel pore blockers but also highlight channel inner pore region as a hotspot to develop novel agents targeting I_{NaR}.

Disclosures: Y. Xiao: None. **Y. Pan:** None. **T. Cummins:** None.

Poster

PSTR166: Cortical Processing in Nociception

Location: MCP Hall A

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Topic: D.01. Somatosensation – Pain and Itch

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Title: The Neurobiological Mechanism underlying Acupuncture Treatment for Vestibulodynia

Authors: *Y. LIU, X.-Y. GAO;
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Abstract: From the perspective view of Chinese medicine, the liver meridian, starting from the hallux and connecting with the genitals along the lower limbs, reflects the vital phenomenon of the somato-somatic relationship. Multiple studies from humans and rodents have shown that the cortical representation of hallux and genitalia is adjacent. In decades, a competitive activity-dependent interaction of the adjacent representations in primary cortex has been demonstrated. How neuronal circuitry in the primary somatosensory cortex (S1) is modified during functional reorganization and the relationship between functional reorganization and the intensity of peripheral noxious stimuli remains to be elucidated. In combination of neurotropic virus tracing, electrophysiological recording and two-photon calcium imaging methods, it was found that in S1, the neurons receiving signals from hallux are neighboring to those areas receiving signals from the genital receptive field separately; the convergent neurons of hallux and genitals were also observed. Following deafferentation of hindlimbs, peristimulus time histograms showed that multiunit responses of S1 to air-puff stimulation of the genitals were increased. By imaging neuronal activity in the mice model of vaginal distention, we found enhanced pyramidal neuron activity and connectivity in genital cortex. But the activity and connectivity of inhibitory neurons responded to hindlimb transiently decreased. Physiologically, cross-correlograms indicated that inhibitory neurons of hindlimb might affect the activity of pyramidal neurons of genitals through monosynaptic inhibition. However, the strength of the monosynaptic interactions decreased under vaginal distention. Electroacupuncture stimulation at hallux could restore these inhibitory connections and downregulate neural hyperactivity in genital cortex. Taken together, our results not only reveal cortical circuit underlying smatosomatic relationship between hallux and genitalia but also provide a potential strategy for altering nociception arising from the female reproductive tract.

Disclosures: Y. Liu: None. **X. Gao:** None.

Poster

PSTR166: Cortical Processing in Nociception

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR166.02/F1

Topic: D.01. Somatosensation – Pain and Itch

Support: STI2030-Major Projects (2022ZD0214400)
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Guangdong Basic and Applied Basic Research Foundation (2022B1515130007)
Guangdong Basic and Applied Basic Research Foundation (2023B1515040015)

Title: Dock4 regulates activity of excitatory neurons in the ventrolateral orbital cortex for antinociception

Authors: *Y. YANG[1], D. GUO[2], B. JIANG[3], L. SHI[4];
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Abstract: Objective Autism spectrum disorder (ASD) is a neurodevelopmental disorder frequently accompanied by altered sensibility to pain such as allodynia and hyperalgesia, but the cause of ASD-related pain has remained unclear. It is of note that ASD patients show pathological changes in orbitofrontal cortex, and the ventrolateral orbital cortex (VLO) is known to regulate pain through projecting to the ventrolateral periaqueductal gray (vlPAG), a key region in the midbrain that produces analgesic effects. Our previous study revealed that mice with knockout of *Dock4*, an ASD candidate gene, display autism-like social deficit, elevated anxiety, and cognitive abnormities, but whether these mice have impaired pain perception has not been explored. The present study aims to elucidate the mechanism of *Dock4* in the regulation of the excitability of VLO neurons and thus the regulation of mechanical allodynia. **Methods** (1) DREADDs-based chemogenetic technology was used to change neuronal excitability. (2) The paw withdrawal threshold (mechanical allodynia) was measured by Von Frey Test. (3) Patch clamp technique was used to record the changes of neuronal function in Layer 5 pyramidal neurons in VLO. **Results** (1) *Dock4* KO mice showed decreased paw withdrawal threshold. (2) DOCK4 protein is highly expressed in VLO excitatory neurons. (3) *Dock4* in excitatory neurons regulated mechanical allodynia whereas *Dock4* in inhibitory neurons was dispensable for pain levels. (4) Whole-cell recording on Layer 5 pyramidal neurons of VLO showed markedly reduced excitation and lowered function of excitatory synapse transmission in *Dock4* KO mice. (5) DREADDs-mediated chemogenetic activation of excitatory neurons in VLO of *Dock4* KO

mice rescued the impaired paw withdrawal threshold. **Conclusions** The deficiency of *Dock4* leads to lowered activity of excitatory neurons in the VLO, resulting in mechanical allodynia of the mice, which can be rescued by the activation of excitatory neurons in the VLO. Together, we found a new mechanism of ASD-related protein DOCK4 who acts on VLO to regulate mechanical pain sensation, revealing that VLO may be a key brain region for the production of pain hypersensitivity associated with ASD. **Keywords:** autism; mechanical allodynia; ventrolateral orbital cortex

Disclosures: Y. Yang: None. **D. Guo:** None. **B. Jiang:** None. **L. Shi:** None.

Poster

PSTR166: Cortical Processing in Nociception

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Topic: D.01. Somatosensation – Pain and Itch

Support: National Natural Science Foundation of China (81971049 and 81671097)
High Education Joint Research Project of Shaanxi Province (2020GXLH-Y-014), China
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Title: Up-regulation of IL-1β and sPLA2-III in the medial prefrontal cortex contributes to orofacial and somatic hyperalgesia induced by malocclusion via glial-neuron crosstalk

Authors: *H. FENG[1,2], L. ZHONG[3], C.-X. XU[4], T.-T. WANG[4], L. WANG[3], R. J. TRAUB[5], X. CHEN[3], D.-Y. CAO[5,6];
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Abstract: The concurrent presence of temporomandibular disorders (TMD) and fibromyalgia syndrome (FMS) is a common clinical problem, yet the mechanisms behind this comorbidity are poorly understood, leading to suboptimal treatment approaches. The medial prefrontal cortex (mPFC) is known to play a critical role in the regulation of persistent pain. Our previous study demonstrated that TMD, induced by unilateral anterior crossbite (UAC), led to the development of somatic hyperalgesia, creating an animal model that represents the comorbidity of TMD and FMS. The present study investigated the involvement of the mPFC in pain modulation within this comorbid context and the underlying pathways. On the 28th day following UAC, rats exhibited increased pain responses in the orofacial area, along with thermal hyperalgesia and mechanical allodynia in the hind paws. RNA sequencing and gene expression analysis of the mPFC revealed 298 differentially expressed genes, with 126 genes increased expression and 172

decreased expression in the UAC group compared to the sham group. Pathway enrichment analysis highlighted the cytokine-cytokine receptor interaction and immune response pathways as significantly affected. Notably, the expression of group III secretory phospholipase A2 (sPLA2-III) significantly elevated in the mPFC. Inhibition of sPLA2-III expression through injection of sPLA2-III-siRNA into the mPFC alleviated both orofacial and somatic hyperalgesia. Immunofluorescence studies showed that sPLA2-III was predominantly expressed in neurons. Additionally, the expression of interleukin-1β (IL-1β) in the mPFC significantly increased after UAC, and IL-1β neutralization via antibody injection blocked the development of hyperalgesia. IL-1β was primarily localized in microglia, with no expression in neurons and astrocytes. Furthermore, administration of IL-1β antibodies post-UAC significantly reduced the expression of sPLA2-III in the mPFC. These findings implicate neuroinflammatory cascade responses mediated by glial-neuronal crosstalk in the mPFC as contributors to the development of TMD and FMS comorbidity. IL-1β and sPLA2-III emerge as potential novel therapeutic targets for the treatment of chronic pain associated with the comorbidity of TMD and FMS.

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Poster

PSTR166: Cortical Processing in Nociception

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR166.04/F3

Topic: D.01. Somatosensation – Pain and Itch

Support: JSPS KAKENHI Grant Numbers JP22K21026,JP22H03257,JP22K09920. Sato Fund, Nihon University School of Dentistry; SATO-2023-27,SATO-2024-27

Title: Cortical responses to electrical stimulation of the periodontal ligament in mice

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Abstract: Previous optical imaging studies with a voltage-sensitive dye (VSD) in rats showed that sensory information of the periodontal ligament (PDL) is processed in two cortical regions: the primary somatosensory cortex (S1) and the border between the secondary somatosensory cortex and the insular oral region (S2/IOR). However, a recent calcium imaging in GCaMP6s transgenic mice has suggested that PDL sensation is processed in three distinguished regions: the S1, S2, and IOR. To explain these contradictory findings between rats and mice, we performed the optical imaging with VSD or mitochondrial flavin fluorescence and immunohistochemistry

for expression of the protein c-Fos, a marker of neuronal activity, in mice. All experiments were performed under urethane anesthesia. In the optical imaging experiments, a craniotomy including the left S1, S2, and IOR was performed, and the intensities of fluorescence were captured with a CMOS camera. c-Fos immunohistochemistry was carried out 60 min after electrical stimulation of the upper or lower right molar PDL. In the VSD imaging, the initial cortical response to electrical stimulation of the upper or lower right PDL occurred in the IOR, and a faint response in the S1 followed. The amplitude of the IOR response was larger than that in the S1 (n = 11~15; one-way ANOVA on ranks). The flavin imaging was performed to observe the responses followed by the initial activities. Cortical responses to upper or lower right molar PDL stimulation were found in the S1, IOR, and S2. However, the signals in S2 and IOR were not to be distinguished clearly. In the immunohistochemical study, c-Fos-immunopositive cells were principally found in the IOR, and some c-Fos-immunopositive cells were found in the S1 and S2. These results suggested that cortical responses to PDL stimulation were initiated from the IOR, but not from the S1 and S2. The S2 might be activated in the later phase of information processing.

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Poster

PSTR166: Cortical Processing in Nociception

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Program #/Poster #: PSTR166.05/F4

Topic: D.01. Somatosensation – Pain and Itch

Support: JSPS KAKENHI Grant Numbers JP23K24515,JP23K18366.

Title: Regulation of nociception in orofacial area by long-term potentiation of inhibitory synapses in the insular cortex using optogenetics

Authors: *S. KOBAYASHI[1], H. OSAKI[2], S. N. FUJITA[3], S. KATO[4], K. KOBAYASHI[5], M. KOBAYASHI[6];
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Abstract: The insular cortex (IC) integrates multiple sensory information such as gustation, nociception, and thermal sensation arising from the trigeminal region. Our previous study showed corticofugal projections from the IC to the trigeminal spinal nucleus caudalis potentiate the nociception of the facial region. Parvalbumin-immunopositive neurons (PVNs) project to pyramidal neurons (PNs) and strongly suppress glutamatergic excitatory outputs from PNs. Therefore, specific activation of PVNs in the IC would suppress nociception from the orofacial

region by suppressing IC activity. In this study, we validated the effect of PVN activation on nociception in the orofacial area using optogenetics. We selectively activated PVNs by blue light using an adeno-associated virus vector [AAV5-EF1α-Flex-hChR2(H134R)-mCherry; AAV] and LE-Tg(Pvalb-cre)2Koba(+/m)PV rats (PV-Cre rats). First, we tested the effect of PNV activation on nociception-related behaviors induced by heat stimulation. As a result, nociception-related behaviors tended to be reduced, but not significantly suppressed by PVN activation. Next, to potentiate inhibitory inputs from PVNs to PNs, we investigated the protocol that induces long-term potentiation (LTP) of PVN->PN synapses. Using the whole-cell patch-clamp technique, we found that repetitive optical stimulation (ROS) similar to theta burst stimulation induced LTP of IPSCs in the IC *in vitro*. Then, we applied ROS to the IC of PV-Cre rats and recorded nociception-related behaviors. As a result, PVN activation significantly reduced nociception-related behaviors after the application of ROS. These results suggest that LTP induction of inhibitory synaptic inputs in synapses in PVN->PN suppresses intractable abnormal pain in the orofacial area.

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Poster

PSTR166: Cortical Processing in Nociception

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR166.06/F5

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant R01-GM115384

Title: Regulatory Role of the Anterior Cingulate Cortex in Chronic Pain Induced by Pancreatitis

Authors: *Q. ZHANG[1], J. WANG[2];
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Abstract: Pancreatitis, characterized by pancreatic inflammation, often manifests in persistent abdominal pain. Chronic pain triggers plasticity in anterior cingulate cortex (ACC) neurons, pivotal in aversive learning associated with chronic pain. However, the impact of chronic pancreatitis on brain activity and behavioral phenotype remains unclear. Here, we induced pancreatitis in rats via Cerulein injection. Behavioral assessment using classical conditioned place aversion (CPA) revealed higher CPA scores in pancreatitis rats compared to the saline control group, indicating increased aversion. Single photon calcium imaging demonstrated heightened neuronal response in the ACC due to pancreatitis. Furthermore, optogenetic inhibition of the ACC lowered CPA scores, suggesting the ACC's potential integral role in encoding pancreatitis-induced pain aversion.

Disclosures: Q. Zhang: None. **J. Wang:** None.

Poster

PSTR166: Cortical Processing in Nociception

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR166.07/F6

Topic: D.01. Somatosensation – Pain and Itch

Title: Lysergic acid diethylamide (LSD) decreases pain aversion

Authors: *J. PLOTKIN;
NYU, New York City, NY

Abstract: Painis the most common chief complaint for hospital visits in the US. It is acomplex experiencerelianton the integration of signals throughout the body and regulatory inputs fromseveral brainregions,including the anterior circulate cortex (ACC) and primary somatosensory cortex(S1).Recentstudies have shown that psychedelics such as lysergic acid diethylamide (LSD)haveefficacyagainst a range of neuropsychiatric conditions including depression andpost-traumaticstressdisorder. Additionally, some studies have suggested LSD could be a potentialtherapeuticoptionin the setting of chronic pain. However, the efficacy and mechanism of LSD as alonglastinganalgesicremains unknown. Here we studied the effect of LSD on both experimentallyinducedacute pain as well as chronic pain, focusing in particular on affective painsymptoms.Firstly,in a conditioned place aversion (CPA) assay, we found rats showed avoidance forthechamberassociated with mechanical or cold pain. After systemic injection of LSD,however, thisaversiveresponse is no longer observed. Further studies indicate that thisanti-aversive effect wasmediatedby the ACC, as direct injection of LSD into the ACC replicated the removal ofpainaversion.This finding was not found with injection of LSD into S1, as this led to normalpainaversionbehavior. In addition, we found that this anti-aversive effect of LSD was alsopresent inachronic neuropathic pain model. Impressively, this effect lasted at least twoweeks. Next, weexaminedthe effect of LSD on neural activity in the ACC, using an endoscopicsingle-photonminiscopeto image calcium activity of individual neurons in freely moving rats. We foundthatLSDreduced ensemble activity of ACC pyramidal neurons in response to a mechanicalpainfulstimulus.Taken together, these results demonstrate a previously undiscovered role forLSD as apotential therapeutic for both acute and chronicaffective pain symptoms.

Disclosures: J. Plotkin: None.

Poster

PSTR166: Cortical Processing in Nociception

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR166.08/F7

Topic: D.01. Somatosensation – Pain and Itch

Support: SNSF 159872
SNSF 182571

Title: Pain-anxiety ensemble dynamics in the mouse anterior cingulate cortex

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Abstract: Pain is a complex experience arising from the intricate interplay of sensory, emotional, and cognitive processes. It has been largely recognized that the maladaptive form of pain has the potential to induce comorbid mental dysfunctions, such as anxiety. This comorbidity may result from morphological and functional alterations in neuronal representations. While the anterior cingulate cortex (ACC) is crucial for both emotional processing and pain perception, the neural mechanisms underlying its influence in the development of anxiety in chronic pain remain poorly understood. Here, we aimed to characterize the representation and functional attributes of pain- and anxiety-activated neuronal ensembles in the mouse ACC using targeted recombination in active populations (TRAP), cFos staining, and population imaging in freely moving animals using miniscopes. We found that these ensembles are functionally segregated and distinct in healthy mice, with minimal overlap between pain- and anxiety-activated neurons. Accordingly, suppression of pain-activated ensembles had no significant impact on pain- or anxiety-related behaviours, and similarly, inhibition of anxiety-activated ensembles did not modulate the affective or anxious behaviour. In the chronic neuropathic pain state, functional miniscope recordings suggest an increased overlap between a supramodal ensemble (i.e., neurons responding to noxious and non-noxious stimuli) and the anxiety ensemble. These results provide evidence for neuronal alterations in chronic neuropathic pain, which might explain the development of an anxiety phenotype. Overall, our study identified a specific neural ensemble that may contribute to both nociception and mood disorders related to chronic pain.

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Poster

PSTR166: Cortical Processing in Nociception

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Topic: D.01. Somatosensation – Pain and Itch

Support: UCI Susan Samueli Integrative Health Institute (SSIHI) Pilot award and a Samueli Scholar award to X.X

Title: Electroacupuncture alleviates chronic inflammatory pain by activating retrosplenial cortex

Authors: *H. ZHANG[1], Z.-L. GUO[1], B. BERACKEY[1], L. CHEN[1], X. XU[2];
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Abstract: Acupuncture is an effective treatment option for patients with chronic pain conditions. While its mechanism is not well understood, it is appealing as an inexpensive, non-addictive medical alternative with a prolonged action. Earlier studies in rodent models indicate that electroacupuncture (EA) activates neurons in multiple cortical and subcortical brain regions, indicating that EA effects are mediated through modulation of brain activity. In the present study, we measured the effect of EA on pain sensitivity in a mouse model of complete Freund's adjuvant (CFA)-induced chronic inflammatory pain. Applying EA (2 Hz, 5 ms, 0.1-0.2 mA for 20 min) in the ST36 (Zu San Li) acupoint in the hindlimb, which is an acupoint frequently used for pain modulation, increased mechanical pain thresholds measured by the von Frey filament test, indicative of pain alleviation. The pain relief effect was confirmed by applying opto-acupuncture in ST36 in Prokr2-Cre:Ai32 mice that express channelrhodopsin-2 (ChR2) in the innervation in deep hindlimb fascia. To elucidate the neural basis of pain alleviation effects of ST36 EA, we mapped the brain activity with EA treatment in a TRAP2 mouse line (that has tamoxifen-dependent, c-fos driven recombinase CreER) crossed with the Cre-dependent Ai9 reporter mouse. Our results showed that EA in the ST36 acupoint significantly increased tdTomato labeled neuron numbers in multiple brain regions, including the secondary motor cortex (M2) and the retrosplenial cortex (RSC). Further immunochemical characterization indicated that the majority of labeled neurons in RSC is vGluT2 positive excitatory neurons. We continued to perform in vivo calcium imaging in Camk2a-Cre; Ai163 mice which express GCamP6s in excitatory neurons, to investigate the effect of EA on RSC neuron activity. Wild field calcium imaging of the cortex indicated elevated activity in RSC when EA was administrated in ST36 acupoint. The increased RSC activity by EA was also confirmed by in vivo two-photon calcium imaging, demonstrating that significantly more proportions of neurons showed stronger activity during EA sessions compared to that of control sessions. In addition, chemogenetic inhibition of excitatory neuronal activity in the RSC eliminated the pain relief effect of EA in the CFA pain model. Together our study reveals that EA alleviates chronic pain by activating RSC excitatory neuron activity, providing new insights into the neural mechanisms underlying acupuncture effect.

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Poster

PSTR166: Cortical Processing in Nociception

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Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant R01NS106301
New York Stem Cell Foundation
Stanford MBCT Program

Title: A cingulate cortico-ponto-cerebellar circuit mediates placebo analgesia

Authors: *C. CHEN[1], J. NIEHAUS[2], F. DINC[3], A. TASSOU[4], A. SHUSTER[5], L. WANG[6], A. LEMIRE[6], V. MENON[7], K. RITOLA[8], A. W. HANTMAN[9], H. ZENG[10], M. J. SCHNITZER[11], G. SCHERRER[12];
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Abstract: Pain is a multidimensional experience with sensory-discriminative, affective-motivational, and cognitive-evaluative components (Melzack and Casey 1968). While numerous neurophysiological and circuit mechanisms underlying the sensory and affective dimensions of pain have been delineated, how cognition modulates pain perception remains poorly understood. Here, we investigate neural mechanisms that mediate placebo analgesia, a widespread biomedical phenomenon during which positive expectations suffice to reduce pain perception. We show that analgesia from the expectation of pain relief is mediated by a distinct population of neurons in the rostral anterior cingulate cortex (rACC) that project to the pontine nuclei (rACC→Pn), a pair of brainstem pre-cerebellar nuclei with no known function in pain processing. We developed a behavioral assay that models placebo analgesia by conditioning mice to expect pain relief upon transitioning from a chamber with a heated floor to a second chamber. Calcium imaging of neural activity in freely moving mice revealed increased activity of rACC→Pn neurons during expectations of pain relief. Electrophysiological studies in cingulate cortical brain slices demonstrated that pain relief expectation alters the excitation-inhibition balance of rACC→Pn neurons, resulting in increased excitation. Transcriptomic studies uncovered an unusual abundance of opioid receptors in Pn neurons, further suggesting a role of the Pn in pain modulation. Optogenetic inhibition of either the rACC→Pn pathway or opioid-receptor-expressing Pn neurons disrupted placebo analgesia and lowered pain thresholds. Furthermore, a subset of cerebellar Purkinje cells exhibits activity patterns similar to those of rACC→Pn neurons during pain relief expectation, providing cellular-level evidence of a role for the cerebellum in placebo analgesia. Altogether, these findings identify a specific neural pathway that mediates expectation-based pain relief and open the possibility of targeting this novel pathway with drugs or neurostimulation methods to treat pain.

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Poster

PSTR166: Cortical Processing in Nociception

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Topic: D.01. Somatosensation – Pain and Itch

Support: NIH NINDS R35NS105076
NIH BRAIN Initiative NCCIH/NIMH R01 AT011447
NIH NINDS R0NS1109947
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NIH NCCIH R01AT010779

Title: Distinct neurons in the secondary somatosensory cortex drive discrete nociceptive somatosensory behaviors.

Authors: *D. G. TAUB[1,2], Q. JIANG[1,2], J. SU[1,2], C. CHUNG[3], A. CARROLL[4], C. CHEN[1,2], Z. HE[1,2], C. J. WOOLF[1,2];
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Abstract: As evident from human lesion mapping studies, the cerebral cortex is vital for the accurate perception of somatosensory stimuli and its dysfunction can lead to a variety of painful conditions. However, exactly which cortical substrates are responsible for processing specific modalities of somatosensory information remains unclear. We have previously shown the importance of the secondary somatosensory cortex (S2) in both male and female mice for the accurate perception of noxious mechanical and heat stimuli, but not cooling. Using fiber photometry, we found that parvalbumin (PV) inhibitory interneurons are specifically tuned to respond to noxious stimuli and optogenetically activating PV neurons produced drastic increases in sensitivity such that traditionally non-noxious stimuli are now perceived as noxious (Taub et al, 2024). Exactly how inhibition produces hypersensitivity and how somatosensory information is organized within S2 is unknown and in this study, we now use a combination of 2-photon tomography and chemogenetic manipulation to characterize the function of distinct layer V pyramidal neuron populations. We find that layer V neurons in S2 project to a variety of downstream cortical and subcortical substrates. Interestingly, we identify the secondary motor cortex (M2) as a key downstream target that mitigates somatosensory sensitivity with inhibition of S2-to-M2 neurons replicating the mechanical and heat hypersensitivity seen with global S2 inhibition. However, using specific layer V Cre-drivers, we find that when neurons expressing the retinol binding protein 4 (Rbp4) are inhibited, that mechanical sensitivity increases by 92% but has no effect on heat sensitivity. Together, these findings suggest that distinct populations of pyramidal output neurons in S2 govern distinct modalities of information, and argues that at the S2 cortical level, somatosensory information is processed via distinct streams of information and aggregated at its downstream target, M2.

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Poster

PSTR166: Cortical Processing in Nociception

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR166.12/F11

Topic: D.01. Somatosensation – Pain and Itch

Support: The OSU startup funds

Title: Breast cancer remotely induces bilateral pain through excitation of the anterior cingulate cortex

Authors: Y. HAYANO, ***H. TANIGUCHI**;
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Abstract: Hyperalgesia or allodynia experienced by cancer patients and survivors needs to be appropriately managed for patients' quality of life and treatment. However, current analgesics are often ineffective and/or associated with severe side effects. Therefore, developing novel pain management strategies stands as urgent clinical needs, and systematic understanding of the mechanisms underlying the cancer-induced pain is required to achieve this end. Previous studies in a rodent model in which cancer cells are unilaterally inoculated into the tibia of the hind limb where the mechanical pain sensitivity is tested at its paw showed that bone cancer induces unilateral pain through neuronal hyperactivation in the anterior cingulate cortex (ACC). However, since in these studies, cancer cells highly likely damage sciatic nerve branches whose manipulations have been traditionally used to induce neuropathic pain in the hind limb, it remains obscure whether they indirectly induce pain through physical nerve damage or directly impact on the pain-related signaling system. It also remains poorly understood whether and how local tumors remotely affect pain sensation across body parts. To address these issues, we first evaluated pain sensation in hind paws of animals that unilaterally have breast cancer cells in the mammary pad. We found that the pain threshold is progressively decreased as tumors develop, implying the remote induction of mechanical allodynia by breast cancer. Immunohistochemical analysis using c-fos, a neuronal activity indicator, showed significant increase of neuronal excitability in several cortical areas including the somatosensory cortex and the anterior cingulate cortex (ACC). Intriguingly, allodynia and cortical hyperexcitation were observed bilaterally without glial activation in the spinal cord at the level of the hind limb, a phenomenon observed in neuropathic and inflammatory pain models with hind limb manipulations. This breast cancer-induced allodynia was significantly ameliorated by bilaterally reducing activity in ACC excitatory neurons with chemogenetic tools. Taken together, these findings suggest that

local breast cancer globally increases cortical activity and remotely induces a bilateral pain syndrome. Moreover, like the findings in bone cancer, the ACC plays a pivotal role in allodynia induced by breast cancer. Our study provides a clue to find novel mechanisms by which peripheral cancer remotely affects pain sensation.

Disclosures: Y. Hayano: None. **H. Taniguchi:** None.

Poster

PSTR166: Cortical Processing in Nociception

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR166.13/F12

Topic: D.01. Somatosensation – Pain and Itch

Title: Alterations in Anterior Cingulate Cortex Circuits by Inflammatory and Migraine-like Pain

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Abstract: The anterior cingulate cortex (ACC) is a cortical region involved in pain processing. The ACC receives input from the Mediodorsal Thalamus and its output influences downstream neural circuits involved in pain. ACC hyperexcitability is shown in chronic pain conditions, and direct inhibition of ACC activity provides relief from pain. ACC activity is regulated by endogenous opioids like enkephalins, which can act at both mu and delta opioid receptors to alter cortical function, however these sites of action are understudied. To address this we used a FosTRAP2 mouseline and viral labeling techniques to identify cortical populations invoked in inflammatory and migraine-like pain. Previously, our lab found that the delta opioid receptor (DOR) is expressed in a majority of parvalbumin (PV) interneurons within the ACC. DOR activation on these interneurons inhibits GABA release, disinhibiting nearby pyramidal cells. While PV cells regulate a majority of inhibitory signaling in the ACC, it is unknown how ACC circuits and PV cells adapt following pain or opioid exposure. To address these unknowns, we used patch clamp electrophysiology, optogenetics and pharmacology in brain slices from mice treated with opioids or pain. Animals used in these studies were treated with chronic morphine via an osmotic minipump, inflammatory pain via a hind paw injection of Complete Freund's Adjuvant (CFA), or with Nitroglycerin (NTG) to invoke migraine-like pain. In both pain states, we observe hyperactivity of the ACC via increases in spontaneous activity at L5 Pyramidal cells. CFA or morphine does not change DOR action on PV interneurons, but there are changes in PV cell intrinsic properties in a treatment-dependent and sex-specific manner. PV cells from CFA-treated male animals are depolarized, have a lower rheobase and higher input resistances as compared to cells from CFA-treated females and naïve groups. These changes in PV cell function may contribute to altered output of the ACC to downstream regions like the amygdala. These CFA-mediated changes appear to be Kir-mediated as they are reversed following treatment with Barium Chloride, a blocker of inward-rectifying potassium channels.

Additionally, the alterations in PV cell properties by CFA are reversed when animals are pretreated with morphine, but cells from females are hyperpolarized following this dual treatment. These data suggests that CFA and morphine disrupt endogenous opioid signaling in the ACC in an opposing manner. Overall, these data suggest that PV cell function is altered in pain and drug-taking states in a treatment-dependent, sex- and synapse-specific manner which may contribute to divergent ACC output.

Disclosures: M. Walicki: None. **W. Birdsong:** None.

Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.01/F13

Topic: D.01. Somatosensation – Pain and Itch

Support: University of Notre Dame College of Science Funding

Title: Can Exosomes in the Saliva Act as a Biomarker for Pain?

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Abstract: Approximately 40 million Americans suffer from chronic pain, with rates steadily increasing due to the aging U.S. population. Current pain treatment plans are highly reliant on patient self-report. However, combining self-report with an effective screen for pain severity will allow better assessment of pain leading to improved pain management and objective assessment of treatment over time. A critical barrier to finding effective biomarkers is that pain is complex and multidimensional. Protective pain, resulting from acute injury, is dramatically different from chronic pain which is known to change neuronal structure and supraspinal processing of nociception. These changes sustain increases in pain without peripheral input from injury or inflammation. Further, the experience of pain is highly subjective, influenced by several factors including stress, anxiety, mood, and sex, varying greatly even for similar etiopathologies. Traditional targets for pain biomarkers have focused on proteins associated with known causes of pain such as pro-inflammatory cytokines (inflammation and physical injury) and neurofilament proteins (nerve injury) that limits their assessment capabilities to specific types of pain. An effective biomarker will have to be universal and change in accordance with pain severity, not the modality causing pain.
Hyperalgesia is associated with increased activity of pronociceptive neurons in the RVM, which is mediated in part by glutamate activation of these cells. Glutamate activity is necessary for release of extracellular vesicles, including exosomes. These vesicles contain numerous biologically active proteins including receptors, mRNA, and miRNA that transfer to different

cell types. We found a decrease in the number of multivesicular bodies (MVB) , organelles that house exosomes, in RVM on-cells following opiate-induced hyperalgesia. Taken together, our work suggests that glutamate-mediated increases in RVM pronociceptive neurons is necessary for heightened pain and sufficient for exosome release. If true, changes in exosome concentrations or content can act as a biomarker that incorporates physical, emotional, and cognitive aspects of pain given that the descending pain system receives inputs from brain regions mediating these aspects of pain. Our study examined the concentration and content of exosomes isolated from participants' saliva samples based on their self-reports of pain. Correlations between pain levels and exosome content is examined, as well as other factors including current pain treatment, age, and sex.

Disclosures: A. Kornaker: None. **S. Leatherberry:** None. **O. Lee:** None. **J. Oliver:** None. **G. O'Malley:** None. **S. Rivera:** None. **S. Staples:** None. **A.G. Suarez:** None. **D.A. Lane:** None.

Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.02/F14

Topic: D.01. Somatosensation – Pain and Itch

Title: Offset analgesia paradigm could reveal abnormalities in the descending pain inhibitory system in Parkinson's disease patients

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Abstract: Approximately 50% of Parkinson's disease (PD) patients report experiencing pain, yet the underlying mechanisms of pain in PD (PD-pain) remain poorly understood. The multifaceted nature of PD-pain poses a significant challenge for clinicians in identifying the specific cause of pain in individual patients and developing targeted treatment strategies. Among the various proposed mechanisms contributing to PD-pain, abnormalities in the descending pain inhibitory system have garnered attention as a potential player. This study aimed to detect abnormalities in the descending pain inhibitory system in PD patients using the offset analgesia (OA) paradigm. (OA is a phenomenon in which the perceived intensity of pain is disproportionately reduced when the temperature of a noxious heat stimulus is temporarily increased by 1℃ and then decreased by 1℃, reflecting the activity of the descending pain inhibitory system.)
Sixteen PD patients (7 females, mean age 68±5.2 years), of whom 5 reported experiencing pain, and twenty-two healthy controls (12 females, mean age 65.8±4.8 years) were included. The

severity of PD (Hoehn-Yahr stage) was 2.4±1.2 (mean±SD). Noxious heat stimuli were applied using a thermal stimulator (TSA-2, Medoc Ltd. Advanced Medical Systems, Ramat Yishai, Israel). OA stimuli and dummy stimuli were pseudo-randomly applied to the participants' upper arms. Participants rated their perceived pain intensity in real-time using a joystick held in the contralateral hand, while viewing a monitor. Pain intensity was assessed using a visual analog scale (VAS) ranging from 0 (no pa in) to 10 (worst imaginable pain). The magnitude of OA (ΔOA) and the duration of OA effect (time of OA) were compared between the two groups.The PD patient group exhibited a significantly diminished OA effect compared to the healthy control group (ΔOA: 2.7±1.1 vs. 4.3±0.8, P<0.01, mean±SD). Furthermore, the duration of OA was significantly shorter in the PD group (time of OA: 10.1±3.1 s vs. 15.2±2.4 s, P<0.01, mean±SD).This study is the first to investigate the effect of OA in PD patients. The results suggest that the OA paradigm can effectively detect abnormalities in the descending pain inhibitory system in PD patients, regardless of their pain status. These findings provide valuable insights into one of the potential mechanisms underlying PD-pain and highlight the need for further research to elucidate the precise role of the descending pain inhibitory system in the development and maintenance of pain in PD. This knowledge may ultimately lead to the development of personalized treatment approaches that address the specific underlying causes of pain in individual patients with PD.

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Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.03/F15

Topic: D.01. Somatosensation – Pain and Itch

Title: Effect of an immersive virtual reality intervention on pain and anxiety associated with peripheral venipuncture in adults. A randomized clinical trial

Authors: *M. I. ORTIZ[1], L. ZAMUDIO[2], I. I. ORDOÑEZ[3], C. BENÍTEZ ARELLANO[4]; [1]Área Académica De Medicina, Inst. de Ciencias de la Salud, Univ. Autónoma del Estado de Hi, Pachuca de Soto, Mexico; [2]Cruz Roja Mexicana Delegacion Pachuca, PACHUCA DE SOTO, Mexico; [3]Univ. Autónoma del Estado de Hidalgo, Pachuca, Mexico; [4]Univ. Autónoma del Estado de Hidalgo, PACHUCA DE SOTO, Mexico

Abstract: Introduction. Venipuncture is an invasive procedure in which a needle is inserted into a peripheral vein to draw blood or administer medication. In general, venipuncture causes pain and anxiety for the patient. Healthcare professionals use pharmacological (anesthetics) and non-pharmacological (distraction, such as virtual reality) alternatives to reduce the anxiety and pain of venipuncture. However, there is no gold standard for reducing these symptoms. **Objective.** To determine whether an immersive virtual reality (VR) intervention reduces anxiety

and pain associated with venipuncture in an adult population compared to standard care. **Methods.** Participants aged 18-65 years were enrolled (after signing informed consent) and randomized to receive standard care (without specific distraction techniques) or an immersive virtual reality intervention. The primary outcomes were patient-reported pain (measured by visual analog scale (VAS)) and patient-reported anxiety (measured by VAS) following peripheral venipuncture. Descriptive and inferential statistical analyses were performed. Student's t-test was used to compare the means of pain and anxiety scores. Statistical significance was considered at $P < 0.05$. The study protocol was approved by an independent institutional ethics committee, registration number ICSa232/2024, and the study was conducted in accordance with the Declaration of Helsinki. **Results.** A total of 98 participants volunteered for the study. Of these, 54 (55.1%) were female and 44 (44.9%) were male. The mean age of all participants was 36.7 ± 11.4 years. Thirty-eight (38.8%) participants with a mean age of 35.5 ± 11.4 years received the immersive virtual reality intervention and 60 participants with a mean age of 37.5 ± 11.0 years received standard care. For the primary outcome, the mean pain score was 2.31 ± 0.3 in the control group and 2.24 ± 0.3 in the experimental group ($P = 0.88$). Similarly, for the other primary outcome, mean anxiety scores were 2.63 ± 0.4 and 1.99 ± 0.3 for the control and experimental groups, respectively ($P = 0.21$). **Conclusions.** The results of this study demonstrated that the use of an immersive virtual reality intervention did not significantly reduce pain and anxiety induced by peripheral venipuncture in an adult populaton compared to standard care.

Disclosures: M.I. Ortiz: None. **L. Zamudio:** None. **I.I. Ordoñez:** None. **C. Benítez Arellano:** None.

Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.04/F16

Topic: D.01. Somatosensation – Pain and Itch

Support: NASU grant 0124U001556
NASU grant 0124U001557
NIH grant #1R01NS113189-01

Title: Descending control of nociceptive processing in spinal lamina X

Authors: I. BLASHCHAK[1], S. V. ROMANENKO[2], O. HALAIDYCH[2], K. KOROID[1], V. KROTOV[1], B. V. SAFRONOV[3], Y. M. USACHEV[4], N. V. VOITENKO[5,6], ***P. BELAN**[7,8]; [1]Mol. Biophysics, Bogomoletz Inst. of Physiol., Kyiv, Ukraine; [2]Biophysics of Sensory Signaling, Bogomoletz Inst. of Physiol., Kyiv, Ukraine; [3]Neuronal Networks, IBMC 503828360, Porto, Portugal; [4]Neurosci. and Pharmacol., Univ. of iowa, Iowa City, IA; [5]Biomedicine and Neurosci., Kyiv Academic Univ., Kyiv, Ukraine; [6]Dobrobut Academy Medical School, Kyiv,

Ukraine; [7]Mol. Biophysics, Bogomoletz Insitute of Physiol., Kyiv, Ukraine; [8]Biomedicine and Neuroscience, Kyiv Academic University, Kyiv, Ukraine

Abstract: Spinal lamina X receives descending projections from several classes of neurons in the rostroventromedial medulla (RVM) that are involved in the control of nociception. However, little is known about how these neurons control the primary afferent input and the local network activity in lamina X. Here we addressed this question by using an *ex-vivo* spinal cord preparation and recording the whole-cell responses of lumbar lamina X neurons to electrical stimulation of primary afferents in the segmental dorsal root (DR) and descending RVM projections in the dorsolateral funiculus (DLF). Stimulation trains (duration, 0.1-8.0 s; frequency, 1-20 Hz) that mimicked discharges in the RVM OFF cells were applied to the DLF before the regular DR stimulation at 0.1 Hz. This allowed us to study both the input from the descending fibers and its effect on the primary afferent-mediated response. We have found that the DLF stimulation inhibits Aδ- and C-afferent inputs and the afferent-driven increase in the network activity in lamina X. Without DLF stimulation, the regular DR stimulation induced a significant many fold increase in spontaneous synaptic activity in lamina X neurons. Mild DLF stimulation partially inhibited this increased network activity without significantly changing the afferent input. With stronger DLF stimulation, further inhibition of local network activity was observed in some cases to the level seen before the regular DR stimulation. In addition, mono- and polysynaptic components of the primary afferent input were presynaptically inhibited. The blocking of primary afferent-driven inputs and of the increase in the network activity also depended on the DLF stimulation pattern. However, the effect of strong and prolonged DLF stimulation on lamina X neurons was transient (20-60 s). This was most likely due to a depletion of the readily releasable pool of vesicles at the terminals of descending axons involved in the presynaptic control of afferent input to lamina X neurons, since the postsynaptic currents induced by DLF stimulation disappeared with the same time course. The inhibition developed progressively during DLF stimulation and lasted 10-100 s after cessation, implying the gradual accumulation and diffusion of volumetric neurotransmitters. Collectively, our findings suggest differential presynaptic volumetric control of primary afferents and local network in lumbar lamina X from the descending projections in the DLF.

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Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.05/F17

Topic: D.01. Somatosensation – Pain and Itch

Support: CONAHCyT 50900
SEP-CINVESTAV 122
CONAHCyT CF-2023-I-2697

Title: Changes in the collective organization and critical dynamics of the functional connectivity between the RVM and the Dorsal Horn neurons during nociception and antinociception

Authors: J. C. TOLEDO-ROY[1], D. CHAVEZ[2], E. HERNÁNDEZ[2], P. REYES[2], A. FRANK[3,4], S. GLUSMAN[5], *P. RUDOMIN[6,4];
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Abstract: The process of central sensitization induced by nociceptive stimulation is an important component of the pain experience. It includes an enhancement of the functional status of neurons and circuits in nociceptive pathways leading to the perception of ongoing pain, hyperalgesia and allodynia. Previous work has shown that the nociceptive-induced state of central sensitization leads to an enduring structured reorganization of the functional connectivity between the dorsal horn (DH) neurons that is modulated by supraspinal influences, among them the rostro-ventral medullary neurons (RVM) as they integrate the information conveyed by other central structures and have direct connections with spinal cord neurons (Contreras-Hernández et al., 2018 and Plamenov et al., 2021). We found in the anesthetized cat that during the capsaicin-induced state of central sensitization, the RVM and DH ongoing neuronal activity enhances its functional coupling while the collective behavior of the DH neurons shifts from a state of low to a state of high synchronization. This process is transiently reverted by the systemic administration of ketamine and lidocaine. These changes are no longer seen after sectioning both dorso-lateral fasciculi (DLF), a finding suggesting they are mediated, to a great extent, by the nociceptive-induced activation of RVM neurons whose axons project to the dorsal horn via both fasciculi. Over the past two decades, an expanding body of research in neuroscience has explored the hypothesis of brain criticality, which posits that neuronal ensembles operate at or near critical points, special points in their dynamical spaces that lie at the edge between order and chaos, offering new ways to explain their capacity for efficient information processing and their highly adaptable responses to stimuli. The rapid and flexible switching between the states of low and high synchronization, observed in our experiments, is one of the features found in systems near criticality, thus suggesting that the DH neurons also operate in this regime. We used a novel technique based on principal component analysis (PCA) eigenspectrum analysis proposed to identify and quantify states of criticality in multi-signal systems (Sánchez-Islas et al., 2021), and found a significant enhancement of the state of collective organization during capsaicin, while the administration of lidocaine and ketamine resulted in a notable reduction of this organization. The new technique shows that both states are consistent with criticality, but that they have different eigenspectrum exponents, indicating that they are different attractor states in the dynamical landscape of the system.

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Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.06/F18

Topic: D.01. Somatosensation – Pain and Itch

Support: NSFC, 31571085

Title: Spinal mechanisms underlying the laterality control of mechanical allodynia

Authors: *J. HUO, D. DONG, F. DU, G. YIN, Q. MA, L. CHENG;
Southern Univ. of Sci. and Technol., Shenzhen, China

Abstract: Mechanical allodynia-pain evoked by innocuous tactile stimuli-is a hallmark symptom of chronic inflammatory and neuropathic pain. For some devastating patients, a local injury can lead to full-blown body-wise pain that lasts for long time, whereas for most patients the pain could be short-lasting or confined to the unilateral injury side. Our recent study reported the brain-to-spinal descending neural circuits that control the laterality and duration of mechanical allodynia. The underlying spinal mechanisms, particularly on laterality control, are, however, still remain unsolved. Here we showed that kappa opioid receptors (KORs) in the spinal dorsal horn control the laterality and duration of mechanical allodynia via modulating the excitability of KOR-expressing neurons following peripheral inflammation or nerve injury. Pharmacologically blocking spinal KORs, or conditional knockout of KORs from dorsal horn neurons (n = 6 mice per group; both male and female were included in this study), prevented the induction of hind paw capsaicin-, formalin-, complete Freund's adjuvant (CFA)-, or spared nerve injury (SNI)-induced mechanical allodynia on the contralateral un-injured, but not the ipsilateral injured side (n = 5-6 mice per group), and caused a relapse, or prolonged lasting duration of the ipsilateral mechanical allodynia. Moreover, we observed increased excitability of dorsal horn KOR-expressing neurons in hind paw capsaicin model mice (versus hind paw vehicle control injected mice), and intersectional genetic ablation/chemogenetic silencing (n = 5-6 mice per group) dorsal horn KOR-expressing neurons could prevent/rescue hind paw capsaicin- and SNI (combined with chemical lesion of the lateral parabrachial nucleus)-induced contra-, but not ipsilateral mechanical allodynia. Conversely, chemogenetic activation of KOR-expressing dorsal horn neurons re-occurred bilateral mechanical allodynia induced by hind paw capsaicin injection (n = 6 mice per group), and opened the gate for the normally gated contralateral mechanical allodynia in SNI model mice (n = 5-6 mice per group). Collectively, our data suggest that dorsal horn KORs could control the laterality and lasting duration of peripheral inflammation and nerve injury-induced mechanical allodynia via modulating the excitability of dorsal horn KOR-expressing neurons. Targeting dorsal horn KORs/KOR-expressing neurons, could therefore, provide preclinical studies and/or clinical trials a mechanism-based strategy to treat mechanical allodynia.

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Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.07/F19

Topic: D.01. Somatosensation – Pain and Itch

Title: Rostral Ventromedial Medulla neurons mediate sleep deprivation-induced pain sensitivity

Authors: *M. ALTINKÖK;
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Abstract: Sleep disorders are known risk factors for chronic pain diseases, and recent studies have indicated the role of sleep deprivation during pain modulation. The descending pathway in the nervous system is involved during the psychological processing of pain, and recent findings suggest its potential role during sleep deprivation-induced pain modulation. However, little is known about the molecular and cellular mechanisms in the descending pathway that are engaged in order to modulate pain perception upon sleep deprivation. Here, we investigated the role of the Rostral Ventromedial Medulla (RVM) neurons in pain modulation upon sleep deprivation in mice. By performing single nuclei RNA sequencing (snRNAseq) and chemogenetic manipulations of the RVM neurons, we show the sufficiency of the RVM neurons during sleep deprivation-induced pain sensitivity. We report that the chemogenetic stimulation of the RVM neurons that are activated during sleep deprivation results in a significant increase in pain sensitization. Moreover, chronic chemogenetic activation of these neurons results in prolonged mechanical sensitivity. Conversely, chemogenetic inhibition of the RVM neurons that are engaged during sleep deprivation appear to increase the mechanical pain thresholds in mice. Finally, we provide insights into the dual role of the RVM ensembles during sleep deprivation and carrageenan-induced persistent pain. Together, our study indicates the RVM as a key region in sleep deprivation-induced pain sensitivity, and highlights the role of the descending pathway in co-morbid sleep and chronic pain disorders.

Disclosures: M. Altinkök: A. Employment/Salary (full or part-time):; Karolinska Institutet.

Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.08/F20

Topic: D.01. Somatosensation – Pain and Itch

Support: KAKENHI 18K17019
KAKENHI 19H03821
KAKENHI 23K09363

Title: Activation of insular cortical projections to the parabrachial nucleus regulates pain-related behaviors in rats

Authors: *Y. NAKAYA[1], K. HIROSE[2], S. KOBAYASHI[3], H. OSAKI[4], M. KOBAYASHI[5], *Y. NAKAYA[1];
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Abstract: The parabrachial nucleus (PBN) receives various sensory inputs including pain, taste, respiration, and blood pressure. The insular cortex (IC) plays a role in processing orofacial nociceptive information and sends corticofugal projections to the PBN. However, little is known about the role of the descending projections from IC to PBN neurons. We aimed to investigate the synaptic relationship between the IC and glutamatergic and GABAergic/glycinergic neurons in the PBN.We performed whole-cell patch clamp recording from slices including the PBN in vesicular GABA transporter-Venus transgenic rats. Monosynaptic excitatory postsynaptic currents were recorded from both excitatory glutamatergic and inhibitory GABAergic/glycinergic PBN neurons, which were induced by selective stimulation of IC axon terminals in the PBN. We found that the amplitude was comparable between glutamatergic and GABAergic/glycinergic neurons. Next, we recorded the frequency of facial grooming after the injection of capsaicin to the right whisker pad under the condition with or without optical stimulation using rats injected with AAV-CAG-ChR2-mCherry in the IC and implanted optical fiber in the PBN. The behavior test showed that activation of IC axons in the PBN increased the frequency of facial grooming in response to nociceptive stimulation. These results suggest that IC projections to the PBN facilitate excitatory outputs from the PBN.

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Poster

PSTR167: Descending Modulation of Pain

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Program #/Poster #: PSTR167.09/F22

Topic: D.01. Somatosensation – Pain and Itch

Support: CIHR Grant PJT-16224

Title: Maximal electroconvulsive shock-induced seizures cause prolonged analgesia and acute hyperoxia in the locus coeruleus

Authors: *L. J. FICK, N. J. VAN DEN HOOGEN, T. TRANG, G. TESKEY;
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Abstract: Postictal hypoxia following a seizure is associated with a variety of comorbidities, such as amnesia and muscle weakness. One of the least understood comorbidity is postictal analgesia, which is a decreased perception of nociceptive stimuli after seizure. Given the temporal overlap between postictal hypoxia and postictal analgesia, we hypothesized that postictal analgesia is associated with postictal hypoxia. Given the protective role of acute pain in the prevention of injury, decreased nociceptive perception can be maladaptive, and increasing our understanding of postictal analgesia could therefore improve the quality of life of people with epilepsy by reducing the risk of post-seizure injury. To assess thermal nociceptive threshold, we use the thermal tail flick test and measure the time taken for a mouse to withdraw its tail from hot water (tail flick latency; TFL). In one group, on three days, each 48 hours apart, the baseline TFL of each mouse was measured, after which mice underwent either a maximal electroconvulsive shock (MES)-induced seizure or sham. Following the seizure/sham, TFL was measured every 15 minutes for 2 hours. In another group, the baseline TFL of each mouse was recorded, after which they underwent either an MES-induced seizure or sham, and their TFL was recorded after 15 minutes, 30 minutes, then every 30 minutes after for a total of 3 hours. The TFL of each mouse was then recorded at two timepoints separated by 15 minutes, 48 hours, 72 hours, and 96 hours after the initial seizure/sham. In a third group, an oxygen-sensing optode was implanted adjacent to the locus coeruleus of each mouse. Local tissue oxygenation was then recorded in each mouse immediately prior to and following an MES-induced seizure or sham for 3 hours on 3 days, each 48 hours apart. All tests were conducted with samples sizes of at least 8 animals, and behavioural tests were completed in both females and males to assess potential sex differences. Both female and male mice displayed an increase in tail flick latency after seizure, indicating a reduction in thermal nociception and verifying the validity of this model of postictal analgesia. This blunted thermal nociceptive response persisted for 48 hrs in female and 96 hrs in male mice. In the period immediately following the seizure in males, oxygen levels in the locus coeruleus significantly increased, before returning to baseline levels after approximately 20 minutes. This research demonstrates that MES-induced generalized seizures result in prolonged decreases in nociceptive perception that differ between females and males, and which may be the result of postictal hyperoxia, rather than hypoxia.

Disclosures: L.J. Fick: None. **N.J. Van Den Hoogen:** None. **T. Trang:** None. **G. Teskey:** None.

Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.10/F23

Topic: D.01. Somatosensation – Pain and Itch

Support: NSFC 32170996

Title: Distinct roles of differential populations of efferent neurons from the parabrachial nucleus in mechanical allodynia

Authors: *D. DONG[1], J. HUO[1], F. DU[1], G. YIN[1], L. CHENG[2];
[1]Southern Univ. of Sci. and Technol., Shenzhen, China; [2]Dept. of Biol., Southern Univ. of Sci. and Technol., Shenzhen, China

Abstract: Mechanical allodynia, evoked by innocuous tactile stimuli represents one prevalent symptom of chronic inflammatory and neuropathic pain. For some devastating patients, a local injury can lead to full-blown body-wise pain that lasts for a long time, whereas for most patients, the pain could be short-lasting or confined to the unilateral injury side. Our recent study reported a descending pathway that controls the latent sensitization induced by chemical irritants, inflammatory reagents, or nerve injury. This circuit starts with neurons located in the lateral parabrachial nuclei (lPBN), which control the activity of hypothalamic neurons in the dorsal medial regions (dmH) that are marked by the expression of prodynorphin (Pdyn) and that send bilateral projections to the spinal dorsal horn (SDH). However, the underlying mechanisms for the initiation of bilateral mechanical allodynia, particularly contralateral allodynia, remain unsolved. Here, we observed that proenkephalin (Penk) neurons were also highly expressed in lPBN and partially overlapped with Oprm1-expressing neurons in el-dvlPB. We did similar behavioral experiments to study their functional roles in modulating mechanical allodynia. In contrast to ablation of Oprm1-expressing neurons, ablating or silencing the el-dvlPBN Penk-expressing neurons, or retro-ablating or silencing of the el-dvlPBN^{Penk+}→dmH projecting neurons had no significant effect on the lasting duration of mechanical allodynia, suggesting cell type-specific roles of lPBN^{Oprm1} neurons in negatively modulating the lasting duration of mechanical allodynia. However, retro-ablation or silencing of the slPBN^{Penk+}→dmH projecting neurons before hind paw capsaicin injection completely prevented the induction of bilateral mechanical allodynia. In contrast, silencing of the slPBN^{Penk+}→dmH projecting neurons post-hind paw capsaicin injection had no significant effect on the expression of bilateral mechanical allodynia, suggesting that this population of neurons is required for the induction, but not expression of bilateral mechanical allodynia. Silencing of slPBN^{Penk} neurons post-hind paw capsaicin injection abolished bilateral mechanical allodynia, suggesting that slPBN^{Penk} neurons are required for the transmission of bilateral mechanical allodynia. In addition, activation of the slPBN^{Penk+}→dmH projecting neurons opened the gate for bilateral mechanical allodynia in naïve mice. Overall, our study suggested distinct roles of differential populations of efferent neurons from the lateral parabrachial nucleus in mediating mechanical allodynia in mice.

Disclosures: D. Dong: None. **J. Huo:** None. **F. Du:** None. **G. Yin:** None. **L. Cheng:** None.

Poster

PSTR167: Descending Modulation of Pain

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Program #/Poster #: PSTR167.11/F24

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH SP0073141

Title: Amygdala VIP interneurons respond to nocifensive-behavior generating stimuli in mice

Authors: *M. KWON[1], S. G. QUADIR[2], S. PATEL[3,4];
[1]Psychiatry and Behavioral Sci., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [2]Dept. of Psychiatry and Behavioral Sci., Northwestern Univ., Feinberg Sch. of Med., Chicago, IL; [3]Psychiatry and Mol. Physiol., Vanderbilt Univ. Med. Ctr., Nashville, TN; [4]Psychiatry and Behavioral Sciences, Northwestern Feinberg School of Medicine, Chicago, IL

Abstract: Alcohol use disorder (AUD) is one of the most prevalent psychiatric disorders associated with substantial morbidity, mortality, and economic burden. The development of AUD addiction cycle is known to encompass the three stages of binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation. The withdrawal stage is associated with physical symptomatic changes such as hyperalgesia. The basolateral amygdala (BLA) is one brain region implicated both in AUD and pain states. The activity of the BLA is coordinated by the interactions of glutamatergic principal neurons and distinct classes of GABAergic inhibitory interneurons. BLA interneurons expressing vasoactive-intestinal peptide (VIP) in particular have been studied in the context of threat response and fear learning. However, the role of BLA VIP interneurons in mediating pain responses remains unclear especially due to the lack of cell-specific approaches used in previous studies. To test whether VIP interneurons in the BLA respond to noxious stimuli we used fiber photometry to measure neural activity during pain responses in mice. More specifically, we examined neural activity during exposure to heat (Hargreaves) and cold (acetone) stimuli and found that the VIP interneurons express dynamic neural activity upon presentation thermal (heat and cold) stimuli. Taken together, these data suggest that the VIP interneurons in the BLA play a significant role in pain-related sensory and behavioral responses. Furthermore, we predict that activity of VIP neurons activity will be specifically magnified during alcohol withdrawal-induced hyperalgesic states. Preliminary data from our lab has shown that mice in alcohol withdrawal have a heightened nocifensive response during thermal stimuli presentation. Our overarching hypothesis is that alcohol withdrawal-induced pain related responses can be modulated by targeting specific neural populations that are recruited during the expression of nocifensive behaviors. Future studies will investigate the role of BLA VIP interneurons in mediating alcohol-withdrawal-induced hyperalgesia and utilizing optogenetics to test a causal role for BLA VIP interneurons in the regulation of alcohol-withdrawal associated hyperalgesia.

Disclosures: M. Kwon: None. **S.G. Quadir:** None. **S. Patel:** None.

Poster

PSTR167: Descending Modulation of Pain

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Program #/Poster #: PSTR167.12/F25

Topic: D.01. Somatosensation – Pain and Itch

Support: NSF CAREER IOS-1652766
NINDS 1 R01 NS111067

Title: Control of Pain Defense by Co-transmission of Oxytocin & Glutamate in a Zebrafish Premotor Network

Authors: *E. DE LA ROSA[1], C. L. WEE[2], S. LUKS-MORGAN[1], M. NIKITCHENKO[3], F. ENGERT[3], W.-C. WANG[4], M. A. JOHNSON[5], A. D. DOUGLASS[1];
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Abstract: Nociception, the physiological component of pain, is an important adaptive mechanism that allows an organism to perceive and respond to threats in the environment. Nociception is processed through multiple neural circuits which trigger behavioral and/or physiological responses. The neuropeptide oxytocin (OXT) is involved in several of these circuits, many of which promote analgesia in response to injury. Our lab has demonstrated a new role for OXT in the pain response. A subpopulation of OXT neurons in the hypothalamus respond to nociception and recruit premotor neurons in the hindbrain to mediate pain-evoked escape behaviors. While activation of OXT-producing neurons alone is sufficient to initiate the escape response, deletion of the oxytocin peptide attenuates but does not altogether ablate defensive behaviors. Published and preliminary data suggested the excitatory neurotransmitter glutamate (GLU) is co-released from OXT-neurons onto premotor neurons. The individual contributions of each molecule to signaling between the OXT-neurons and the hindbrain premotor neurons presented a new avenue of inquiry. Recent advances in the field of fast-scan cycle voltammetry (FSCV) allowed for the direct measurement of OXT release in the hindbrain throughout the premotor array. I have measured OXT release in the larval hindbrain in response to both a strong nociceptive stimulus and direct activation of the OXT-neurons via optogenetics. This led to the findings that OXT release is fast, spatially precise, and prolonged in this circuit. **I hypothesize that this extended OXT signaling primes the premotor neurons to robustly respond to GLU**, whose release is yet to be characterized in this circuit. Moving forward, I intend to continue using FSCV as well as whole-cell patch clamp electrophysiology to explore the dependence of OXT and GLU release on stimulus intensity and how release of each molecule impacts activity within the hindbrain premotor neurons.

Disclosures: E. De la Rosa: None. **C.L. Wee:** None. **S. Luks-Morgan:** None. **M. Nikitchenko:** None. **F. Engert:** None. **W. Wang:** None. **M.A. Johnson:** None. **A.D. Douglass:** None.

Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

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Program #/Poster #: PSTR167.13/F26

Topic: D.01. Somatosensation – Pain and Itch

Support: K01 1K01DA058543-01
R01 HL166317

Title: Disentangling descending pain modulation and respiratory control at the level of the rostral ventromedial medulla using intersectional genetic strategies

Authors: *R. PHILLIPS[1], N. A. BAERTSCH[2];
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Abstract: Opioid medications are pivotal in pain management but are often accompanied by severe side effects such as opioid-induced respiratory depression (ORID), a primary cause of death in opioid overdoses. The respiratory side effects of opioid use occur due to interacting and overlapping neuronal circuits that regulate pain and/or breathing. Understanding the brain regions and cell types that link pain and breathing is essential for developing safer pain management strategies. Opioid analgesia involves altered firing patterns of neurons within the rostral ventromedial medulla (RVM). Although the RVM is known for its role in descending pain modulation, local administration of opioids within the RVM induces potent respiratory depression in addition to analgesia. The RVM has two main classes of neurons, identified based on their response to pain stimuli. As their name suggests, “ON-cells” are activated during pain and “OFF-cells” are inhibited. ON-cells are known to express the μ-opioid receptor (μOR), as they respond directly to opioids, and can be excitatory or inhibitory. Moreover, ON-cell activity facilitates pain and is thought to contribute to respiratory drive. Therefore, opioid suppression of ON-cells is thought to contribute to ORID. In contrast, OFF-cells are mostly inhibitory, and do not appear to express μOR or contribute to respiratory drive. Moreover, activation of OFF-cells is required for opioid-mediated analgesia. Therefore, selective activation of OFF-cells is predicted to evoke potent pain relief without respiratory side effects. However, due to a lack of selective pharmacological agents, directly testing these predictions has not been possible. Using intersectional genetic techniques to manipulate neuron subtypes in mice, we explore the specific roles of excitatory and/or inhibitory neurons based on their expression of Oprm1 (the gene encoding μOR) in modulating pain and respiration. Initial results indicate that activating certain RVM neuron types distinctly affects respiration and pain, pointing towards potential targets that could either reverse ORID or generate pain relief without respiratory depression. These findings contribute to the understanding of opioid regulation of descending pain modulation and respiratory control at the level of the RVM and complement ongoing research into the broader neurophysiological and molecular mechanisms underlying opioid effects.

Disclosures: R. Phillips: None. **N.A. Baertsch:** None.

Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.14/F27

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant NS038261
NIH Grant NS118731

Title: Involvement of impaired GluD1-Cbln1 trans-synaptic signaling and autophagy in pain-related behaviors and neuroplasticity in central amygdala PKCδ neurons in a neuropathic pain model

Authors: *T. KIRITOSHI[1], K. S. NARASIMHAN[2], G. JI[1], S. DRAVID[2], V. NEUGEBAUER[1];
[1]Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; [2]Texas A&M Univ., College Station, TX

Abstract: Neuroplasticity in the central nucleus of the amygdala (CeA) plays a key role in pain modulation and emotional-affective aspects of pain. Our previous study revealed dysfunction of synaptic organizer glutamate delta 1 receptor (GluD1) and its signaling partner cerebellin1 (Cbln1) at the synapse between parabrachial nucleus (PB) and protein kinase C delta (PKCδ)-expressing neurons in the CeA in pain models. Accumulating evidence suggests that autophagy is an important mechanism for synaptic plasticity, including long-term depression (LTD), under neurological and psychiatric disorders. Furthermore, GluD1 has been shown to regulate autophagy mechanisms in multiple brain areas. However, the contribution of autophagy to neuroplasticity in the CeA and pain-related behaviors remains to be determined. Here we examined the involvement of GluD1-Cbln1-related autophagy mechanisms in pain-related behaviors and neuroplasticity in CeA-PKCδ neurons in a neuropathic pain model. Recombinant Cbln1 or an autophagy inducing peptide Tat-Beclin 1 was stereotaxically injected into the right and left CeA of sham control or neuropathic mice (4 weeks after L5 spinal nerve ligation, SNL model). Mechanical hypersensitivity was measured with von Frey test. Whole-cell patch clamp recordings were obtained from identified CeA-PKCδ neurons in brain slices from sham and SNL transgenic *Prkcd*-cre mice. In current clamp mode, neuronal excitability was measured by injecting depolarizing currents. In voltage clamp mode, excitatory postsynaptic currents (EPSCs) were evoked by electrical stimulation of presumed PB input. Intra-CeA injection of recombinant Cbln1 or Tat-Beclin 1 significantly alleviated mechanical hypersensitivity in SNL. Our preliminary electrophysiological results show no significant change in spontaneous and miniature EPSCs (sEPSCs and mEPSCs) and excitability but potentially impaired LTD in CeA-PKCδ neurons in SNL compared to sham controls. These results suggest that GluD1-Cbln1-related autophagy mechanisms are impaired in neuropathic pain and that neuroplastic changes in CeA-PKCδ neurons may involve the impairment.

Disclosures: T. Kiritoshi: None. **K. S. narasimhan:** None. **G. Ji:** None. **S. Dravid:** None. **V. Neugebauer:** None.

Poster

PSTR167: Descending Modulation of Pain

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Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant NS038261
NIH Grant NS106902
NIH Grant NS109255
NIH Grant NS120395

Title: Kappa opioid receptor signaling in hypothalamus promotes stress-induced functional pain

Authors: *V. YAKHNITSA[1], G. JI[1], N. ANTENUCCI[1], E. NAVRATILOVA[2], F. PORRECA[2], V. NEUGEBAUER[1];
[1]Pharmacol. and Neurosci., Texas Technol. Univ. Hlth. Sci. Ctr., Lubbock, TX; [2]Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: Nociplastic pain conditions include a number of so-called functional pain syndromes (FPS) that are often female-prevalent and hormone-dependent. Stress, a key trigger of FPS, promotes the release of hypothalamic dynorphin, an endogenous kappa opioid receptor (KOR) agonist. Stress or systemic dynorphin elevate prolactin (PRL) blood levels predominantly in females. Tuberoinfundibular dopamine (TIDA) neurons in the hypothalamic arcuate nucleus (Arc) provide tonic inhibition of PRL release from pituitary lactotrophs. In this project we test the hypothesis that repeated stress inhibits activity of KOR-TIDA neurons resulting in increased PRL release that produces an FPS-like condition.The FPS model was induced in KOR-Cre and KOR-tdTomato mice using a repeated restrained stress (RRS) paradigm. RRS is an established model demonstrated to induce PRL dependent FPS in female mice. After assessment of periorbital mechanosensitivity with von Frey filaments and anxiety-like behaviors in the elevated plus maze and light-dark test, brain slice physiology experiments were performed in two groups: stressed group (3 days after stress) and stress-resolved group (14 days post stress). Whole-cell patch-clamp recordings were made from KOR-positive Arc-TIDA neurons in brain slices. KOR-positive cells were labeled in KOR-Cre mice with inhibitory DREADD 3 weeks before slice preparation. TIDA neurons were labeled by i.v. injection of fluorogold 1 week before recordings. Stages of the estrous cycle were monitored in female mice for 3-4 days prior to electrophysiology experiments. Three neuronal phenotypes were observed in both stressed and stress-resolved groups: spontaneous bursting, regular firing, and silent neurons. Number of silent neurons was increased in the stressed female group compared to the stressed male group and stressed-resolved male and female groups. Spontaneous firing rate was reduced in the stressed female group compared to stress-resolved female and both male groups. In stressed mice, superfusion of a KOR agonist (U69,593) suppressed firing in bursting and regular firing neurons more strongly in females than males. In stress-resolved mice, U69,593 caused moderate suppression of neuronal firing in males and females. U69,593 decreased excitability of all neuronal phenotypes in stressed and stress-resolved groups but predominantly in female mice. These results suggest that hypothalamic KOR signaling inhibits the activity of Arc-TIDA

neurons and becomes more sensitive to exogenous KOR activation in acute stress conditions in females, which may play a role in promoting FPS.

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Poster

PSTR167: Descending Modulation of Pain

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Program #/Poster #: PSTR167.16/F29

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH grant NS038261 (VN)
SLU startup funds (DS)

Title: Effects of P2Y14 receptor activation on amygdala neurons

Authors: *V. NEUGEBAUER[1], D. SALVEMINI[2], M. MAZZITELLI[1], N. ANTENUCCI[1]; [1]Texas Technol. Univ. Hlth. Sci. Ctr., Lubbock, TX; [2]St. Louis Univ. Dept. of Pharmacol. and Physiological Sci., Saint Louis, MO

Abstract: Pain is a multidimensional experience with a significant averse-affective component. Because of its complexity, pain remains an important medical challenge. The amygdala is a limbic brain structure critically involved in the emotional-affective aspects of behaviors and in pain modulation. The central nucleus of amygdala (CeA) serves major output functions and receives nociceptive information via the external lateral parabrachial nucleus (PB). Increasing evidence from preclinical and clinical studies has provided direct support for amygdala involvement in pain. Neuroplastic changes in the amygdala have been detected in different models of pain and mechanistically linked to pain behaviors, yet underlying synaptic, cellular and molecular mechanisms remain to be determined. P2Y purinergic receptors are G-protein coupled receptors that have emerged as potential therapeutic targets in different models of chronic pain. Specifically, P2Y14 receptor (P2Y14R) is a Gαi/o-protein coupled receptor expressed in glial cells and neurons, and has pro-inflammatory and pro-nociceptive functions during pain development and maintenance. P2Y14R has several endogenous ligands, but UDP-glucose is the most potent agonist. Our proteomic analysis of P2Y14R expression reveals abundant presence of the receptor in the amygdala. In this study, we assessed the effects of the pharmacological activation of P2Y14R using UDP-glucose on amygdala neuronal functions under normal conditions. Whole-cell patch-clamp recordings were performed from neurons in the laterocapsular division of the central nucleus of amygdala (CeLC) obtained from naive rats. UDP-glucose bath application resulted in facilitatory effects on excitatory synaptic responses at the CeA-PB synapse without significantly affecting neuronal excitability. These data suggest a potential contribution of P2Y14R to the development of pain-related amygdala neuroplasticity. It

remains to be determined if the endogenous activation of P2Y14R in pain conditions drives neuroplasticity and pain behaviors.

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Poster

PSTR167: Descending Modulation of Pain

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Topic: D.01. Somatosensation – Pain and Itch

Support: NIH grant NS038261 (VN)
SLU startup funds (DS)

Title: Beneficial effects of amygdala P2Y14 receptor blockade a in a model of neuropathic pain.

Authors: *M. MAZZITELLI[1], N. ANTENUCCI[1], D. SALVEMINI[2], V. NEUGEBAUER[1]; [1]Texas Technol. Univ. Hlth. Sci. Ctr., Lubbock, TX; [2]Pharmacol. and Physiological Sci., St. Louis Univ. Dept. of Pharmacol. and Physiological Sci., Saint Louis, MO

Abstract: Pain is a multidimensional experience with a significant averse-affective component. Because of its complexity, pain remains an important medical challenge. The amygdala is a limbic brain structure critically involved in the emotional-affective aspects of behaviors and in pain modulation. The central nucleus of amygdala (CeA) serves major output functions, and neuroplasticity in the CeA is mechanistically linked to pain-related behaviors in different pain conditions. Strategies to mitigate amygdala maladaptive plasticity may be desirable approaches for pain relief. P2 purinergic receptors have emerged as novel therapeutic targets for chronic pain. P2Y14 receptor (P2Y14R) is one of the eight purinergic G-protein coupled receptors and is linked to Gαi/o protein signaling. P2Y14R is expressed in immune cells and glia in the central nervous systems. Importantly, we and others have reported critical roles of spinal P2Y14R in inflammatory and neuropathic pain states. However, the role of P2Y14R in brain mechanisms of chronic pain remains unknown. Here we address this knowledge gap by determining the effects of pharmacological P2Y14R blockade in the amygdala on pain-like behaviors in a rodent model of neuropathic pain.Our proteomic analysis of P2Y14R expression reveals abundant presence of the receptor in the amygdala. Selective PY214R blockade in the CeA was achieved by the stereotaxic application of 4-(4-(piperidin-4-yl)-phenyl)-7-(4-(trifluoromethyl)-phenyl)-2-naphthoic acid (PPTN), a highly selective and potent competitive P2Y14R antagonist. Emotional responses (evoked vocalizations), mechanical withdrawal thresholds, anxiety-like and spontaneous (grimace score) behaviors were used to measure the effects of intra-CeA PPTN in adult rats 2 weeks after the induction of the spared-nerve injury (SNI) neuropathic pain model. Selective P2Y14R inhibition resulted in the decrease of evoked vocalizations, amelioration of mechanical withdrawal responses, and grimace score in a neuropathic condition (SNI).These

data suggest a critical contribution of P2Y14R in the amygdala to pain-related behaviors in a neuropathic pain model and provide evidence to support P2Y14R as a desirable therapeutic target for pain relief.

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Poster

PSTR167: Descending Modulation of Pain

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.18/F31

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant NS038261

Title: Exogenous BDNF modulates electrophysiologic changes in neurons of the amygdala

Authors: *Z. J. HURTADO, V. A. YAKHNITSA, P. D. PRESTO, N. ANTENUCCI, T. KIRITOSHI, M. MAZZITELLI, G. JI, V. NEUGEBAUER;
Texas Technol. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Exogenous BDNF modulates electrophysiologic changes in neurons of the amygdala

Zachary J. Hurtado[1], Peyton Presto[1], Takaki Kiritoshi[1], Nico Antenucci[1], Vadim Yakhnitsa[1] Guangchen Ji[1,2], and Volker Neugebauer[1,2,3]
Department of Pharmacology and Neuroscience[1], Center of Excellence for Translational Neuroscience and Therapeutics[2], Garrison Institute on Aging[3], Texas Tech University Health Sciences Center, Lubbock, TX

Neuropathic pain has a strong emotional component and is often comorbid with anxio-depressive disorders. The amygdala, a limbic brain region, has been shown to play critical roles in the modulation of pain, fear, and anxiety behaviors. Neuroplastic changes in the amygdala have been linked to pain behaviors, but the specific mechanisms are not fully understood. Brain derived neurotrophic factor (BDNF) plays an important role in neuroplasticity and there is evidence for downregulation of BDNF in neuropsychiatric diseases such as anxio-depressive disorders while increasing BDNF has been implicated as a mechanism of antidepressant therapeutic interventions. The role of BDNF signaling in the brain, and the amygdala in particular, in pain modulation is not yet known, but expression of the TrkB receptor, a known target of BDNF, has been shown in the amygdala. The goal of this study is to examine the effects of exogenously delivered BDNF on amygdala plasticity in neuropathic pain, using brain slice physiology. Chronic pain model was induced in CRF+/tdTomato+ mice through spinal nerve ligation surgery (SNL). Whole-cell patch-clamp recordings were made from neurons in the capsular division of the central amygdala. The application of BDNF decreased excitability measured as action potential firing is response to depolarizing current injection. BDNF also decreased excitatory

synaptic transmission of parabrachial inputs. These results would suggest that in chronic pain, exogenous BDNF delivery can inhibit amygdala neuronal activity.
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Poster

PSTR167: Descending Modulation of Pain

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Program #/Poster #: PSTR167.19/F32

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant NS038261

Title: Sex differences in the contribution of microglia in the amygdala to neuropathic

Authors: *N. ANTENUCCI[1], P. D. PRESTO[2], M. MAZZITELLI[2], V. NEUGEBAUER[2]; [1]Texas Technol. Univ. Hlth. Sci. Ctr., Lubbock, TX; [2]Pharmacol. and Neurosci., Texas Technol. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Chronic pain imposes a substantial burden on individuals and healthcare systems worldwide, necessitating novel therapeutic strategies to alleviate its debilitating effects. Emerging evidence implicates neuroinflammation in the amygdala as a key contributor to the maintenance of chronic pain states. The central amygdala (CeA), known for its pivotal role in emotional processing and pain modulation, serves as a critical node in pain processing pathways. In this study, we aimed to elucidate the contribution of microglia in the amygdala (CeA) to neuropathic pain behavior and neuroplasticity using pharmacological inhibition of colony-stimulating factor 1 receptor (CSF1R) with PLX3397. PLX3397 was administered stereotaxically into the CeA of female and male rats in a neuropathic pain model induced by L5 spinal nerve ligation (SNL). Stereotaxic administration of PLX3397 into the CeA of female and male rats in a neuropathic pain model induced by L5 spinal nerve ligation (SNL) revealed significant improvements in pain behaviors, particularly notable in female rats, as evidenced by decreased mechanosensitivity assessed through von Frey and paw compression tests, attenuated emotional responses measured as evoked vocalizations, and decreased anxiety-like behavior in the open field test and elevated plus maze. Additionally, we used brain slice electrophysiology to measure the effects of microglia depletion in the amygdala and explore the underlying synaptic and cellular mechanisms. We found decreased neuronal excitability in brain slices from neuropathic rats preincubated with PLX3397 for 1 hour. In summary, our study suggests and important role of neuroinflammation in the amygdala in chronic pain conditions and underscores the promise of microglia inhibition as a targeted therapeutic approach.

Disclosures: N. Antenucci: None. **P.D. Presto:** None. **M. Mazzitelli:** None. **V. Neugebauer:** None.

Poster

PSTR167: Descending Modulation of Pain

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Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grants NS038261
NS106902
NS109255

Title: Microglia depletion blocks the induction of pain behaviors by chemogenetic activation of amygdala CRF neurons

Authors: *G. JI, T. KIRITOSHI, V. A. YAKHNITSA, V. NEUGEBAUER;
Texas Technol. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: The bidirectional interactions between the immune system and the CNS have emerged as key players implicated in the development of pain. Microglia perform immune surveillance in the CNS, and the complex interactions with neurons contribute to synaptic modulation and neuroplasticity. Neuroimmune signaling has been implicated in peripheral and spinal pain mechanisms. In the spinal cord, microglia are one of the first cell types to be activated in pain conditions, and direct inhibition of microglial activation effectively attenuates both developing and established pain hypersensitivity. However, relatively little is known about neuroimmune signaling in brain regions associated with different aspects of pain. Our previous studies demonstrated that amygdala with its central nucleus (CeA) is an important brain region involved in the emotional-affective component of pain and pain modulation, and the amygdala CRF system has been found to be a critical modulator of pain-related plasticity and behavior. In this study, we addressed the question if microglia activation is involved in pain-like behaviors induced by the chemogenetic activation of amygdala CRF neurons in naïve animals. For chemogenetic activation of amygdala CRF neurons, a Cre-inducible viral vector encoding Gq-DREADD (hM3D) was injected stereotaxically into the right CeA of transgenic CRF-Cre rats. Mechanosensitivity (electronic von Frey anesthesiometer), emotional-affective behaviors (vocalizations), and spontaneous pain-like behaviors (grimace score) were assessed in naïve CRF-cre rats. Chemogenetic activation of amygdala CeA-CRF neurons with clozapine-N-oxide (CNO, 1.0 mg/kg, i.p. 7 days) induced long-lasting pain-like behaviors and microglia activation in CRF-cre rats, which was blocked by an inhibitor of microglia colony-stimulating factor-1 receptor CSF1R (PLX3397, oral gavage, 30 mg/kg, 7 days).The data suggest that microglia activation is downstream of CRF signaling in the amygdala, providing evidence for an important role of the amygdala CRF system in neuroimmune pain mechanisms in the brain. Keyword (s): CRF CeA, chemogenetics, amygdala, pain Support Contributed By: NIH Grants NS038261, NS106902, NS109255

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Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.21/F34

Topic: D.01. Somatosensation – Pain and Itch

Support: NSERC Grant (486901)
CIHR Grant (RGPIN-2020-06777)

Title: Neural signalling and network connectivity in the brainstem and spinal cord: An fMRI investigation of the individualized pain experience in healthy humans

Authors: *J. S. MERLETTI, H. A. ALGITAMI, S. HASSANPOUR, B. K. KEAST, M. UMRAW, P. W. STROMAN;
Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada

Abstract: Pain is more than a warning signal; it is a subjective and complex experience involving interactions between peripheral sensitization and central processing. Although prior functional magnetic resonance imaging (fMRI) studies exploring pain have largely focused on the brain, research has shown that to fully understand the pain experience, the entire integrated network must be examined, including the brainstem and spinal cord. Investigating these regions is critical as a key process, descending pain modulation, occurs here. Descending pain modulation involves brainstem and spinal cord regions where the periaqueductal gray (PAG) and rostroventromedial medulla (RVM) integrate descending signals from higher brain regions and modulate pain perception. This allows cortical and subcortical areas to influence the pain experience by regulating the transmission of nociceptive signals in a network. Expanding on this, our confirmatory study aims to investigate how varying intensities of stimuli, from innoxious to noxious, correlate with differences in blood oxygenation-level dependent (BOLD) signal patterns and region connectivity across the integrated network. We hypothesize that connectivity and BOLD responses in the brainstem and spinal cord will vary with stimulus intensity (innoxious vs. noxious), and individual differences in sensitivity will be reflected by variances in input or output signalling to the distinctive regions.
To examine this, healthy humans aged 20-60 underwent thermal stimulation at temperatures 38°C, 46°C, and 51°C while we acquired fMRI scans in the brainstem and spinal cord, tracked eye movements, and obtained verbal pain ratings. A novel network analysis method that models input and output signalling was then used to identify how BOLD responses and connectivity vary in relation to different temperatures, and in which anatomical regions these relationships occur. Results revealed variations in network connectivity, input and output signaling from each region, and BOLD responses across temperatures. The key differences observed were in the connectivity between specific regions involved with descending pain modulation such as the PAG and locus coeruleus. Additionally, the variety of regions which formed connections in the network increased as the stimulus went from a non-painful (38°C), to painful temperature (51°C). These findings support existing literature on connectivity differences between noxious and innoxious

stimuli, however, further investigation is needed to understand how individual differences in input and output signaling, among BOLD responses, correspond with innoxious and noxious temperatures.

Disclosures: J.S. Merletti: None. **H.A. Algitami:** None. **S. Hassanpour:** None. **B.K. Keast:** None. **M. Umraw:** None. **P.W. Stroman:** None.

Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.22/F35

Topic: D.01. Somatosensation – Pain and Itch

Support: NSERC
CIHR

Title: An investigation of the neural correlates of affective modulation of pain perception in fibromyalgia by means of fMRI in the brainstem and spinal cord

Authors: *H. ALGITAMI[1], S. HASSANPOUR[2], J. MERLETTI[2], B. K. KEAST[3], M. UMRAW[2], P. W. STROMAN[4];
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Abstract: Fibromyalgia (FM) is a chronic pain condition whose symptoms involve both sensory disturbances, such as increased pain sensitivity, and affective disturbances, such as negative mood. Although FM involves both pain and negative affect, it is unclear how they influence each other. Moreover, due to FM's increasing burden on society, it is necessary to investigate it from all angles, both the psychological and the physiological, to create a comprehensive understanding of the disorder. Hence, the aim of this study was to examine the neural correlates of negative affective modulation of pain perception in FM using fMRI of the brainstem and spinal cord. Although the brainstem and spinal cord are often ignored in investigations of pain perception, they play an integral role in nociceptive processing. Inputs to the brainstem from regions in the brain and spinal cord are integrated to modulate pain perception. Our lab has developed specialized fMRI methods to extract information about nociceptive processing in these areas. We hypothesize that there are differences in observed BOLD signal fluctuations between negative and neutral conditions, and that these differences are more prominent in FM compared to healthy controls (HC).
Data were obtained from female participants, with (FM) and without (HC) fibromyalgia. The study involved fMRI of the brainstem and spinal cord, noxious heat stimulation of the right hand at a calibrated temperature, and a visual display of negative and neutral images from the International Affective Picture System (IAPS). Each MRI run involved either negative or neutral

images and thermal stimulation, with participants being asked to rate their pain and affect at the end. A novel network connectivity analysis method was used to assess differences between negative and neutral affective conditions, in both FM and HC participants.
The results indicate that FM participants may have greater variability in connectivity values across participants than HC participants. However, there is no strong evidence for differences in neural activity involved with affective modulation between FM and HC. This is supported by analyses of covariance (ANCOVAs) that did not reveal significant relationships between pain ratings, study conditions, and study groups. The results at the present stage do not suggest that there are differences in affective modulation of pain in people with FM.

Disclosures: H. Algitami: None. **S. Hassanpour:** None. **J. Merletti:** None. **B.K. Keast:** None. **M. Umraw:** None. **P.W. Stroman:** None.

Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR167.23/F36

Topic: D.01. Somatosensation – Pain and Itch

Support: NSERC Grant RGPIN-2020-06777
CIHR Grant 486901

Title: Conditioned pain modulation and its effect on descending pain regulation within the human brainstem and spinal cord

Authors: *B. K. KEAST, S. HASSANPOUR, H. A. ALGITAMI, J. MERLETTI, M. UMRAW, C. D'SOUZA, E. BRANDER-WHITTINGHAM, P. W. STROMAN;
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Abstract: Pain involves complex neural mechanisms that regulate how we perceive and respond to nociceptive signals. Conditioned pain modulation (CPM) has garnered considerable attention for how it demonstrates descending regulation of pain. Stemming from diffuse noxious inhibitory control (DNIC) studies in animals, previous research has established the presence of the CPM effect in humans and its importance in pain modulation. However, the precise neural pathways and mechanisms underlying this phenomenon are still not fully understood. Recent neuroimaging studies, particularly using techniques like functional magnetic resonance imaging (fMRI), have provided some insight into the neural mechanisms underlying CPM, although the full extent of its relationship with descending pain regulation has not yet been revealed. This has been attributed partially to difficulties surrounding functional imaging of the brainstem and spinal cord regions involved in these processes. Therefore, the present study aimed to investigate the effects of CPM within the brainstem and spinal cord of healthy humans by means of fMRI, using our established methods. Data were collected from healthy males and females at a 1:1 ratio, aged between 20-65 years. Participants underwent multiple trials within the MR system in

which they experienced a predictable painful stimulus on their right hand, randomly interleaved with trials in which they experienced a second stimulus simultaneously, on their left calf. Through the use of Structural and Physiological Modeling (SAPM), a novel connectivity analysis method, we compared the two study conditions in relation to verbal subjective pain intensity and unpleasantness ratings, and investigated the neural mechanisms that underlie the differences between the conditions. Results revealed significant connections between regions in the brainstem and spinal cord and connectivity values were identified which are correlated with pain ratings. These findings help to further our understanding of CPM and the mechanisms involved in descending pain regulation in humans.

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Poster

PSTR167: Descending Modulation of Pain

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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Topic: D.01. Somatosensation – Pain and Itch

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Neuroscience Scholars Program

Title: Long-term spinal cord imaging in behaving animals before and after nerve injury

Authors: *M. ROSA CASILLAS[1], A. J. CROWTHER[2], B. O. AHANONU[3], A. I. BASBAUM[4];
[1]Neurosci., UCSF, San Francisco, CA; [2]Univ. of California San Francisco, San Francisco, CA; [3]Dept. of Anat., Univ. of California, San Francisco, San Francisco, CA; [4]Univ. of California, San Francisco, San Francisco, CA

Abstract: Optical imaging of spinal cord neural activity in the awake, behaving animal has clear and significant advantages (Cheng et al., 2019; Nelson et al., 2019; Iseppon et al., 2022). However, due to technical difficulties, few studies have recorded spinal cord neural activity, long-term, in awake animals (Cheng et al., 2016; Sekiguchi et al., 2016; Ju et al., 2022; Shekhtmeyster et al., 2023). We recently solved the limitation posed by post-laminectomy fibrosis, which makes long-term imaging impossible (Ahanonu et al, 2023). The impact of

excessive spinal cord movement has also been addressed, allowing us to record long-term from the same population of spinal cord neurons in behaving mice. With this new ability, we can now track cellular changes in the organization of dorsal horn circuits that process injury messages, before and after nerve injury, and can correlate these changes with behavior. The spinal optical imaging approach provides an expansive view of both sides of the spinal cord, within multiple segments of the lumbar enlargement. By incorporating fluoropolymer membranes, which provide long-term fibrosis regrowth inhibition, we reliably maintain clear optical access to the spinal cord, for many months to over a year. To handle the complex spinal cord motion that occurs during awake spinal cord imaging, we developed novel image registration pipelines, which are available within CIAtah, our existing Ca^{2+} imaging analysis pipeline (Corder et al., 2019). Using this method, we longitudinally recorded the activity of genetically-defined dorsal horn lamina I projection neurons (SCPNs; using Phox2a-Cre floxed-GCaMP6 mice) across the development of chronic pain phenotypes in the SNI (Spared Nerve Injury) model. Repeated imaging of single-cell neural activity of large numbers of SCPNs revealed that SCPNs predominantly display polymodal responses in awake animals. Individual response profiles were consistent over months before injury. Further, in contrast to anesthetized mice, in the awake state, lamina I projection neurons exhibit significant spontaneous and movement-evoked activity. Our ability to follow the same population of neurons over time will illuminate the tissue and nerve injury-induced changes during the transition from acute to chronic pain. In progress are studies that evaluate tissue and nerve injury-induced changes in spontaneous and evoked excitability of SCPNs. Ongoing studies are also assessing the impact of existing and novel analgesics on the activity of these projection neurons, at different times during the development of chronic pain.

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Poster

PSTR168: Peripheral Somatosensory Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR168.01/G1

Topic: D.02. Somatosensation – Touch

Support: NIH Grant MH122987
NIH Grant GM140130

Title: Identifying Gating Mechanisms of Thermoreceptors

Authors: *A. HUDA, L. NI;
Sch. of Neurosci., Virginia Tech., Blacksburg, VA

Abstract: Temperature significantly influences physiological processes and behaviors, impacting chemical reactions, biomolecule activity, and species distribution. Insect vectors of diseases, like mosquitoes, are responsive to temperature cues, shaping survival, reproduction,

and host-seeking behaviors. *Drosophila melanogaster* (fruit flies) is a model for studying insect thermosensory processes. Its thermosensory systems rely on manipulable sensory neurons and a balance between high- and low-temperature activated neurons. Specific neurons, including aristal heating and cooling cells, guide avoidance responses to temperature shifts. The warm receptor GR28B(D), essential for avoiding high temperatures, is poorly understood.

Disclosures: A. Huda: None. **L. Ni:** None.

Poster

PSTR168: Peripheral Somatosensory Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR168.02/G2

Topic: D.02. Somatosensation – Touch

Support: NIH Grant GM140130

Title: The Expression of Ionotropic Receptors Impacts the Sensitivity of Drosophila Larval Cool Cells under Hypertonic Conditions

Authors: *H. BAI, L. NI;
Sch. of Neurosci., Virginia Technol., Blacksburg, VA

Abstract: *Drosophila melanogaste*r exhibits multiple highly sophisticated temperature-sensing systems, enabling its effective response and navigation to temperature changes. Previous research has identified three dorsal organ cool cells (DOCCs) in fly larvae, consisting of two A-type and one B-type cell that displays distinct calcium dynamics. When subjected to hypertonic conditions, A-type DOCCs maintain their responses to cool temperatures, while the responses of B-type DOCCs are greatly diminished or completely eliminated. The activation of both A-type and B-type DOCCs depends on the same three members of the ionotropic receptor (IR) family: IR21a, IR93a, and IR25a. A-type DOCCs exhibit a higher somal level of IR93a than B-type DOCCs. In larvae with *Ir93a* overexpression, B-type DOCCs exhibit a comparable level of IR93a expression to A-type cells. Under hypertonic conditions, B-type calcium responses to cool temperatures are also increased, similarly to A-type responses. These findings suggest that IR expressions may alter receptor responses to environmental stimuli, and B-type DOCCs may serve as a pivotal integrator for temperature and tonicity.

Disclosures: H. Bai: None. **L. Ni:** None.

Poster

PSTR168: Peripheral Somatosensory Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR168.03/G3

Topic: D.02. Somatosensation – Touch

Support: NIH Grant GM140130

Title: Defining molecular mechanisms underlying thermoreceptors IR21a and IR68a

Authors: *T. J. VADEN, L. NI;
Sch. of Neurosci., Virginia Tech., Blacksburg, VA

Abstract: Temperature affects nearly every aspect of an organism's life, from general survivability to efficiency of biological functions. To navigate to preferred temperature ranges, animals use thermoreceptors: a diverse group of temperature sensitive molecules located in specialized sensory neurons. While several classes of thermoreceptors have been identified across a vast array of species, the molecular mechanism responsible for thermosensation is not well understood. This project focuses on two closely related thermoreceptors found in *Drosophila melanogaster*, IR21a and IR68a, a cool and warm receptor respectively, to elucidate the molecular mechanism underlying the response to temperature stimuli. We first utilized the computational protein modeling software AlphaFold to understand the structures of IR21a and IR68a. We then generated fly lines containing chimeric IR21a/IR68a proteins using CRIPSPR/Cas9 technology. We are now in the process of testing these fly lines for changes in phenotype using techniques such as immunofluorescent staining, calcium imaging, and temperature preference behavioral assays. We expect to identify at least one fly line showing an altered response to temperature which will be further analyzed to determine critical amino acid residues, as well as residue interactions, required for thermosensation. This project will not only answer a fundamental question regarding sensory systems but will also provide valuable insight about thermosensation in insect vectors of disease, which will aid in the development of methods to minimize the spread of infectious diseases harbored by these insects.

Disclosures: T.J. Vaden: None. **L. Ni:** None.

Poster

PSTR168: Peripheral Somatosensory Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR168.04/G4

Topic: D.02. Somatosensation – Touch

Support: NIH Grant DE018661
NIH Grant DE023090

Title: Acid-sensing ion channels drive the generation of tactile impulses in Merkel cell-neurite complexes of the glabrous skin of rodent hindpaws

Authors: *J. GU, A. YAMADA, J. LING;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Merkel cell-neurite complexes (MNCs) are enriched in touch-sensitive areas, including whisker hair follicles and the glabrous skin of the rodent's paws, where tactile stimulation elicits slowly adapting type 1 (SA1) tactile impulses to encode for the sense of touch. Recently, we have shown with rodent whisker hair follicles that SA1 impulses are generated through fast excitatory synaptic transmission at MNCs and driven by acid-sensing ion channels (ASICs). However, it is currently unknown whether, besides whisker hair follicles, ASICs also play an essential role in generating SA1 impulses from MNCs of other body parts in mammals. In the present study, we attempted to address this question by using the skin-nerve preparations made from the hindpaw glabrous skin and tibial nerves of rodents and applying the pressure-clamped single-fiber recordings. We showed that SA1 impulses elicited by tactile stimulation to the rat hindpaw glabrous skin were largely diminished in the presence of amiloride and diminazene, two ASIC channel blockers. Furthermore, using the hindpaw glabrous skin and tibial nerve preparations made from the mice genetically deleted of ASIC3 channels ($ASIC3^{-/-}$), we showed that the frequency of SA1 impulses was significantly lower in $ASIC3^{-/-}$ mice than in littermate wildtype $ASIC3^{+/+}$ mice, a result consistent with the pharmacological experiments with ASIC channel blockers. Our findings suggest that ASIC channels are essential for generating SA1 impulses to underlie the sense of touch in the glabrous skin of rodent hindpaws.

Disclosures: J. Gu: None. **A. Yamada:** None. **J. Ling:** None.

Poster

PSTR168: Peripheral Somatosensory Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR168.05/G5

Topic: D.04. Interoception

Support: RS-2023-00254795

Title: Molecular Mechanism of Tentonin 3 inhibition by NMB-1

Authors: *S. LIM, S. PAK, P. LEE, G. HONG, U. OH;
KIST, Seoul, Korea, Republic of

Abstract: Tentonin3 (TTN3/TMEM150c) is a mechanosensitive channel activated by mechanical stimuli, whose inactivation kinetics follow the slow-adapting (SA) type of mechanically activated (MA) currents found in dorsal root ganglion (DRG) neurons. TTN3 is known to mediate physiological functions including proprioception, baroreceptor reflex, and insulin release from pancreatic cells. NMB-1 is a mutant of a rho-conotoxin known to inhibit SA-type MA currents in DRG neurons. Given that TTN3 confers SA-type MA currents in DRG neurons, we hypothesized that NMB-1 might block TTN3 MA currents. Indeed, NMB-1 inhibits TTN3 with an IC50 of 0.92 μM. NMB-1 is a peptide consisting of 19 amino acids, characterized

by two cysteine knots and many positively charged residues. Through mutational studies, we identified positively charged residues whose mutation ablated its inhibitory action on TTN3. Utilizing state-of-the-art deep learning software, the structure of TTN3 has been predicted in combination with mutational experiments. The predicted structure exhibits a rectangular shape with a pore in the center of four subunits. At the entrance of the pore, the negative charge ring which is observed in many cation channels is also positioned. The positive charges of NMB-1, key residues for its inhibitory action, appear to interact with the negative charges at the entrance of the pore. As TTN3 mediates numerous physiological functions, the identification of a specific blocker and its interaction mechanism could lead to the development of useful tools for clinical applications.

Disclosures: S. Lim: None. **S. Pak:** None. **P. Lee:** None. **G. Hong:** None. **U. Oh:** None.

Poster

PSTR168: Peripheral Somatosensory Mechanisms

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Topic: D.04. Interoception

Support: National Research Foundation of Korea Grant RS-2023-00254795
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National Research Foundation of Korea Grant 2020R1C1C101024514
National Research Foundation of Korea Grant 2022M3E5E801739512

Title: Tentonin 3/TMEM150C activation is modulated by the focal adhesion and mechanosensitive adaptive protein, Stomatin-like protein-3 (STOML3)

Authors: *S. PAK[1], S. LIM[1], P. LEE[2], G. HONG[3], U. OH[4];
[1]Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of; [2]Brain Sci. Inst., Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of; [3]Neurosci., Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of; [4]Brain Sci. Inst., KIST, Seoul, Korea, Republic of

Abstract: Tentonin 3/TMEM150C (TTN3) is a slowly-inactivating mechanosensitive channel involved in various physiological roles, including proprioception, hypertension, and type 2 diabetes. Its representative slowly-inactivating property is distinctive from the rapidly-inactivating Piezo channels. Structure prediction algorithms, coupled with mutational studies, predict a homo-tetrameric structure with a rectangular shape and a central pore. Previously, TTN3 was considered a regulator of Piezo1 because TTN3's mechanosensitivity was abolished in Piezo1-knockout cells. However, when we treated Piezo1-knockout cells with jasplakinolide, an actin assembly enhancer, TTN3 mechanosensitivity was reproduced. This suggests that cytoskeleton integrity is highly linked to TTN3 mechanosensitivity. Therefore, we studied the functional role of focal adhesion complex proteins in TTN3 channel response. We found that TTN3 mechanosensitivity is highly regulated by focal adhesion proteins, as its response was

markedly attenuated when focal adhesion proteins were knocked down. Additionally, we identified STOML3, a homolog of the mechanosensitive mec-2 from C. elegans, augmenting TTN3 activity, leading to an increase in current response. Results from a pull-down assay with STOML3 indicate that TTN3 is physically associated with STOML3. We conclude that the functional interaction with STOML3 bridges TTN3 to focal adhesion proteins, which further modulates the mechanosensitivity of TTN3. As TTN3 mediates many physiological functions, the molecular interaction will contribute to an in-depth understanding of the gating mechanism of TTN3.

Disclosures: S. Pak: None. **S. Lim:** None. **P. Lee:** None. **G. Hong:** None. **U. Oh:** None.

Poster

PSTR168: Peripheral Somatosensory Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR168.07/G7

Topic: D.04. Interoception

Support: NIH Grant 1F31NS134241-01A1
NIH Grant 5T32GM099608-10

Title: Differential encoding of mammalian proprioception by voltage-gated sodium channels

Authors: *C. ESPINO[1,2], C. NAGARAJA[3], S. ORTIZ[4], J. DAYTON[3], A. MURALI[3], Y. MA[3], E. MANN[3], S. GARLAPALLI[3], R. WOHLGEMUTH[5], S. BRASHEAR[5], I. J. VILLEGAS[6], J. A. HALMAI[7], L. SMITH[5], K. FINK[8], K. A. WILKINSON[9], T. GRIFFITH[10];
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Abstract: Our sense of body and limb position in space -proprioception- is required for accurate movement execution and is initiated by a subclass of sensory neurons called proprioceptors. Despite the essential nature of proprioception to motor output, our understanding of the mechanisms that govern proprioceptive activity is incomplete. We previously found that the voltage-gated sodium channel (Na_V) $Na_V1.1$ is required for normal proprioception and predicted that other Na_V isoforms expressed within proprioceptors have distinct roles in electrical signaling. Here we focus on the $Na_V1.6$ subtype. Mice lacking $Na_V1.6$ in somatosensory neurons ($Pirt^{Cre};Na_V1.6^{fl/fl}$, $Na_V1.6^{cKO}$) exhibited extreme motor coordination deficits that were distinct from the ataxic-like phenotype in our $Na_V1.1$ conditional knockout model. Unlike $Na_V1.1$, which we previously showed is only required for reliable proprioceptive transmission during static

muscle stretch, we found a complete loss of all stretch- and vibratory responses in *ex vivo* muscle-nerve recordings from $Na_V1.6^{cKO}$ animals, suggesting $Na_V1.6$ plays a dominant role signal propagation. The proprioceptor-mediated monosynaptic spinal cord reflex in $Na_V1.6^{cKO}$ mice was nearly absent, further highlighting an important role for $Na_V1.6$ in proprioceptor signaling. In addition to cell autonomous deficits, our analyses of skeletal muscle found proprioceptor-induced nonautonomous impairments. Analysis of myofibers from $Na_V1.6^{cKO}$ mice found a significant decrease in fiber area and diameter compared to controls. Ongoing experiments are investigating if $Na_V1.1$ is also required in proprioceptors for skeletal muscle growth. We hypothesized the differential function of Na_Vs in proprioceptors was due to distinct cellular localization patterns. Thus, we immunolabeled against $Na_V1.1$ and $Na_V1.6$ in muscle spindle end organs and found $Na_V1.6$ channels are densely expressed at discrete AnkyrinG-positive clusters. $Na_V1.1$ occupied a completely distinct cellular region; it was observed more diffusely along equatorial, but not distal, muscle spindle wrappings. Finally, we developed an intersectional CRISPR/Cas9 approach to delete Na_V channels selectively in proprioceptors *in vivo* by viral delivery of single guide RNAs (sgRNAs). Temporally controlled deletion of $Na_V1.1$ in proprioceptors induced significant impairments in rotarod performance 3 weeks post sgRNA injection, demonstrating an acute requirement of $Na_V1.1$ for proprioceptor function. We are currently investigating the acute requirement of $Na_V1.6$. Together, these studies provide mechanistic insight into the different roles of Na_Vs in mammalian proprioception.

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Poster

PSTR168: Peripheral Somatosensory Mechanisms

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR168.08/G8

Topic: D.04. Interoception

Support: NIH Grant UF1NS115817

Title: Mechanisms of proprioception in the soft-bodied octopus arm

Authors: *C. S. OLSON[1], C. W. RAGSDALE[2];
[1]Univ. of Chicago, Chicago, IL; [2]Dept Neurobio., Univ. of Chicago Committee On Neurobio., Chicago, IL

Abstract: Octopuses have the profound ability not only to control a single highly flexible arm, but also to coordinate movements efficiently across eight arms. These arms lack skeletal structure and are composed solely of muscle, nervous tissue, connective tissue, and skin, increasing the complexity of the motor control problem. Proprioceptive feedback is essential for

effective motor control. However, the mechanisms by which proprioception is detected in the soft-bodied octopus arm are unclear. A classic study using silver staining by Graziadei (ProcRoySocB, 1965) describes muscle receptors embedded in the lateral edges of the arm musculature. These neurons extend their processes onto muscle and send their axons to the nearby intramuscular nerve cords, which are small nerve cords located in the four corners of the arm. We employed modern molecular techniques of gene expression and immunohistochemistry to confirm Graziadei's anatomy conclusions. In addition, we found a novel candidate proprioceptive organ in the octopus arm. Similar to where Graziadei (1965) describes muscle receptors, this proprioceptive organ is located laterally in the arms, sandwiched between two muscle groups: the outer oblique and longitudinal muscles. Structurally, it is composed of acetylated alpha-tubulin-positive (acTUBA+) nerve cells with fibers wrapped around a rod enriched in F-actin and message for myosin heavy chain. Some of these cells send projections into the neighboring muscle, matching the structure of the proposed muscle receptors of Graziadei (1965). This lateral arm territory is also enriched in the expression of *PIEZO*, a mechanosensitive ion channel marker, and *DRGX*, the dorsal root ganglion homeobox gene. The organ itself is at an angle to the muscle groups it is situated between, and traverses the full oral, which is towards the sucker, to aboral, which is opposite the sucker, extent of the main body of the arm. This candidate proprioceptor organ is repeated down the proximal-distal axis of the arm, with two organs for each sucker length. By its location between muscle groups, this proprioceptor appears competent to detect deflections of the arm as a whole, as opposed to movements of individual muscle groups, and its location at an eccentric position should maximize the amount and quality of the information it collects. We conclude that the proprioceptive organ is situated to provide information about the local configuration of the arm in response to the muscle commands and external forces acting upon it. This architecture may inform some of the challenges in soft-body robot development.

Disclosures: C.S. Olson: None. **C.W. Ragsdale:** None.

Poster

PSTR168: Peripheral Somatosensory Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR168.09/G9

Topic: D.02. Somatosensation – Touch

Support: NIDCD grant R01 DC015799

Title: Identification of putative oral mechanosensory end-organs

Authors: *D. DUTTA BANIK[1], T. TANG[2], B. PIERCHALA[3];
[1]Indiana Univ. Sch. of Med., Dept. of Anat., Cell Biol. & Physiol., Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN; [2]Indiana Univ. Sch. of Med., Dept. of Anat., Cell Biol. & Physiol., Indiana Univ. Sch. of Med., Indianapolis, IN; [3]Anatomy, Cell Biol. & Physiol., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Oral mechanosensation plays a critical role in feeding behaviors, social grooming, and speech. The mechanosensory neurons innervating the tongue and oral cavity convey the textural properties of the food such as crispiness, creaminess, and graininess to the central nervous system. These properties of food bestow appetitive or aversive characteristics resulting in it either consumed or rejected. Recent studies have identified RET+ neurons in both Geniculate and Trigeminal ganglia that are indispensable for oral mechanosensation. RET is the signal-transducing receptor for the Glial Cell Line-Derived Neurotrophic Factor (GDNF) Family Ligands (GFLs). There are four GFLs (GDNF, Artemin, Neurturin, Persephin) which bind with high affinity to co-receptors comprised of one of the GFRαs (GFRα1-4) which then bind and activate RET. In this study, we characterized the expression of GFLs on the lingual epithelium using recently developed reporter lines and found GDNF and Artemin expression in lingual epithelium in and around Fungiform taste papillae, whereas GDNF and Persephin are expressed at the base of Filiform papillae. Using intersectional genetics and immunolabelling, we found that RET+ fibers originating from Geniculate ganglia innervate mostly the extragemmal region of the Fungiform papillae and express GFRα1, the receptor for GDNF. These RET and GFRα dual positive fibers originating from Geniculate ganglia make contacts with both GDNF+ and Artemin+ cells in the lingual epithelium. Finally, using FM 1-43 dye labelling, we found activated Piezo2+ mechanosensory fibers in the tongue colocalize with GFRα1 just under the lingual epithelium. Our data indicate that the GDNF+ and Artemin+ cells on the lingual epithelium might act as putative oral mechanosensory end organs. We are currently using GFL knock-out mice to characterize how the loss of GDNF and Artemin affects tongue innervation and the oral mechanosensory responses to mechanical stimuli like chewing.

Disclosures: D. Dutta Banik: None. **T. Tang:** None. **B. Pierchala:** None.

Poster

PSTR168: Peripheral Somatosensory Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR168.10/G10

Topic: D.02. Somatosensation – Touch

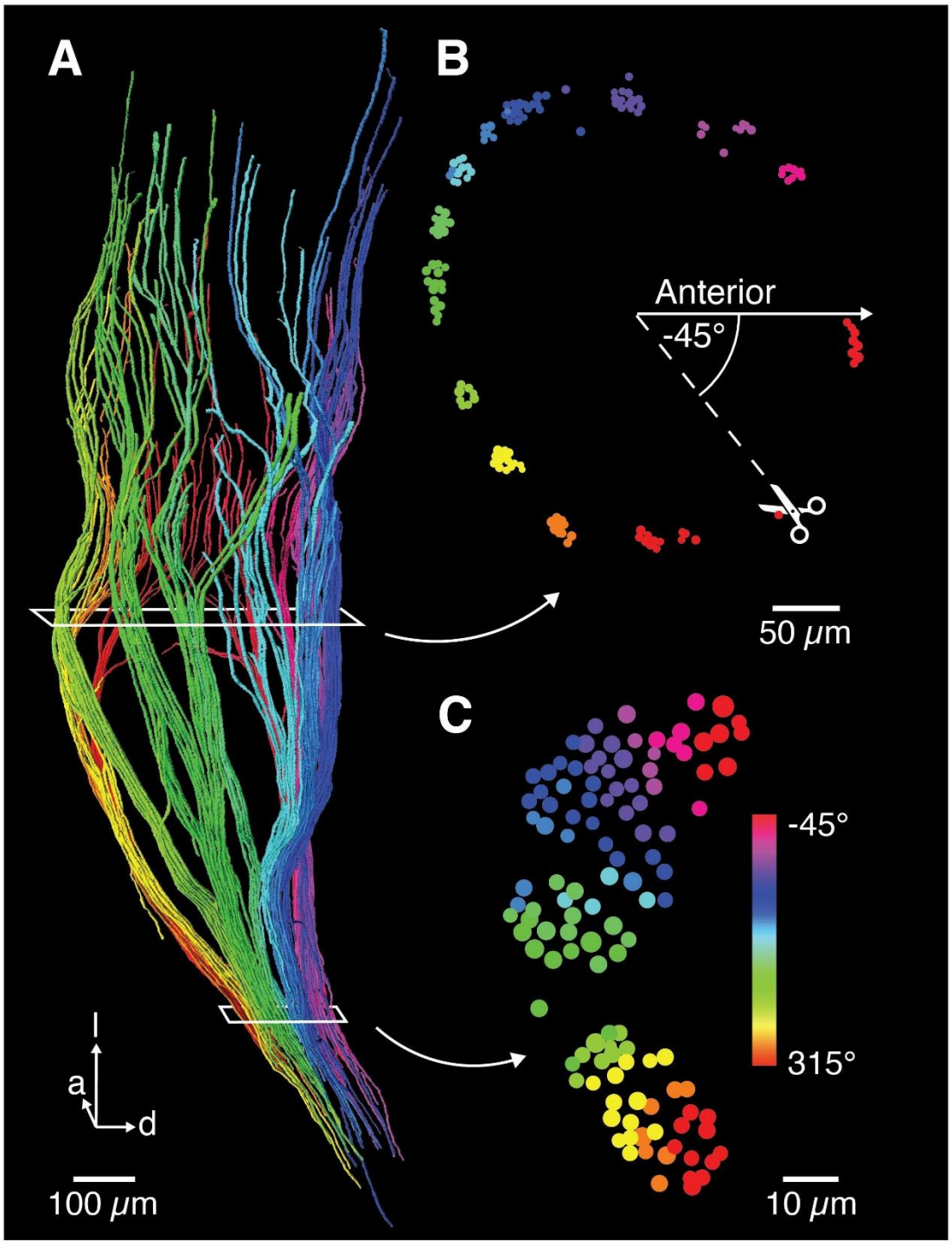
Support: EXC-2049—390688087
European Research Council (BrainPlay ERC Synergy Grant)
DESY Photon Science

Title: Three-dimensional architecture and linearized mapping of vibrissa follicle afferents

Authors: *B. GERHARDT[1], J. ALFKEN[2], J. REICHMANN[2], T. SALDITT[2], M. BRECHT[1]; [1]BCCN Berlin, Berlin, Germany; [2]Georg-August-Universität Göttingen, Göttingen, Germany

Abstract: Understanding the vibrissa follicle has been challenging, given the intricate afferent innervation, which maps onto an encapsulated and complex internal structure. While serial sections and identified afferent recordings have clarified overall anatomy and response

properties, the precise mapping of the afferent population onto the vibrissa follicle remains elusive. Here, we reveal the three-dimensional architecture of the rat C2-vibrissa follicle innervation along with the accessory mechanosensory machinery by synchrotron X-ray phase contrast tomograms. Axons ascend in straight trajectories and differ in ending location, morphology, trajectory, branching, axon diameter and internode length. We distinguish four afferent types (below-ringwulst-, club like-, Merkel-, and lanceolate-afferents), of myelinated innervation, out of which merkel and club-like endings are most abundant. Innervation is radially polarized to the posterior circumference - presumably to sample contacts from vibrissa-protraction. Afferents are organized in axon-arms innervating discrete angular territories. Axon-arm tracing revealed elaborate vibrissal nerve microtopography: The radial axon-arm arrangement around the vibrissa maps into a linear representation of axon-arm-bands in the nerve. Such linearization of follicle representations presumably instructs the downstream linear (cylindric) brainstem barrelettes and thalamic barreloids. We conclude synchrotron X-ray tomograms can elucidate follicle architecture and afferents mapping into the nerve in unprecedented detail.



Disclosures: B. Gerhardt: None. **J. Alfken:** None. **J. Reichmann:** None. **T. Salditt:** None. **M. Brecht:** None.

Poster

PSTR168: Peripheral Somatosensory Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR168.11/G11

Topic: D.02. Somatosensation – Touch

Support: Seoul National University (550-20230121)

Title: Tactile hypersensitivity and the upregulation of mechano- and chemo- sensitive ion channels in Cxcl5 KO mice

Authors: *Y. SEO, R. SHARMA, S. YANG, S. LEE, J. PARK;
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Abstract: The tactile sensation plays a critical role in normal physiology and is facilitated by mechanically activated ion channels responsible for converting mechanical stimuli into electrical signals. Tactile hypersensitivity due to numerous pathophysiological conditions, including trauma to the central or peripheral nervous system, has been well studied. However, the physiological factors that control tactile hypersensitivity in non-pathological conditions are poorly understood. Here, we show that the basal level of CXC ligand 5 (CXCL5), a key inflammatory mediator, unexpectedly controls physiological tactile sensitivity through a non-inflammatory pathway. In the present study, CRISPR/Cas9-generated *Cxcl5* knockout (KO) mice exhibited a higher paw withdrawal response in the von Frey filament test, indicating tactile hypersensitivity, compared to the wild type mice (7-8 weeks old, male, C57BL/6N). On the other hand, *Cxcl5* knockout had a minimal effect on other essential brain functions, including motor and cognitive function. Meanwhile, mRNA expression of mechano- or chemo-sensors, including *Piezo2, Trpv1,* and *Trpa1* channels, was upregulated in the spinal cord of the *Cxcl5* KO mice, while in skin tissue, *Piezo2* expression was increased. In addition, immunoreactivity of Piezo2 was also increased in the Merkel cells of the hind paw epidermis of *Cxcl5* KO compared to wild type mice. Taken together, our results suggest that basal CXCL5 essentially controls physiological tactile sensation and thus may represent a promising therapeutic option for treating spontaneous tactile hypersensitivity.

Disclosures: Y. Seo: None. **R. Sharma:** None. **S. Yang:** None. **S. Lee:** None. **J. Park:** None.

Poster

PSTR168: Peripheral Somatosensory Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR168.12/G12

Topic: D.02. Somatosensation – Touch

Support: Korea Grant NRF-2022R1C1C2003317

Title: Transcriptional profiling of dental and masseter nerves

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[1]Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of; [2]Brain Sci. Inst., Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of; [3]Univ. of Pennsylvania, Philadelphia, PA; [4]Sch. of Dent., Seoul Nat'l Univ., Seoul, Korea, Republic of

Abstract: Dental primary afferent (DPA) sensory neurons and mesencephalic trigeminal nucleus (MTN) proprioceptive neurons, situated within the trigeminal ganglion and the brainstem respectively, play essential roles in the regulation of masticatory functions. Despite the extensive transcriptomic inquiries into various somatosensory neuron types, the molecular signatures of these specific neuron populations remain unknown due to technical challenges in isolating them within their validated circuits. In this study, we employed single-cell RNA sequencing combined with retrograde tracing strategies in mouse models to elucidate the intrinsic transcriptional features of DPA and MTN neurons. Our transcriptome analysis revealed distinct subtypes of DPA neurons with unique gene expression patterns, some of which manifest mechano-nociceptive attributes, specialized for transmitting pain signals elicited by innocuous mechanical stimuli in sensitive teeth. Moreover, we identified cellular diversity within MTN neurons, potentially contributing to their capacity to sense mechanical stretch in the masseter muscle spindles. Thus, our study provides new biological insights regarding the highly specialized mechanosensory functions of dental and masseter nerves in pain and proprioception.

Disclosures: P. Lee: None. **U. Oh:** None. **J. Kim:** None. **S. Oh:** None.

Poster

PSTR169: Interoception: Peripheral Signals to Regulation of Physiology

Location: MCP Hall A

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Program #/Poster #: PSTR169.01/G13

Topic: F.01. Neuroethology

Support: Serrapilheira 01/2017
Brazilian Federal Agency for Support and Evaluation of Graduate Education
Brazilian National Council for Scientific and Technological Development

Title: Respiration modulates frontal brain neural activity across the rat behavioral repertoire in the open field

Authors: *D. DRIESKENS[1,2], J. BELO[2], A. DIAS[2], E. DUARTE[2], A. FURTUNATO[3], A. B. TORT[2], D. A. LAPLAGNE[2];

[1]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD; [2]Brain Inst., [3]Program of Psychobiology, Federal Univ. of Rio Grande do Norte, Natal, Brazil

Abstract: Breathing is an essential rhythm of life, and each breathing cycle serves as a unit of odor sensation. In the past decade, several studies have investigated the modulation of brain activity by respiration in cortical and subcortical areas, and across taxa. The majority of research has been conducted in contexts in which respiratory rates are narrowed. There remains a gap in our understanding regarding the manifestation of this phenomenon across natural behaviors and varied respiratory rates. To address that, we recorded synchronized respiration, depth videography, and brain local field potentials as rats behaved spontaneously in an enriched open field arena. We implanted a 2x7 custom-manufactured array of single 50µm microelectrodes organized anteroposteriorly and dorsoventrally in the rat medial prefrontal cortex. We recorded field potentials activity from the medial orbitofrontal, prelimbic, and anterior cingulate cortex, as well as ECoG activity from the olfactory bulb. We show evidence that nasal breathing evokes waves of activity across the frontal brain for all respiratory rates and across the rat's behavioral repertoire. Low and high-frequency components of distributed brain activity aligned with constant latency to landmarks of the nasal breathing cycle. The magnitude of these brain respiratory potentials is highest for low respiratory rates, while being further influenced by the animal behavioral/cognitive state. Our results support that respiratory potentials are salient in the frontal brain of the rat across its spontaneous behavioral repertoire.

Disclosures: D. Drieskens: None. **J. Belo:** None. **A. Dias:** None. **E. Duarte:** None. **A. Furtunato:** None. **A.B. Tort:** None. **D.A. Laplagne:** None.

Poster

PSTR169: Interoception: Peripheral Signals to Regulation of Physiology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR169.02/G14

Topic: D.04. Interoception

Support: European Commission, Horizon MSCA Program (grant n° 101151118)

Title: Brain-heart dynamics at near-death can predict successful resuscitation

Authors: *D. CANDIA-RIVERA, S. CARRION-FALGARONA, M. CHAVEZ, F. DE VICO FALLANI, S. CHARPIER, S. MAHON;
Paris Brain Institute: Inst. du Cerveau, Paris, France

Abstract: Previous research in experimental models of anoxia has identified a consistent cortical activity pattern post loss of brain oxygenation. Initially, there is a surge in beta-gamma band activities, followed by a gradual decline in all frequency bands. Occasionally, this decline is interrupted by late delta oscillations before reaching a complete cessation of spontaneous electrical activity, transitioning to a prolonged isoelectric state. However, timely reoxygenation can reverse this electro-cerebral silence, restarting a gradual recovery in physiological activity.

Interestingly, changes in EEG activity co-fluctuates with cardiac electrophysiology at the anoxia onset in rodents. Moreover, investigations in humans reveal relationships between brain-heart interaction and the severity of post-anoxic brain injuries in cardiac arrest patients.In this study, we investigated the functional relationship between cardiac responses (differentiating the potential contribution of sympathetic and vagal systems) and brain responses to asphyxia. Our aim was to determine whether the dynamics of brain-heart interaction could serve as a predictor for the outcome of a rescue procedure. We conducted continuous electrocardiography along with multi-site cortical field potential (LFP) recordings in anesthetized and curarized rats (n=30). Anoxia was induced by interrupting artificial ventilation, with resuscitation involving the resumption of oxygen supply after 3-4 minutes. We quantified the strength of brain-heart interactions by computing Spearman correlation coefficients between LFP frequency bands and cardiac sympathetic-vagal activity envelopes during the post-asphyxia periods, up to 50 seconds.Following the restoration of brain oxygenation, 60% of cases witnessed a resumption of cerebral activity (successful outcome), while the rest succumbed within minutes (fatal outcome). We found that the onset of asphyxia coincided with an increase in heart rate variability, particularly pronounced in rats with successful outcomes. We found that a more gradual decline in cortical activities also correlated with a higher likelihood of successful outcome. Additionally, beta-gamma decline preceding the isoelectric state aligned with an increase in cardiac vagal activity, which exhibited strong predictive value for the recovery of brain activity post-resuscitation.Our findings underscore the potential of further investigating brain-heart interactions, offering promise in using LFP/EEG alongside cardiac dynamics as a prognostic tool for estimating outcomes in patients recovering from prolonged cerebral anoxia.

Disclosures: D. Candia-Rivera: None. **S. Carrion-Falgarona:** None. **M. Chavez:** None. **F. De Vico Fallani:** None. **S. Charpier:** None. **S. Mahon:** None.

Poster

PSTR169: Interoception: Peripheral Signals to Regulation of Physiology

Location: MCP Hall A

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Program #/Poster #: PSTR169.03/G15

Topic: D.04. Interoception

Support: VA Merit Review Award (PULM-024-17S)

Title: Function of rapidly adapting baroreceptors in regulation of hemodynamics

Authors: B. MOLDOVEANU[1,2], J. GUARDIOLA[1,2], S. WICHMANN[2], J. YEUNG[2], *J. YU[2,1]; [1]Louisville VA MC, Louisville, KY; [2]Univ. of Louisville, Louisville, KY

Abstract: Large myelinated baroreceptors (BRs), such as carotid and aortic ones, are thought to send beat-by-beat blood pressure information to the brain for hemodynamic control. However, after a century of intensive study, how their information is processed remains unclear. Conventionally, one afferent axon is believed to transmit a single BR signal (one-sensor theory

or OST). Under OST, these sensors are slowly adapting, having both dynamic and static components. Recently, a multiple-sensor theory (MST) has been advanced in the respiratory mechanosensory system. Here, a sensory unit consists of many heterogeneous sensors that intercept different aspects of mechanical forces and work together for mechanosensory function. Our recent electrical recordings of aortic BRs support the MST, demonstrating the BR unit contains many rapidly and slowly adapting BRs responsible for dynamic and static components, respectively. They are activated at different instants during a cardiac cycle to give complete and precise cyclic information. To seek morphological support for MST, we used a double staining approach in whole mount aortic tissue of rabbits. We labeled the whole sensory structure with Na^+/K^+ ATPase (α3 subunit) and the myelin sheath with myelin basic protein (MBP) and found 2 types of complex unencapsulated sensory endings (compact and diffuse). The compact ones are tightly packed. After demyelination of the axon, they immediately form end formations covering an area of 994.9 ± 90.4 μm^2, with an axon fiber diameter of 4.3 ± 0.16 μm (n=113). On the other hand, the diffuse structures are sparsely packed. After demyelination, they continue to divide with fibrillar branches covering a large area (8,124.2 ± 683.5 μm^2) with an axon diameter of 3.3 ± 0.17 μm; n=115). In many cases, a parent axon can connect to many sensors. It is not uncommon for the two types to exist in the same region and even appear to share a parent axon. These morphological data perfectly match the electrophysiological data, supporting MST, i.e., the existence of numerous 2 different types of sensors (rapidly and slowly adapting ones) in a single unit. Under the MST, with a new interpretation of how the mechanosensory signal is processed and decoded, many previously unsolved mysteries regarding BRs' control of circulation (such as sensory resetting, discharge pattern shifting, and heterogeneous sensing function) can be better explained and understood.

Disclosures: B. Moldoveanu: None. **J. Guardiola:** None. **S. Wichmann:** None. **J. Yeung:** None. **J. Yu:** None.

Poster

PSTR169: Interoception: Peripheral Signals to Regulation of Physiology

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Program #/Poster #: PSTR169.04/G16

Topic: D.04. Interoception

Support: NIH SPARC 3OT2OD023867
NIH HL 141560

Title: Transcutaneous auricular vagus nerve stimulation parameter specific responses in the nucleus of the solitary tract and spinal trigeminal nucleus compared to cervical vagus nerve stimulation

Authors: *M. OWENS[1], V. JACQUEMET[2], V. NAPADOW[3], N. LEWIS[4], E. BEAUMONT[1]; [1]Biomed. Sci., East Tennessee State Univ., Johnson City, TN; [2]Pharmacol. and Physiol., Univ.

de Montréal, Montreal, QC, Canada; [3]Physical Med. and Rehabil., Harvard Med. Sch., Charlestown, MA; [4]Med. Educ., East Tennessee State Univ., Johnson City, TN

Abstract: Transcutaneous auricular vagus nerve stimulation (taVNS) involves the electrical activation of subcutaneous axons in the auricular branch of the vagus nerve located at the outer ear. Its non-invasive nature makes it a potential alternative to the traditional invasive cervical vagus nerve stimulation (cVNS) in the treatment of various disorders. taVNS induces neuromodulatory effects within brainstem regions like the nucleus of the solitary tract (NTS) and the spinal trigeminal nucleus (Sp5). However, the specific pathway through which taVNS influences these regions is unclear. This study aimed to evaluate single-neuron electrophysiological responses in NTS and Sp5 using various stimulation parameters in Sprague Dawley rats under α-chloralose anesthesia. The parameters assessed included different frequencies ranging from 20 Hz to 250 Hz and intensities ranging from 0.5 mA to 1.0 mA. Neurons were categorized as positive, negative, or non-responders based on increased activity, decreased activity, or no response during stimulation, respectively. Stimulation at frequencies of 20 Hz and 100 Hz resulted in a higher proportion of positive responders in both NTS and Sp5 ($p<0.05$). Moreover, a higher intensity (1.0 mA) elicited stronger responses in both brain regions ($p<0.05$). Notably, Sp5 exhibited stronger responses and a higher proportion of responders compared to NTS ($p<0.05$), suggesting that taVNS may provide greater input to Sp5. Comparisons between taVNS and cVNS revealed similar parameter specific activation patterns for NTS neurons. However, individual neurons displayed varying activation profiles, suggesting that cVNS and taVNS may engage different neuronal pathways to elicit NTS neuromodulation. This study demonstrated distinct parameter-specific responses of taVNS in both NTS and Sp5 and confirmed the ability to generate comparable neuromodulation with both taVNS and cVNS. Understanding how adjusting stimulation parameters influence neuronal activity will facilitate the development of more personalized taVNS treatments for diverse clinical disorders.

Disclosures: M. Owens: None. **V. Jacquemet:** None. **V. Napadow:** None. **N. Lewis:** None. **E. Beaumont:** None.

Poster

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Program #/Poster #: PSTR169.05/G17

Topic: D.04. Interoception

Support: NIH R01 AT011653
NIH R01 DK092246

Title: Liver-innervating vagal sensory neurons play an indispensable role in anxiety-like behavior in mice fed a high-fat diet.

Authors: *Y.-H. JO[1], J. HWANG[2];
[1]Albert Einstein Col. of Med., bronx, NJ; [2]Endocrinol., Albert Einstein Col. of Med., New York, NY

Abstract: The visceral organ-brain axis, mediated by vagal sensory neurons, is essential for maintaining various physiological functions. These neurons transmit interoceptive signals from visceral organs to the medulla via the vagus nerve. Despite the important contribution of the liver to energy metabolism, glucose homeostasis, energy storage, immunity, and detoxification, surprisingly, there is little information about the vagal sensory neurons innervating the liver. Recent studies have focused on identifying the neural circuits of the liver and how these neural circuits regulate liver functions. However, individuals with nonalcoholic fatty liver disease (NAFLD) have a higher risk of psychiatric disorders including anxiety, depression, bipolar disorder, schizophrenia, and dementia, suggesting that impaired hepatic lipid metabolism may be closely associated with mental illness. Therefore, this study investigated the impact of liver-projecting vagal sensory neurons on energy balance, hepatic steatosis, and anxiety-like behavior in mice under obesogenic conditions.We performed single-nucleus RNA sequencing of vagal sensory neurons innervating the liver. Based on our snRNA-Seq results, we used the advillin (Avil)^{CreERT2} strain to identify vagal sensory neurons that innervate the liver. We found that vagal sensory neurons innervating the liver exhibited distinct molecular characteristics compared to other types of vagal sensory neurons. Second, both left and right vagus nerve ganglia contained vagal sensory neurons that innervate the liver. Third, these sensory neurons sent peripheral nerve projections primarily to the periportal area of the liver, and their central axonal terminals were localized to the NTS and AP, and to a lesser extent, in the DMV. Fourth, the loss of liver-innervating vagal sensory neurons prevented the development of diet-induced obesity in both males and females largely due to increased energy expenditure. Fifth, male and female mice without vagal sensory innervation of the liver displayed lower blood glucose levels and improved systemic glucose tolerance. Sixth, deleting liver-innervating vagal sensory neurons prevented HFD-induced hepatic steatosis in male and female mice. Finally, mice lacking liver-innervating vagal sensory neurons exhibit less anxiety-like behavior compared to the control mice.Our research has yielded highly original findings that the liver-brain axis contributes to the regulation of energy balance, systemic glucose tolerance, insulin sensitivity, hepatic steatosis, and anxiety-like behavior depending on the nutrient status in healthy and obesogenic conditions.

Disclosures: Y. Jo: None. **J. Hwang:** None.

Poster

PSTR169: Interoception: Peripheral Signals to Regulation of Physiology

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Topic: D.04. Interoception

Support: U01NS113868
U01DK116311
OT2OD023854

Title: Piezo2 is required for mechanotransduction in nodose afferent fibers innervating the esophagus.

Authors: *S. NAIR[1], S. HADLEY[2], M. PATIL[1], T. TAYLOR-CLARK[3];
[1]Univ. of South Florida, Tampa, FL; [2]Univ. of South Florida, TAMPA, FL; [3]Mol. Pharmacol. & Physiol., Univ. of South Florida, Tampa, FL

Abstract: The esophagus is densely innervated by polymodal sensory afferents from both the dorsal root ganglia as well as the vagal ganglia. Previous research has demonstrated that the esophagus is innervated by low threshold mechanoreceptors (LTMRs) arising from the nodose ganglia, which provide critical peripheral feedback that is required for proper swallowing and peristaltic reflexes. PIEZO ion channels are the bona fide proteins in mammals responsible for sensing mechanical stimuli. Moreover, nodose PIEZO2 has been implicated in important functions like airway stretch sensation and aortic blood pressure sensing. Hence, we hypothesize that PIEZO2 is the mechanotransducer in these nodose esophageal low threshold mechanoreceptors. We used a two-pronged approach to elucidate PIEZO2's role in esophageal mechanosensation: first, GCaMP6s imaging and then single fiber electrophysiology to understand the kinetics of nodose esophageal afferent fiber activation. We used our ex vivo intact vagal ganglia-vagus-esophagus preparation in both approaches. Our proof-of-principle studies were conducted in PIRT-GCaMP6s mice, obtained through the crossbreeding of Pirt-Cre (Cre expressed in all sensory neurons) mice with Cre-dependent, ROSA26 based, GCaMP6s reporter (B6.129S6-Gt(ROSA)26Sor-tm96(CAG-CGaMP6s)Hze). Our data in these mice showed a profound increase in the Ca^{2+} influx in a subset of nodose neurons in response to mechanical distention of the esophagus at 5, 10, and 30mmHg pressures. Our control studies were conducted in $Piezo2^{fl/fl}$ mice. Nodose-specific knockout of PIEZO2 was obtained by crossing $P2X_2$-Cre mice ($P2X_2$ is expressed in all nodose neurons) with $PIEZO2^{fl/fl}$ mice ($P2X_2^{PIEZO2}$ KO mice). Comparing the fluorescence intensities of all esophageal neurons between the control and KO groups, we observed a reduction of 3%, 41% and 52% at 5, 10 and 30mmHg intraesophageal pressure (n=2 mice; 120 neurons and 4 mice; 402 neurons in control and KO groups, respectively). The population of mechanosensitive neurons also reduced from 40% in the control group to 15% in the $P2X_2^{PIEZO2}$ KO mice. In our single fiber electrophysiology experiments, we were able to successfully record action potentials from A-fiber mechanosensitive esophageal neurons. The number of APs recorded from KO group was reduced at all three intraesophageal pressures when compared to the control $PIEZO2^{fl/fl}$ mice. Our findings support the hypothesis that PIEZO2 is the mechanotransduction protein involved in vagal nodose afferent fibers innervating the esophagus.

Disclosures: S. Nair: None. **S. Hadley:** None. **M. Patil:** None. **T. Taylor-Clark:** None.

Poster

PSTR169: Interoception: Peripheral Signals to Regulation of Physiology

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Program #/Poster #: PSTR169.08/G19

Topic: D.04. Interoception

Support: Intramural grants from the NIDDK, NIH

Title: Regulation of glucose sensing and insulin secretion by vagal sensory neurons

Authors: *S. KUMAR, Y. BAHN, J. LEE, M. J. KRASHES, S. G. RANE;
NIH, Bethesda, MD

Abstract: Glucose is the primary energy source for all mammalian cells. Levels of blood glucose are under tight regulation to preclude hypoglycemia and hyperglycemia, both of which are damaging to cells. Minute fluctuations in glucose levels get detected via intricate sensing mechanisms that relay the information to cells that either uptake glucose or produce hormones involved in glucose metabolism. Pancreatic islet beta cells (β-cells) produce the hormone insulin in response to elevations in glucose levels and insulin release is suppressed under conditions of hypoglycemia. We recently identified a distinct subset of neurons in the paraventricular hypothalamus that are activated in response to hypoglycemia and suppress insulin secretion. The sensory neurons of the vagus also communicate information regarding the peripheral glycemic state to the brain. However, the precise vagal circuits that rapidly sense and regulate glucose levels are unclear. Here, we show that vagal sensory neurons sense glucose and regulate its levels via communication with β-cells. Neuronal tracing in combination with *in-situ* hybridization and immunofluorescence revealed that vagal sensory neurons expressing cocaine and amphetamine regulated transcript (VSN^{CART}) are anatomically connected to β-cells. Via chemogenetic stimulation in combination with automated glucose telemetry, we observed that VSN^{CART} activation suppressed glucose levels and increased the levels of insulin (c-peptide) and the neurotransmitter, acetylcholine. In contrast, glucose tolerance tests revealed that silencing of VSN^{CART} led to increased blood glucose levels and suppression of glucose-stimulated insulin secretion. In agreement, VSN^{CART} responded differentially to fed and fasted states via changes in the transcriptome and in neuronal activity. Ongoing *ex vivo* experiments are further investigating the glucose sensing features of the VSNs. Taken together, we propose that the VSN^{CART} - β-cell circuit plays metabolic-state dependent roles in glucose homeostasis, hormone secretion, and feeding behavior. Deciphering the central-peripheral glucose sensing and hormone release circuits will unravel important details about glucose homeostasis of physiological and pathological relevance.

Disclosures: S. Kumar: None. **Y. Bahn:** None. **J. Lee:** None. **M.J. Krashes:** None. **S.G. Rane:** None.

Poster

PSTR169: Interoception: Peripheral Signals to Regulation of Physiology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR169.09/G20

Topic: D.04. Interoception

Support: Simons Foundation
HHMI

Title: <Investigating how lung cancer alters behavior via regulating the internal sensory system>

Authors: *A. ZAVITSANOU[1], S. DOWNES TONEY[1], A. KNIGHT[1], J. KOTHANDARAMAN[2], S. DURÁN[3], S. OGUNDARE[1], I. ABDUS-SABOOR[4]; [1]Columbia Univ., New York, NY; [2]Columbia Univ., New York City, NY; [3]Columbia Univ., ABQ, NM; [4]Biol., Columbia Univ., NEW YORK, NY

Abstract: The brain receives vital sensory information from internal organs within our bodies and regulates critical autonomic functions such as breathing. Lung cancer patients display an array of behavioral alterations, traditionally regulated by the nervous system, with pain being the most common one. We hypothesize that lung tumors dysregulate interoceptive signals from the lung to the brain driving cancer-induced visceral pain.

Given that rodents cannot articulate their experiences, we use an array of behavioral tools to assess their pain state. We assess spontaneous behaviors, including locomotion, body posture and facial grimace in $Kras^{G12D/+}$; $p53^{-/-}$-driven lung adenocarcinoma mice across tumor development. Using a tracking software, we perform open field locomotor analysis and show that lung cancer mice display decreased locomotion as tumors progress. To measure body posture changes, we use a 3D imaging and behavioral platform termed Keypoint-MoSeq. We discovered that cancer mice show more hunching and stationary movement. They also engage in “turning” behaviors requiring upper body movement less frequently than healthy mice likely because they are causing them discomfort. We also employed PainFace, a deep neural network, to assess grimace in healthy and lung tumor bearing mice as another measure of spontaneous pain. Our approach involves scoring four distinct facial action units, namely orbitals, nose, ears, and whiskers. We find that lung cancer mice exhibit significantly higher grimace scores in comparison to healthy controls. Moreover, the grimace score is higher in mice at advanced tumor stages, suggesting a correlation between tumor progression and heightened grimace expression.

To assess the role of specific neuronal populations in driving lung cancer-induced pain, we combine transcriptomic and chemogenetic approaches. We are currently performing transcriptomics in lung innervating neurons of healthy and lung cancer mice to identify genes and neuronal populations dysregulated during cancer progression. Using chemogenetics, we show that activation of sensory lung innervating neurons drives pain behaviors similar to those induced by lung cancer.

In conclusion, we are integrating advanced behavioral tools, cellular biology methods, and lung cancer mouse models to unravel the mechanisms behind cancer-induced visceral pain, offering a novel perspective on cancer biology. Our goal is to deepen our understanding of how visceral pain signals are transmitted and to pave the way for innovative therapies in lung cancer treatment.

Disclosures: A. Zavitsanou: None. **S. downes toney:** None. **A. Knight:** None. **J. Kothandaraman:** None. **S. Durán:** None. **S. Ogundare:** None. **I. Abdus-Saboor:** None.

Poster

PSTR169: Interoception: Peripheral Signals to Regulation of Physiology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR169.10/G21

Topic: D.04. Interoception

Support: Shenzhen Medical Research Fund B2302011
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T2394532

Title: The amygdala integrates mechanical signals and regulate bone fracture repair

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Abstract: Bone fracture is a commonly occurring traumatic injury with high morbidity and mortality rates. The reconstruction of the local bone mechanical structure during fracture healing is a long-term process, and the associated changes in the central nervous system in this process remain largely unknown. Our previous results have shown that the amygdala could rapidly integrate mechanical signals and regulate bone homeostasis. In this study, we aim to elucidate how the amygdala responds to the healing process of two distinct bone injury models (drill-hole injury and femur fracture). We have used safranin O with fast green staining and immunostaining of bone samples at different time points to characterize the growth features of nerves during bone healing. We found that the regeneration of nerves at the bone fracture site accompanies blood vessel growth and gradually increases during osteogenesis. Open field test, social interaction rate, and tail suspension test were used to evaluate the locomotion ability and depression state at specific bone healing stages. In these experiments, mice had undergone significantly decreased locomotion ability immediately after fracture surgery, and the depressive-like behavior was then observed during bone healing. A 3D-motion capture system was employed to explore the typical spontaneous behavior during the recovery state, which revealed that grooming time largely increased in the process. We also used *in-vivo* fiber photometry recording in the basolateral amygdala (BLA) area to show that the BLA^{CAMKII} neuronal activity is activated in mice with fracture-induced mechanical hypersensitivity (with von Frey filament). Similarly, long-term *in-vivo* two-photon calcium imaging of BLA neurons subjected to treadmill running revealed a sustained elevation of spontaneous calcium response. Through the above findings, we propose that different types of fractures induce distinct neural innervation patterns at specific periods; the amygdala displays different neural activity dynamics in the recovery process of different fracture models.

Disclosures: B. Li: None. **N. Liu:** None. **L. Zhang:** None. **F. Yang:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.01/G22

Topic: D.05. Auditory and Vestibular Systems

Title: Speech understanding characteristics in individuals with normal hearing and hearing loss: an fNIRS study

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Abstract: Hearing loss (HL) has a negative impact on communication that requires integration of auditory and visual information. Previous research has utilized objective and behavioral measures to examine speech understanding ability of individuals with HL. However, behavioral tests could yield different results depending on the examiner or the test materials used, while objective measures have limitations such as radiation exposure and longer testing time. Among various objective measures, functional near-infrared spectroscopy (fNIRS) has the advantage of being non-invasive and requiring shorter testing time. This study explored speech understanding in individuals with NH and HL using fNIRS, representing the first investigation to utilize fNIRS across different modalities to examine speech understanding characteristics. A total of 17 (10 with NH and seven with HL) individuals completed puretone audiometry and fNIRS testing. The fNIRS testing was conducted under three conditions: auditory only (AO), visual only (VO), and audiovisual (AV). A total of five trials per condition were conducted. For the experiment, a video of a male speaker speaking the Seoul National University Hospital Everyday Sentence Test was created. Each trial consisted of a task where the participant repeated two sentences. The stimulus was presented for four seconds and the participant repeated the sentence for the following four seconds. After repeating two sentences, a 30-second post-task break phase followed. Percent-correct scores were obtained. The average speech performance for the NH group were 100% in AO, 0% in VO, and 99% in AV while for the HL group, it was 16.4% in AO, 0.6% in VO, and 44.1% in AV. In the NH group, oxygenated and reduced hemoglobin concentration was highest in AO, followed by AV and then VO, while in the HL group, the concentration was highest in AV, followed by AO and then VO. This suggests that in the NH group, auditory stimulation alone activates the brain most, whereas in the HL group, auditory and visual stimulation elicits the highest brain activity. Comparing both groups, the oxygenated hemoglobin concentration levels were higher in the HL group than in the NH group in all conditions. The results of the pilot study align with prior research indicating that individuals with HL show lower speech performance, more reliance on visual cues, and higher listening effort compared to those with NH. However, given the variability in experimental setups and participant characteristics across fNIRS studies targeting individuals with HL, subsequent studies with a larger sample size and diverse characteristics of participants are necessary to generalize and objectify the findings.

Disclosures: H. Seol: None. **H. Kim:** None. **S. Kim:** None. **J. Shin:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.02/G23

Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant P01-AG055365
NIH Grant R01-DC019394

Title: Neuroplasticity Changes in Older Adults Following Auditory-Cognitive Training

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Abstract: Listening in noisy environments is a common challenge for older adults, even those with clinically normal hearing. Moreover, compared to younger adults, older adults report higher listening effort when listening to competing speakers. This suggests that auditory scene segregation is affected by more than just hearing loss and may also be influenced by cognitive processing, both of which decline with age. In this study, we aim to determine if auditory-cognitive training (of two types, with different levels of required cognition) can improve speech-in-noise listening in normal-hearing, older adults. To analyze the effects of auditory-cognitive training, we collected behavioral and neural data from older adults pre- and post-training, along with younger adults who did not undergo training. Magnetoencephalography (MEG) was used to record brain responses while subjects listened to narrated audiobooks under four different noise conditions. For each audio presentation, we logged listener-reported intelligibility and listening effort. All neural data was analyzed using the temporal response function (TRF) framework. Additional behavioral data obtained include various tasks of working memory and audio segregation, such as stochastic figure-ground ("tone cloud") detection, the quick speech in noise test (QuickSIN), a speech perception in noise task (SPIN), and tests of working memory (RSPAN and N-back). Preliminary results for older adults show a post-training reduction in listening effort with competing speakers. Additionally, some neural measures, such as the reconstruction of stimulus speech features, were generally reduced—an indication that maladaptive overcompensation typically observed in older adults may be decreased. This reduction of stimulus speech feature reconstruction demonstrates neuroplasticity that brings the older adults closer to the younger adults. Critically, one pre-training behavioral measure may predict the level of neuroplasticity benefit, performance in the tone cloud detection task: lower tone cloud detection pre-training scores were associated with larger reductions in stimulus reconstruction measures post-training. These results are promising for the incorporation of auditory-cognitive training in older adults who experience difficulty understanding speech in noise.

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Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.03/G24

Topic: D.05. Auditory and Vestibular Systems

Support: AB Nexus Research Collaboration Grant from University of Colorado and Demant Foundation Grant awarded to Dr. Anu Sharma & Dr. Vinaya Manchaiah

Title: Cortical neuroplasticity after intervention in hearing loss

Authors: *K. CORMIER[1], C. SCHIMMEL[1], V. MANCHAIAH[2], A. SHARMA[3];
[1]Speech Language and Hearing Sci., Univ. of Colorado Boulder, Boulder, CO; [2]Univ. of Colorado, Anschutz Med. Campus, Denver, CO; [3]Univ. of Colorado Boulder, Boulder, CO

Abstract: Background: We are examining neuroplastic changes in the cortex in patients with hearing loss who receive treatment with hearing aids. Recent studies have suggested that hearing loss may be linked to cognitive decline and treatment with hearing aids may improve cognitive deficits. However, more research is needed to examine the underlying brain changes associated with treatment of hearing loss. Specifically, research examining both short-term and long-term neuroplastic brain changes with hearing loss treatments is needed. **Methods:** A 128 channel EEG net was used to record cortical visual evoked potentials, visual oddball P300s, and auditory oddball P300s. Along with examining responses in the time domain, responses were also examined in the spectral and time-frequency domains, as well as examining EEG source localization. Two groups of participants were recruited. The first group of participants included first time hearing aid users with mild to moderate high frequency hearing loss, who self-fit their hearing aids. These first-time hearing aid users were tested within five days of starting hearing aid use, and returned for testing following one month, three months, six months, and one year of hearing aid use. The second group of participants were experienced hearing aid users with any degree of hearing loss. Participants were considered experienced hearing aid users if they had used hearing aids for at least six months. Participants in both groups also self-reported the presence of tinnitus (ringing in the ears) and daily amount of hearing aid use. The amount of sensory restoration (hearing aid amplification) was measured in all participants. **Results:** Preliminary results show an initial increase in auditory P300 amplitudes followed by a return towards baseline. No significant changes were noted in visual P300 responses. In individuals with tinnitus visual P300 amplitudes were found to be decreased in comparison to those without tinnitus. Controlling for the daily use of hearing treatments did not change these findings. Source density reconstruction of cortical visual evoked potentials suggest a changed, but continued presence of crossmodal plasticity, in which auditory cortical regions are responding to visual motion stimuli. **Conclusion:** These preliminary results suggest evidence of neuroplastic changes

in the auditory cortex with hearing loss, as well as hearing treatments. Our results may reflect an initial upregulation in neurocognition followed by a return to baseline, suggestive of adaptation to increased audibility. Based on these results, future research may wish to examine how amount of sensory restoration impacts cortical neuroplasticity.

Disclosures: K. Cormier: None. **C. Schimmel:** None. **V. Manchaiah:** None. **A. Sharma:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.04/G25

Topic: D.05. Auditory and Vestibular Systems

Title: Sound-evoked pupil-linked arousal predicts misophonia

Authors: *J. DE GEE, L. ALONSO-MARMELSTEIN, K. L. SCHWARZ-ROMAN, R. ROUW;
Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: Individuals with misophonia experience strong negative emotions like rage or disgust in response to everyday, often human-made, sounds (e.g., chewing or throat clearing). The ubiquitous nature of these “trigger sounds” makes misophonia a devastating condition. Furthermore, a remarkably large number of individuals in general population report 'mild' or 'moderate' misophonic complaints. This points at a contentious topic of debate, not only in misophonia research, but also in the pluriform and extensive research on sound sensitivity in related research areas: (e.g., autism, tinnitus, hyperacusis, or PTSD: what underlies the large individual differences in sound sensitivity? To this end, we sought to establish a sensitive measurement tool to objectively quantify the physical response to trigger sounds. We hypothesized that pupillometry might be that tool. Pupil size fluctuations at constant luminance have previously been shown to reflect neuromodulatory activity and the ensuing cortical arousal state; importantly, pupil size is also highly correlated to activity of the anterior insula, a key brain region in misophonia. Thirty participants were recruited from the general population. Their misophonic complaints, as measured with the Amsterdam Misophonia Scale (AMS), ranged from sub-clinical to extreme. On each trial they listened to either a misophonia trigger sound or a generally unpleasant sound and rated their experienced annoyance level on a 4-point scale. We observed larger pupil dilation time-locked to the trigger sounds versus generally unpleasant sounds. Within participants, pupil dilation correlated with the subjective (rated) severity of the negative experience of both trigger and generally unpleasant sounds. Across participants, pupil response magnitude, but not subjective ratings, significantly predicted AMS scores. A leave-one-out cross-validation analysis furthermore showed that pupil data predicted AMS score within granularity of its severeness categories. In summary, this study demonstrates, for the first time, the sensitivity of pupillometry to variations in the strength of the misophonic response. The

method holds promise as an additional instrument for investigating between-group or inter-individual differences, and may potentially add a diagnostic tool at the level of a single individual.

Disclosures: J. de Gee: None. **L. Alonso-Marmelstein:** None. **K.L. Schwarz-Roman:** None. **R. Rouw:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

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Program #/Poster #: PSTR170.05/G26

Topic: D.05. Auditory and Vestibular Systems

Support: DFG grant 523344822
BMBF SEMECO 03ZU1210FB

Title: Attentional modulation of the cortical contribution to the frequency-following response evoked by continuous speech

Authors: A. SCHÜLLER[1], A. SCHILLING[2], P. KRAUSS[3], S. RAMPP[4], ***T. REICHENBACH**[1];
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Abstract: Selective attention to speech allows us to communicate with other people and turn out distracting background noise. Research on the neural mechanisms of selective attention to speech has primarily explored low-frequency responses to amplitude modulations in the auditory cortex. However, EEG studies revealed, in addition, attentional modulation of subcortical neural responses at the fundamental frequency of speech (speech-FFR). Recent MEG studies have identified cortical contributions to the speech-FFR, but it remains unclear if these cortical contributions are also modulated by selective attention.
Here we acquired MEG data from 22 healthy individuals with normal hearing who listened to 40 minutes of two competing male speakers, one with a higher and one with a lower pitch (Schüller et al., J. Neurosci. 43:7429, 2023). Participants were asked to sometime attend the higher-pitch and sometimes the lower-pitch speaker. We then determined the cortical speech-FFRs through source reconstruction of the MEG data followed by the computation of source-level Temporal Response Functions (TRFs). This allowed to assess the effects of selective attention on these neural responses.
We found a significant impact of selective attention on the cortical contribution to the speech-FFR. The neural response increased when the participants attended a speaker compared to when ignoring it, evident at both population and individual subject levels. Moreover, irrespective of attention, the lower-pitch speaker consistently triggered a larger cortical contribution to the

speech-FFR than the higher-pitch speaker. Attentional modulation of speech-FFRs hence occurs not only on the subcortical but also on the cortical level.

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Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

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Program #/Poster #: PSTR170.06/G27

Topic: D.05. Auditory and Vestibular Systems

Support: PAPIIT Grant BG200424

Title: Population dynamics in the primate audiomotor system during tapping synchronization

Authors: *J. MARQUEZ GUTIERREZ[1], Y. A. AYALA[2], L. PRADO[3], G. MENDOZA[4], H. MERCHANT[5], *J. P. MARQUEZ GUTIERREZ[1];
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Abstract: Audiomotor synchronization depends on the interaction between auditory and motor areas of the brain, including the pre-supplementary motor area (pre-SMA) and the basal ganglia. This auditory information may reach the pre-SMA and then the putamen through the posterior parietal cortex. Hence, in this work we recorded the activity on primary auditory cortex(A1), posterior belt auditory cortex (A2), parietal area 7a, putamen, pre-SMA, and primary motor cortex (M1) simultaneously with single-neuron resolution while Rhesus monkeys performed an auditory synchronization task, a passive metronome listening task, and a tonotopic mapping procedure. Using a warping method, we identified cells whose responses were aligned to the metronome, to the tapping, or to a mix between the stimuli and movements. There was a gradient of increasing motor cell density and a decrease in sensory cell density from A1 to M1. Notably, A1 and A2 showed cells with responses associated with a top-down signal associated with the internal beat representation. Next, we projected the activity of the overall population for each area in low dimension state-space using principal component analysis. We found that there are motor and timing related signals in all areas and low-latency responses related to the stimulus in the auditory cortices. As a next step we will identify how the interaction between these areas correlates with the perception of time and the execution of synchronized movements to a given stimulus.

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Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

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Program #/Poster #: PSTR170.07/Web Only

Topic: D.05. Auditory and Vestibular Systems

Support: National Social Science Fundation of China [Grant No. 22&ZD299]
Shenzhen University Humanities and Social Sciences High-level Innovation Team Project for Leading Scholars [Grant No. 24LJXZ02]

Title: Speech perception triggers language network plasticity by hub reconstruction in children with cochlear implant

Authors: *Q. LUO[1], S. LU[2];
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Abstract: SfN Abstract**Speech perception triggers language network plasticity by hubreconstruction in children with cochlear implantAuthors*Q. LUO[1, 2]**, S. LU[1]; [1] Neurolinguistics Laboratory, College of International Studies,Shenzhen University, Guangdong, China; [2]Department of Chinese Language andLiterature, The Chinese University of Hong Kong, Hong Kong SAR, China**DisclosuresQ. LUO:** None. **S. LU:** None.**Abstract**The significance and underlying mechanism of varied auditory stimuli on the perceptualneurological rehabilitation of prelingually deaf children with cochlear implants (CIchildren) remain unclear. Employing functional near-infrared spectroscopy (fNIRS),we probed the disparities in neural activity of 106 CI children in perceiving speech,pure tones and animal vocalizations, and revealed how diverse stimuli function on theirneural network plasticity by comparing with 30 normal developing children. Resultsshowed that the normalization the oxygenation activity of CI children with longerduration of cochlear implantation was particularly evident in the comparison betweenspeech and pure tones. This observation underscores a robust association betweennatural speech perception and the normalization of auditory function in this cohort ofchildren. Furthermore, the hubs of the speech perception network of CI childrenconverged more towards the normal pattern as the duration of cochlear implantationincreases, highlighting the importance of hub development in the reorganization oflanguage networks in CI children. Intriguingly, CI children demonstrated a notablesensitivity towards “speech-like” animal vocalizations, as evidenced by more robustconnectivities and the normalization of hubs. These findings underscore the neuralspecificity of speech and speech-like auditory stimuli in CI children and help deepenour understanding on the mechanism of language network plasticity.

Disclosures: Q. Luo: None. **S. Lu:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

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Program #/Poster #: PSTR170.08/G28

Topic: D.05. Auditory and Vestibular Systems

Support: DGAPA
CONACYT: A1-S-8430
PAPIIT: IG200424

Title: Monkeys have rhythm

Authors: *V. G. RAJENDRAN[1,2], J. P. MARQUEZ GUTIERREZ[3], L. PRADO[4], H. MERCHANT[5];
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Abstract: Synchronizing movements to music is one of the hallmarks of human culture whose evolutionary and neurobiological origins remain unknown. The ability to synchronize movements requires 1) detecting a steady rhythmic pulse, or beat, out of a stream of complex sounds, 2) projecting this rhythmic pattern forward in time to predict future input, and 3) timing motor commands in anticipation of predicted future beats. Here, we demonstrate that the macaque is capable of synchronizing taps to a subjective beat in real music, and even spontaneously chooses to do so over alternative strategies. This contradicts the influential “vocal learning hypothesis” that musical beat synchronization is only possible in species with complex vocalizations such as humans and some songbirds. We propose an alternative view of musical beat perception and synchronization ability as a continuum onto which a wider range of species can be mapped depending on their ability to perform and coordinate the general abilities listed above through association with reward.

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Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.09/G29

Topic: D.05. Auditory and Vestibular Systems

Title: Neural underpinnings of the continuity illusion for speech and music in musicians

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Abstract: The natural world is noisy, and listeners frequently encounter speech that is masked by competing sounds. Decades of research have observed that the brain can make inferences about degraded sensory information to improve perception. One such auditory phenomenon is the *continuity illusion* whereby a sound stimulus is perceived as continuous through noise-filled interruptions. The continuity illusion has been observed using interrupted pure tones, speech, and music, and listeners' enhanced perception of continuity has been indexed neurophysiologically by reduced auditory theta (4-8 Hz) power and phase-locking following onsets/offsets of the noise interruption. In this study, we examine the behavioral and neurophysiological differences between musicians (n=16) and non-musicians (n=16) to noise-interrupted trisyllabic words and classical music segments matched in length to assess two competing hypotheses: (H1) Musicians would be less likely to perceive the continuity illusion because of their enhanced ability to detect acoustic gaps, thereby exhibiting more theta power and phase-locking to interruption boundaries compared to non-musicians. (H2) Musicians would be more likely to perceive continuity because specialized cortical networks quickly take over processing and induce a perception of continuity (i.e., fill-in missing representations), demonstrated by reduced theta power and phase-locking. While recording EEG, individuals identified whether speech and music stimuli (presented in random order) sounded continuous or interrupted on each trial. Behaviorally, musicians and non-musicians had similar rates of perceived continuity for music, but musicians perceived more continuity to speech compared to non-musicians, although this difference was not significant. Neurophysiologically, musicians exhibited more suppressed theta spectral power and phase-locking to interruption onsets, followed by enhanced alpha (8-14 Hz) and beta (15-30 Hz) power for speech and music. Our results indicate that musicians engage greater audio-motor inhibitory (gating) neural mechanisms that are indexed by reduced theta and enhanced alpha and beta power, to maintain an equal level of perceived continuity through acoustic interruptions compared to non-musicians.

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Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.10/G30

Topic: D.05. Auditory and Vestibular Systems

Title: Pupil response reflects selection of task-relevant and (unsuccessful) suppression of task-irrelevant background sounds

Authors: *L. FIEDLER[1], T. CHRISTIANSEN[2], I. S. JOHNSRUDE[3], D. WENDT[1,4];
[1]Eriksholm Res. Ctr., Snekkersten, Denmark, Snekkersten, Denmark; [2]R&D, EPOS Audio, Copenhagen, Denmark; [3]Brain and Mind Inst., Univ. of Western Ontario, London, ON, Canada; [4]Hearing Systems, Technical University of Denmark, Lyngby, Denmark

Abstract: Auditory attention can be voluntarily focused on a sound source, but it may also be automatically captured by off-focus sounds. The latter can be either relevant or irrelevant for a listener. The ability to switch attention towards a relevant sound source (but to suppress an irrelevant one) requires attentional control and is crucial for navigating in a complex auditory scene. In a dual-task paradigm, we investigated whether pupil responses reflect relevance-dependent selectivity in the processing of background sounds and whether this selectivity correlates with behavioral performance. We asked 21 participants with self-reported normal hearing (N = 21, Age: 27 to 66 years, pure tone average: -4 to +26 dB HL) to listen to continuous speech presented from the front (primary task). At random order and unpredictable times, additional speech sounds were presented from the left or right side (background sounds). Each of these background sounds consisted of a name followed by a two-digit number. While the name served as a cue, the secondary task was to memorize numbers from either the right or left side (i.e., relevant), which was instructed before each one-minute trial. Afterwards, participants were asked to pick three relevant numbers from a board of nine, which also contained three irrelevant numbers and some random numbers that were not presented on that trial. We found increased pupil responses to relevant background sounds compared to irrelevant ones (i.e., selectivity). This selectivity predicted behavioral performance in the secondary task: Participants who exhibited stronger selectivity were able to recall more numbers correctly. Interestingly, pupil responses did not significantly differ between correctly recalled and missed relevant background sounds. However, they were stronger for stream-confused compared to correctly rejected irrelevant background sounds. This suggests that participants were more challenged by suppressing irrelevant sounds than switching attention to relevant sounds. Importantly, neither hearing thresholds nor age predicted behavioral performance in the secondary task. Our findings demonstrate that pupillometry reflects auditory attentional control abilities, which are meaningful for hearing diagnostics as well as the development of intelligent noise management in hearing aids and communication devices.

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Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

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Program #/Poster #: PSTR170.11/G31

Topic: D.05. Auditory and Vestibular Systems

Support: Postdoctoral fellowship from the Fondation pour l'audition to EA (FPA RD-2022-1)
Grand Prix Scientifique from the Fondation Pour l'Audition to RZ (2021)

Title: The representation of speech conversations in the human auditory cortex

Authors: *E. ABASSI, R. J. ZATORRE;
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Abstract: Auditory perception is likely shaped by human social nature. Indeed, we spend a significant part of our lives talking with others, relying heavily on hearing to navigate our social world, including not just participating in conversations but also listening to others' conversation. While the neural basis of speech understanding has been largely studied at the word or sentence low-scale level, the role of social context in processing speech, and how it interacts with semantics at the large scale of a whole conversation, remains barely explored. We conducted a fMRI study focusing on how the brain processes dual-speaker conversations from a third-person perspective. We manipulated both social and semantic contexts using AI-generated auditory stimuli, consisting of two-speaker dialogues or one-speaker monologues (social context factor) presented in either intact or sentence-scrambled order (semantic context factor), to healthy young adult in a 7T fMRI scanner. Whole-brain analyses showed that semantic context had significant effect on brain activity in the left superior temporal sulcus (STS) and right Angular Gyrus, with stronger activity for scrambled than intact conversations in these regions. Social context did not show a direct effect. However, an interaction between semantic and social contexts was observed in the left STS, with larger activity differences between scrambled and intact dialogues as compared to monologues. Results in the left STS were confirmed with an ROI analysis showing similar effect of semantic context and interaction with social context in the independently functionally localized speech-selective auditory cortex. In addition, we trained a multivariate classifier in this region to discriminate neural representations of sentences presented individually, and then used it to compare the neural representations of these sentences when presented in their whole context. We found a more accurate classification of the sentences when embedded in dialogues than monologues, suggesting an effect of social context on perceptual representation of individual sentences. Overall, our study emphasizes the influence of both semantic and social aspects on neural speech processing. It suggests specialized mechanisms in the left STS favoring the processing of prototypical conversations, such as intact dialogues, highlighting the need to consider social and semantic components in understanding speech processing at the large-scale level of a whole conversation. The findings also raise questions regarding the predictive or other neural mechanisms that may be present when perceiving speech in naturalistic contexts.

Disclosures: E. Abassi: None. **R.J. Zatorre:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.12/G32

Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant R01DC016267

Title: Auditory-motor entrainment and listening experience shape the perceptual learning of concurrent speech

Authors: *J. A. MACLEAN, J. STIRN, G. M. BIDELMAN;
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Abstract: Background: Plasticity from auditory experiences shapes the brain's encoding and perception of sound. Though prior work has shown that neural entrainment (i.e., brain-to-acoustic synchronization) aids speech perception, how long- and short-term plasticity influence entrainment to concurrent speech has not been investigated. Here, we explored neural entrainment mechanisms and interplay between short- and long-term neuroplasticity for rapid auditory perceptual learning of concurrent speech sounds in young, normal-hearing musicians and nonmusicians. **Method**: Participants (n = 27) were separated into musician (n=13) and nonmusician (n=14) groups based on the extent of their formal music training (musicians: ≥10 years, nonmusicians: ≤5 years). Participants learned to identify double-vowel mixtures (/a/ + /e/, /i/ + /e/, /i/ + /a/) during ~45 min training sessions recorded simultaneously with high-density EEG. We examined the degree to which brain responses entrained to the speech-stimulus train (~9 Hz) to investigate whether entrainment to speech just prior to behavioral decision predicted task performance. Source and directed functional connectivity analyses of the EEG probed whether behavior was driven by group differences in coupling between auditory and motor cortices.**Results**: While both groups showed rapid perceptual learning in accuracy and reaction time with speech training, musicians showed faster behavioral decisions than nonmusicians overall. Interestingly, listeners' neural entrainment strength prior to target speech mixtures predicted their behavioral identification performance; stronger neural synchronization was observed preceding incorrect compared to correct trial responses. We also found stark hemispheric biases in auditory-motor coupling during speech entrainment, with greater auditory to motor connectivity in the right hemisphere for musicians. **Conclusions**: Our findings confirm stronger neuroacoustic synchronization and auditory-motor coupling during speech processing in musicians. Stronger neural entrainment to rapid stimulus trains preceding incorrect behavioral responses supports the notion that alpha-band (~10 Hz) arousal/suppression in brain activity is an important modulator of trial-by-trial success in perceptual processing.

Disclosures: J.A. MacLean: None. **J. Stirn:** None. **G.M. Bidelman:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.13/G33

Topic: D.05. Auditory and Vestibular Systems

Title: Relative encoding of speech intensity in the human temporal cortex

Authors: *I. BHAYA-GROSSMAN[1], Y. OGANIAN[2], E. F. CHANG[3];
[1]Bioengineering, UC Berkeley / UCSF, San Francisco, CA; [2]Univ. of Tuebingen, Tübingen, Germany; [3]Univ. of California, San Francisco, Piedmont, CA

Abstract: Lexical stress, the perceived emphasis placed on syllables within words, critically facilitates word segmentation as well as word recognition processes (e.g distinguishing between the noun 'the PRE-sent' and the verb 'to pre-SENT'). In English, lexical stress is prominently cued by relative speech intensity, with the stressed syllable exhibiting the greatest intensity relative to other syllables in the word. Prior work has shown that the human speech cortex on the superior temporal gyrus (STG) encodes speech intensity as a series of discrete acoustic landmarks marking moments of peak intensity change (peakRate). Building on this finding, a key question arises: Is there a neural encoding of relative intensity in the STG that supports the perception of lexical stress? To address this question, we performed intracranial recording (n=9 ECoG patients) while participants performed two experiments. In Experiment 1, participants were asked to make lexical stress decisions for a set of synthesized two-syllable pseudo-words (e.g. hu-ka, ma-lu). The intensity of the first syllable in each pseudo-word varied while the intensity of the second syllable was fixed, allowing us to experimentally test whether neural responses to the second syllable depended on the intensity of the first. We found that a subset of cortical sites on the human STG only responded to the second syllable when it was greater in intensity than the first, suggesting that these sites encode relative intensity. Critically, we found that cortical sites that encoded relative intensity were distinct from those that encoded peakRate, and that neither of these two sets of cortical sites encoded perceived stress when participants were presented with ambiguous pseudo-words, where both syllables had identical intensity. In Experiment 2, we used a passive listening paradigm to extend our findings to a naturalistic speech stimulus. Our results indicate that relative and absolute intensity of speech is encoded in two distinct neural populations on the STG and further, that these populations do not encode stress percepts in cases where the speech intensity cue to lexical stress is removed.

Disclosures: I. Bhaya-Grossman: None. **Y. Oganian:** None. **E.F. Chang:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.14/G34

Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant 1R01DC018579

Title: Neural Encoding of Acoustic Features Across Speech and Music in the Human Brain

Authors: *R. AGRAVAT[1], M. DESAI[2], G. FOOX[3], A. FIELD[4], A. ANDERSON[5], E. C. TYLER-KABARA[6], A. WATROUS[7], H. L. WEINER[5], L. S. HAMILTON[8];

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Abstract: Neural Encoding of Acoustic Features Across Speech and Music in the Human Brain The human brain's ability to process complex auditory signals, such as speech and music, relies on extracting and representing various embedded information. Speech contains linguistic information (phonology, semantics, lexical properties) and acoustic information (spectral patterns, pitch structures, rhythmic elements). This study aims to advance our understanding of the neural representations of speech and music in the auditory cortex by addressing key questions: 1) How do the neural representations of acoustic features in speech compare to those in music within the auditory cortical hierarchy? 2) How does the neural representation of pitch and spectral features in speech and music change with development from early childhood to late adolescence? We recorded brain activity via stereo-electroencephalography (sEEG) from 24 participants (14M/10F) while they listened to movie trailer stimuli containing both speech and music. We extracted the high-gamma band (70-150 Hz) activity in bilateral auditory-related regions, including Heschl's gyrus (HG), superior temporal gyrus (STG), and middle temporal gyrus (MTG). These movie trailers were then split into speech and music-only content for analysis using a neural network audio separation algorithm. We then fit linear encoding models that predicted high gamma activity from either the original spectrogram (mixed speech and music), the music-only spectrogram, or the speech-only spectrogram. In all cases, the patients heard the original (mixture) of speech and music, so if the separate spectrograms more effectively predict neural activity, this suggests a preferential representation of that information. Preliminary findings indicate higher pitch-related selectivity for speech than music in areas such as HG, STG, and MTG. We also looked at spectral features for speech and music and where these are encoded in the brain. The data points for this analysis will follow a general positive correlation, suggesting that electrodes with higher spectral responses to music tend to also have higher responses to speech and vice versa. The model demonstrates enhanced acoustic selectivity for speech when evaluating speech-only spectrograms compared to the original spectrograms extracted from the movie trailers. This suggests that removing non-speech auditory sources, such as background music or sound effects, can improve the model's performance in representing and processing the speech components. Further work could examine the roles of auditory attention and encoding differences between sung speech and regular speech.

Disclosures: R. Agravat: None. **M. Desai:** None. **G. Foox:** None. **A. Field:** None. **A. anderson:** None. **E.C. Tyler-Kabara:** None. **A. Watrous:** None. **H.L. Weiner:** None. **L.S. Hamilton:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.15/G35

Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant 1R01DC018579

Title: Understanding speech and language development using intracranial recordings in pediatric epilepsy

Authors: *M. DESAI[1], A. M. FIELD[2], G. FOOX[3], N. NUSSBAUM[4], R. DELEON[5], E. C. TYLER-KABARA[6], A. WATROUS[7], H. L. WEINER[8], A. E. ANDERSON[8], L. S. HAMILTON[9];
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Abstract: Intracranial recordings have provided valuable insights into investigating the neural circuitry of speech perception in adults. Similar research in pediatric populations is rare due to the difficulty of recordings and, in many cases, the inability of younger patient participants to tolerate monotonous experimental sessions. We addressed this gap by determining whether movie trailer stimuli could be used to replace more typical, less engaging sentence stimuli to derive auditory receptive fields in children undergoing invasive surgical monitoring for epilepsy. We additionally incorporate age and neuropsychological measures in efforts of identifying speech and language responses in the brain across the lifespan. We recorded stereoelectroencephalography (sEEG) from 30 patients (age 4-21, 17M/13F) at Dell Children's Medical Center in Austin, Texas and Texas Children's Hospital in Houston, Texas. Electrode coverage included right hemisphere or bilateral coverage of auditory and language-related areas. All patients listened to and watched audiovisual children's movie trailer stimuli. A subset of patients also listened to sentences from the TIMIT acoustic-phonetic database. We fit linear encoding models to describe the relationship between acoustic and linguistic stimulus features and the high gamma power of the local field potential (70-150 Hz). Predicting neural activity from phonological features and the spectrogram demonstrated robust model performance in bilateral primary and non-primary auditory regions such as Heschl's gyrus, superior temporal gyrus and middle temporal gyrus (r_{avg_phn}=0.12, r_{max_phn}=0.68, r_{avg_spec}=0.11, r_{max_spec}=0.53). To determine whether phonetic and spectral selectivity changes with development, we categorized our patient population into young childhood (age 4-5), middle childhood (6-11), early adolescence (12-17), and late adolescence (18-21). We found that phonetic selectivity was more robust in the early and late adolescent groups compared to younger ages, whereas spectral tuning emerged earlier in development. In addition, latency of speech responses decreased with age. These differences appear to be age-related rather than related to speech and language ability, as we observed no correlation between pre-operative neuropsychological measures of receptive language nor attention on phonetic and spectral selectivity. Overall, our use of an engaging audiovisual stimulus allowed us to derive acoustic and phonetic selectivity using a task that is appropriate for a wide age range.

Disclosures: M. Desai: None. **A.M. Field:** None. **G. Foox:** None. **N. Nussbaum:** None. **R. DeLeon:** None. **E.C. Tyler-Kabara:** None. **A. Watrous:** None. **H.L. Weiner:** None. **A.E. anderson:** None. **L.S. Hamilton:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.16/G36

Topic: D.05. Auditory and Vestibular Systems

Title: Brain activity predicting auditory communication during REM sleep

Authors: *D. MORRIS, K. KONKOLY, K. A. PALLER;
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Abstract: To what extent is sensory input blocked out during sleep? Why are some sounds incorporated into ongoing dreams while others are not? Comparing transient periods of sensory connection and disconnection during sleep may provide insights into sensory integration and awareness during sleep. During wakefulness, sensory experiences are usually constrained by environmental inputs, whereas during dreams, any experience within the realm of imagination is possible. Lucid dreams, characterized by understanding that one is dreaming while remaining asleep, provide a unique method for addressing these questions. Lucid dreamers are capable of providing real-time feedback on the incorporation of sounds into dreams, such that investigators need not rely only on dream reports given after awakening, which are often subject to forgetting and distortion. We analyzed data from 6 participants across 24 sessions in which dreamers responded to sounds presented during REM sleep. Responses consisted of pre-arranged patterns of eye movements or sniffing signals executed volitionally as an indication that the sound was heard. We analyzed EEG brain activity and eye movements preceding stimuli that were or were not followed by responses (89 response trials, 612 non-response trials). Sleep was scored according to standard physiological criteria and trials were categorized according to the nature and timing of any post-stimulation sleep disruption. Theoretically, responsiveness could be predicted by differences in REM sleep microarchitecture, EEG power spectra, neural complexity, or connectivity. Preliminary analyses revealed higher theta activity preceding cues that elicited a response compared to those that did not. Further tests of such findings could utilize machine learning and automated closed-loop stimulation algorithms to attempt to facilitate effective communication with dreamers. Additional perspectives on sensory disconnection during sleep from this research could shed light on how information processing differs between waking and REM sleep. Furthermore, effective communication with dreamers would facilitate additional experimentation during dreaming that could reveal more about its possible adaptive value.

Disclosures: D. Morris: None. **K. Konkoly:** None. **K.A. Paller:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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Topic: D.05. Auditory and Vestibular Systems

Support: NIH Informational Masking Grant (RO1-DC019126)
NIH T90 Training Grant (T90DA060116)

Title: Effects of sustained auditory selective attention on cortical and subcortical representations of sound

Authors: *Y. LI[1], V. FIGAROLA[1], A. L. NOYCE[1], A. T. TIERNEY[2], R. K. MADDOX[3], F. DICK[4], B. SHINN-CUNNINGHAM[1];
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Abstract: Auditory selective attention, the ability to focus on specific sounds while ignoring competing sounds, enables communication in complex auditory environments. Previous studies have demonstrated that attention strongly modulates cortical representations of sound, but whether and where this modulation occurs in subcortical structures remains unclear.
Here, we use electroencephalography (EEG) to record event-related potentials (ERPs), an index of cortical activity, as well as auditory brainstem responses (ABRs, subcortical responses to sound) during a selective listening task. Using a previously developed paradigm (Laffere et al 2020; 2021), subjects attend to a 3-note melody presented to one ear in one range of pitches while ignoring an interleaved, competing melody played to the other ear in a different pitch range. To test brainstem attention modulation, we utilized pitch-evoking pseudo-tones formed by convolving a periodic impulse train with a tone pip, after Polonenko et al (2019; 2021). With these stimuli, each individual tone pip within a pseudo-note elicits one ABR, while the pseudo-note onset elicits a strong cortical response. An earlier version of this combined paradigm presented notes at a rate of 4 Hz; however, overlap of the cortical ERPs from each note hindered quantification of cortical responses. In this current study, we present the stimuli at an across-melody presentation rate of 3 Hz so that cortical responses are temporally isolated, allowing us to better assess cortical neural activity.
Initial results produced clear cortical ERPs to each note and subcortical ABRs to each pip. From the cortical responses, we analyzed how attention modulated both the ERP phase and the inter-trial phase coherence (ITPC) at 1.5 Hz. With the slower stimulus presentation, we could see that attention enhanced the ERP evoked by the note onset when it was the “target” stream (as quantified by the cortical P1-N1 peak difference). Additionally, the ITPC showed peaks at the within-melody repetition rate of 1.5 Hz. Importantly, the best performing listeners showed nearly a 180 degree phase separation between conditions. Similar to results from the companion study using the faster stimulus rate, we also found robust ABRs evoked by each tone pip. Consistent with our earlier results, we also see a post wave V peak in the ABR that is modulated by attention. By simultaneously recording ERPs and ABRs, these results allow us to track attention-mediated changes in the neural signals in the cortex and brainstem, respectively.

Disclosures: Y. Li: None. **V. Figarola:** None. **A.L. Noyce:** None. **A.T. Tierney:** None. **R.K. Maddox:** None. **F. Dick:** None. **B. Shinn-Cunningham:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.18/H1

Topic: D.05. Auditory and Vestibular Systems

Title: Behavioral differences between left and right handed on spatial ability: an exploratory study on sound localization task.

Authors: *M. CASTRO GONZÁLEZ[1], Y. DEL RÍO-PORTILLA[2];
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Abstract: Differences in brain organization between right and left-handed have been described, nevertheless is continue to be an issue of fact. There is also known that laterality has an influence on evolution and human being development including sensory and motor systems like eye movements and relation with sound localization. The aim of this study was to analyze if laterality has an influence on eye movements and tipping response on a sound localization task. We used 120 stimuli (musical note A, 2s each). Stimuli were presented in a classical random block design, for each group (homogeneous left-handed and right-handed/ 80-100% of hand preference; and left and right-handed / 60-75% of hand preference). After each run, subjects (n=20 male) respond on a keyboard according to where they heard the stimuli (right, middle or left side) and at the same time to gaze on the direction they heard the stimuli (right or left side and not eye movement if it is at the middle). For eye movements recording, we placed electrodes according EOG. Preliminary results between 20 males (10 homogeneous right-handed and 10 homogeneous left-handed) showed laterality influence for eye movements and tipping response for left-handed, it means that left-handed move their eyes and tipping to the left on the sound localization task. In addition, right-handed also tipping to the left on some sounds but also has a laterality influence to the right. Nevertheless, we also found contralateral responses specially for eye movements related to tipping response for both groups. Contralateral responses on eye movements and motor response, may be related to TR between saccades and tapping and also may the type of sound.

Disclosures: M. Castro González: None. **Y. del Río-Portilla:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.19/H2

Topic: D.05. Auditory and Vestibular Systems

Support: NIH DC020097

Title: Interference of mid-level sound statistics predicts human speech recognition in natural noise

Authors: *A. CLONAN[1,2], X. ZHAI[3], I. H. STEVENSON[4,2], M. A. ESCABI[5,2];
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Abstract: Recognizing speech in noise is a critical task of the human auditory system. For example, humans can attend to speech in a busy restaurant, where automated speech recognition (ASR) systems, such as Alexa and Siri, would often fail. This ability is driven by critical bottom-up acoustic features interference (power, spectrum, modulation statistics) between the foreground and background. Yet, current modelling approaches are unable to predict recognition sensitivity in distinct, real-world backgrounds.

First, we assess how the spectrum and modulation statistics of natural sounds mask the recognition of spoken digits (0 to 9). We enrolled participants in a psychoacoustic study where digits were presented in various natural background sounds (e.g., water, construction noise, speaker babble; tested for SNR=-18 to 0 dB) and their perturbed variants. We perturbed the backgrounds by either 1) phase randomizing (PR) the sound spectrum or 2) spectrum equalizing (SE). PR retains the power spectrum but distorts the modulation statistics while SE distorts the power spectrum and retains modulation statistics. To further quantify this interference between foreground and background spectrum/modulation content, we used texture synthesis (McDermott & Simoncelli 2011) to manipulate individual modulation statistics from the backgrounds. Even at a constant noise level (-9 dB SNR), the ability to recognize foreground digits was substantially helped or harmed by these background perturbations, depending on the original background sound.

We next developed a physiologically inspired model of the auditory system to predict perceptual trends. Sounds were decomposed through a cochlear filter bank (cochlear stage) and a subsequent set of spectrotemporal receptive fields that model modulation selectivity in auditory midbrain (mid-level stage). Logistic regression was performed on these features to estimate perceptual transfer functions and predict human accuracy. This approach was extended to ASR systems to contrast the acoustic features machine perception relies on relative to human perception, which exhibits higher accuracy. We found that our midbrain representation outperformed the cochlear model, accounting for more than 90% of the variance in the human perceptual data, compared to 62%. Perceptual transfer functions allow us to identify spectral and modulation cues critical to recognition in noise while constrained to a low feature space. Further, comparing the transfer functions for human and machine perception, provides preliminary evidence that the acoustic invariances are distinctly different.

Disclosures: A. Clonan: None. **X. Zhai:** None. **I.H. Stevenson:** None. **M.A. Escabi:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.20/H3

Topic: D.05. Auditory and Vestibular Systems

Support: NIH DC020097

Title: Using word-level acoustic interference of spectrum and modulation statistics to predict human speech recognition behavior in natural environmental noise

Authors: A. CLONAN[1,2], ***M. ESCABI**[3,2], I. H. STEVENSON[4,2], X. ZHAI[5];
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Abstract: Recognizing speech in noisy environments is a critical task of the human auditory system where the unique spectrum and modulation content of speech and backgrounds interfere to influence perception. Natural backgrounds can be quite diverse, with high degrees of spectrotemporal variability that arises from highly varied environmental acoustic generators. For speech, articulation imposes unique acoustic idiosyncrasies (i.e.: fundamental frequency, intonation) that influence our vocal quality, pronunciation, and phonetic implementation.
Here we assess how the spectrum and modulation statistics of natural sounds mask the recognition of spoken digits (0 to 9) and investigate speaker and word driven foreground effects. We enrolled participants in a psychoacoustic study where digits were presented in various natural background sounds (e.g., water, construction noise, speaker babble; tested for SNR=-18 to 0 dB) and their perturbed variants. We perturbed the backgrounds by either 1) phase randomizing (PR) the sound spectrum or 2) spectrum equalizing (SE). PR retains the power spectrum but distorts the modulation statistics while SE distorts the power spectrum and retains modulation statistics. Even at a constant noise level (-9 dB SNR), the ability to recognize foreground digits was substantially helped or harmed by these background perturbations, depending on the original background sound. Yet, each word had a unique interaction with a delivered background's statistics, varying across spectro-temporal perturbations. This indicates that word level acoustics interfere with the background statistics in unique to each digit.
To identify the acoustic cues that underlie speech and noise interference, we next developed a physiologically inspired model of the auditory system to predict perceptual trends with a bottom-up representation of word acoustics. Sounds were decomposed through a cochlear filter bank (cochlear stage) and a subsequent set of spectrotemporal receptive fields that model modulation selectivity in auditory midbrain (mid-level stage). Using logistic regression, we then estimated perceptual transfer functions that provide insight to the acoustic cues driving speech perception from the vantage point of different speakers and vocalized words. Preliminary results suggest

that these word specific transfer functions interfere with background sounds in predictable ways, explaining ~60% of the perceptual variance for single digit-in-noise identification.

Disclosures: A. Clonan: None. **M. Escabi:** None. **I.H. Stevenson:** None. **X. Zhai:** None.

Poster

PSTR170: Auditory Processing: Perception, Speech, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR170.21/H4

Topic: H.10. Human Learning and Cognition

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NSF/UKRI 2342847

Title: Decoding single unit and mass neural signatures of sequence maintenance and replay in the human auditory cortical mnemonic system

Authors: *R. M. CALMUS[1,2], Z. KOCSIS[3], J. I. BERGER[1], H. KAWASAKI[1], T. D. GRIFFITHS[4], M. A. HOWARD III[1], C. I. PETKOV[1,2];
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Abstract: To understand how the human cognitive system establishes internal models of the world, there is substantial interest in identifying neuronal signals that carry traces of the sensory past and predictions about the future. However, outside of animal models, we lack insights into how site-specific neurophysiological activity within the cortical mnemonic system carries information reflecting maintenance activity to sounds over delays, and on the signals that reflect prospective preplay or retrospective replay of a learned sensory sequence. To study these signals in a controlled manner, we conducted an auditory statistical learning task with a cohort of neurosurgery patients during presurgical intracranial monitoring of refractory epilepsy. Patients listened to perceptual sequences of 3 nonsense words containing a dependency between two sounds in each sequence. Words were drawn from sets (X, A and B), with regularities between pairs of relevant sounds (A-B) often separated in time by uninformative (X) words, forming either an adjacent or non-adjacent dependency. We first analyzed site-specific single-unit activity and local field potentials (LFPs) from auditory cortex, hippocampus and frontal cortex using traditional methods, demonstrating engagement of fronto-temporal auditory sites and the hippocampus in the processing of the sequencing regularities. In addition to univariate analyses,

a novel multivariate decoding analysis applied to both single-unit and LFP responses revealed evidence of auditory hippocampal replay, suggesting that time-compressed replay occurs after key sounds in the sequence. Building on these findings, we characterized a variety of single unit responses that, in concert with our decoding analyses, provide evidence for a distributed neural code underlying prospective and retrospective auditory sequence item representation in the human hippocampus. Our results elucidate critical roles for the mnemonic system in transforming sensory events into mental structures, providing insights into the single-neuron and mesoscale contributions to the maintenance and replay of sequential information in the human brain.

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Poster

PSTR171: Higher Visual Areas

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR171.01/H5

Topic: D.06. Vision

Support: BBSRC: BB/V003917/1
The School of Philosophy, Psychology and Language Science, University of Edinburgh

Title: Retinotopically driven negative and positive receptive fields within people and place memory regions of medial parietal cortex

Authors: *C. L. SCRIVENER, E. H. SILSON;
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Abstract: Generating mental images of people and places has been found to activate four spatially separate regions within the medial parietal cortex (MPC) (Silson et al., 2019). In contrast, during perception these regions typically demonstrate a *decrease* in activation, which likely reflects their proximity to the default mode network (Raichle et al., 2001). Here we used population receptive field mapping (pRF) to quantify the pRF properties (amplitude, size, and eccentricity) within these MPC recall regions, and compared them with their perceptual counterparts in ventral temporal cortex (VTC; parahippocampal place area, PPA and fusiform face area, FFA). To estimate pRFs, we presented participants with four runs of a bar aperture that made eight sweeps through the visual field, whilst revealing scene fragments (Silson et al., 2015). PRFs were estimated using AFNI's non-linear fitting algorithm (3dNLfim). We quantified the percentage of suprathreshold (R^2>0.08) -ve pRFs within each ROI, meaning that the activity *decreased* when the bar aperture was presented in that voxels preferred spatial location. In addition, we extracted the median eccentricity, pRF size and visual field coverage of each ROI. All ROIs within both MPC and VTC contained a significant proportion of -ve pRFs

(t-test vs zero, $p<0.05$). However, the average proportion was greater in MPC (53%) than VTC (18%). Within MPC, ROIs engaged in people recall had significantly larger proportions of -ve pRFs than ROIs engaged in place recall, although there was no effect of posterior-anterior position within MPC. A similar finding was present in VTC with FFA containing larger proportions of -ve pRFs than PPA. Although ventral temporal regions had greater contralateral biases in their visual field coverage, we also found evidence for contralateral biases in MPC. These results mirror findings from place recall regions recently identified on the lateral and ventral surfaces (Steel et al., 2021; Steel, Silson et al., 2024). When comparing pRF size, place recall regions within MPC contained larger pRFs than people recall regions – a pattern consistent with pRF size differences within FFA and PPA often attributed to their different functional specialisations. Interestingly, there was no effect of amplitude on pRF sizes within MPC. The pRF properties of people and place recall regions within MPC show some striking similarities with those of face and scene-selective regions of VTC, suggesting a functional coupling. Overall, these data further demonstrate the ubiquitous representation of retinotopy throughout cortex and the need to better understand the relationship between +ve / -ve pRFs and functional specialisation.

Disclosures: C.L. Scrivener: None. **E.H. Silson:** None.

Poster

PSTR171: Higher Visual Areas

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DBT-IISc Partnership Programme
KVPY Fellowship

Title: Encoding of light direction and object identity in the monkey inferior temporal cortex

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Abstract: Objects in the real world produce a wide variety of images depending on the viewing condition, making their recognition an extremely challenging problem. Among these variations is illumination or lighting direction, whose neural basis has received relatively little attention. Here, we performed wireless brain recordings from the inferotemporal (IT) cortex of the macaque monkey (Macaca radiata, 1 male, aged 9 years) while the monkey fixated on images of 3-dimensional objects with varied lighting directions. In Experiment 1, we presented images of naturalistic objects lit at the same intensity but from different directions, such that while the visual appearance of the objects is sufficiently modulated by the lighting variation, the overall

object structure is preserved. In Experiment 2, we extend this to bas-relief objects with ambiguous 3D structure, which can equivalently be perceived as either concave or convex.The main findings are as follows: (1) In Experiment 1, multi-unit neural responses were strongly modulated by object identity and only weakly by light direction, as evidenced by 30% (38/128) of channels showing a main effect of only object identity, and 9% (12/128) of channels showing a main effect of only light direction; 26% (34/128) of channels are modulated significantly by both factors. This was also confirmed by modelling the neural response as a multiplicative/additive combination of object identity and light direction tuning; (2) Neural selectivity for object identity attained a peak slightly later than light direction tuning (peak latency: 127ms for object identity, 111ms for light direction). This was further corroborated by decoding analyses, which showed that object identity and lighting direction can be reliably extracted from neural responses, with a similar delay in peak decoding accuracy; (4) In Experiment 2, we observe that the light direction tuning curve for concave and convex objects are mirrored with respect to each other, suggesting that lighting direction tuning for perceptually ambiguous objects is biased by a shape prior. Taken together, our results elucidate how light direction is encoded at the neural level so as to achieve invariant object representations.

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Poster

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Title: Memory-dependent perceptual processing for transformation from first-person perspective to allocentric spatial representation in the primate inferotemporal cortex

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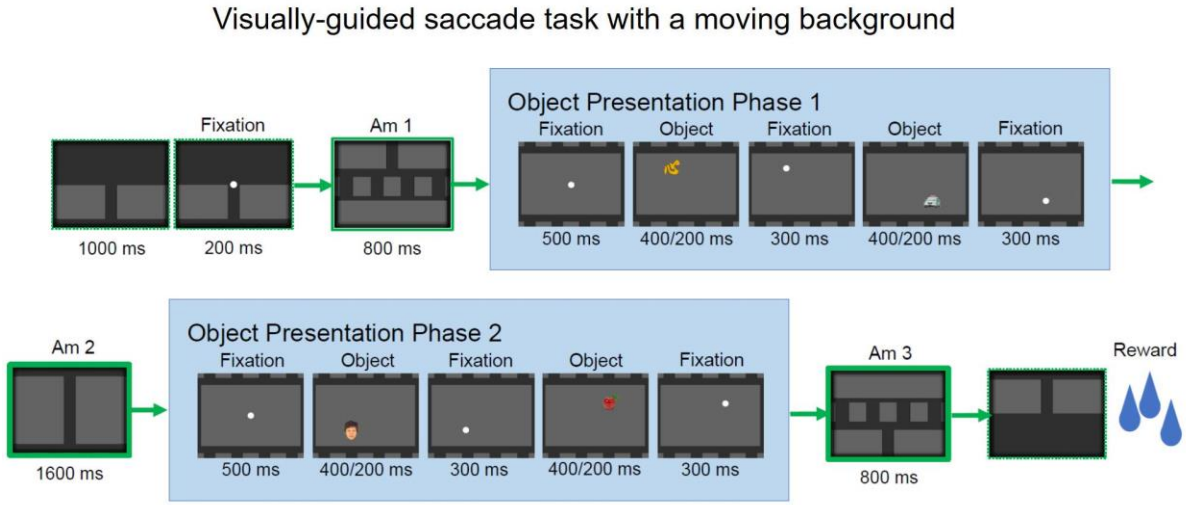
Abstract: To encode an object's location, a transformation from the first-person perspective to allocentric space is necessary. Our recent study showed that view-center-background signal in the ventral pathway may address this problem because the viewing image from the first-person perspective could specify the current gaze location in a scene in allocentric manner by similarity of images when a subject gazes on an object (Chen & Naya, 2020). In the present study, we first examined a presence of view-center-background signal by conducting single-unit recording from the posterior inferotemporal (PIT) cortex of two macaque monkeys during a newly devised

saccade task requiring them to gaze at objects, which were sequentially presented at one of four positions on a particular background stimulus. In each trial, we also presented moving background animations prior to the object presentations to indicate the current frame position on the entire background. Only a part of the entire background was presented to the animals as a background stimulus at each time point. Out of 377 recorded neurons, 119 neurons showed different responses to 16 visual input patterns determined by gaze locations and background stimuli ($p < 0.01$, One-way ANOVA), which could specify the current retinal image except for object information. These neurons ('space cells') showed an interaction effect between background and gaze-location in population ($p < 0.001$, KS-test). These results indicate that the space cells can represent particular combination patterns of gaze locations and background stimuli, suggesting a presence of view-center background signal in the PIT cortex. We next examined the prior animation effect on the space representation. The space cells showed a significant prior-animation effect depending on the current background stimulus ($p < 0.001$, Signed-rank test), which would allow them to signal an allocentric location of a gazing object on the entire background. This memory-dependent visual perception might relate view-center background signals at multiple gaze locations for scene construction.



Disclosures: Y. Naya: None. **A. Li:** None. **H. Chen:** None.

Poster

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Topic: D.06. Vision

Support: BioTechMed-Graz

Title: Variability in claustrum responses to natural videos is best explained by self-perceived video arousal

Authors: *A. COATES[1,2], P. SEDLMAYR[1], A. WASTIAN[1], A. BARTELS[3], D. LINHARDT[4], C. WINDISCHBERGER[4], A. ISCHEBECK[1,2], N. ZARETSKAYA[1,2];
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Abstract: The claustrum is a subcortical structure, which is densely interconnected with the cortex, but its function remains poorly understood. Consistent with its connectivity with the visual cortex and responses to visual stimulation in animal models (Remedios et al., 2010), we recently identified a region of the human claustrum that responded to visual naturalistic stimuli in the form of movie clips (Coates et al., 2023). In the current study, we investigated which visual stimulus properties the visual claustrum zone has a preference to.
Firstly, we extracted each participant's (n = 12) average response to each of the 48 video clips. We observed that some videos elicited a consistently higher response in the claustrum compared to other videos. We then extracted low- and mid-level visual features for each video: variation in luminance, contrast, colour and motion (Bartles, Zeki & Logothetis, 2008). Finally, in a separate experiment we asked a group of 68 participants for their subjective ratings of the videos in terms of arousal, interest and emotional valence. To compare effects observed in the claustrum to those in other regions, we also analysed responses of visual areas hMT and V4, adding the auditory cortex as a control region. To relate variability in claustrum responses across videos to physical movie features and subjective ratings, we performed linear mixed models (LMM) analysis with the stimulus features or subjective ratings as predictors and claustrum activity as a dependent variable.
We found no relationship between the claustrum responses and the low-level feature content of the videos. Unlike previous reports in animal models (Sherk & LeVay, 1981), we did not observe a preference for motion in the claustrum, which was present in hMT. Claustrum responses were not significant for subjective ratings of interest or valence. However, claustrum responses were significantly associated with subjective arousal ratings of the videos. This association was also present in other analysed visual areas, but not in the auditory cortex, ruling out that unspecific physiological arousal effects are driving fMRI activity. Our results suggest that the visual claustrum activity is associated with arousal evoked by the visual stimuli. Given the close link between arousal and attention, this result is consistent with the recently proposed role of the claustrum in selective attention (Atlan et al., 2018) and provides grounds for a more detailed investigation of this topic.

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Poster

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Title: Effects of partial occlusion on response dynamics in the primate middle and anterior ventral Superior Temporal Sulcus

Authors: *A. BOGNÁR, G. GHAMKHARI NEJAD, R. VOGELS;
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Abstract: Humans and non-human primates can seemingly effortlessly recognize objects even when they are partially concealed by occluders, yet only little is known of the underlying mechanisms. To investigate the effect of various levels of occlusion (ranging from 5 to 60 percent) on selectivity and the temporal dynamics of neuronal responses, we conducted single-unit recordings in body-responsive regions in both the middle and anterior ventral bank of the Superior Temporal Sulcus (STS) while the monkeys performed a passive fixation task. Across both monkeys and regions, several key findings emerged: 1, The average response strength decreased as occlusion levels increased. 2, In the population responses selectivity was preserved even for the highest levels of occlusions. 3, Response onset and peak latency gradually shifted by approximately 70 ms with higher degrees of occlusion, with mid-STS responses preceding those of anterior STS. 4, Following the first response peak, a trough was observed, succeeded by a stronger second peak under occlusion conditions. To investigate the role of visual information loss on the observed latency shifts, reduced responses, and peak-valley amplitude changes, we presented partially occluded bodies alongside the same stimuli presented on top of the occluding pattern and with an invisible occluding pattern, creating bodies with cut-outs. Intriguingly, onset latency shifted by only approximately 20 ms for the highest cut-out levels and remained unaffected by the background occluding pattern, suggesting that onset latency shifts with occlusion may result from the simultaneous presence of occluding pattern and the occluded stimuli. Despite the weakening of responses induced by cut-outs, those with highest information loss maintained selectivity similar to that observed during occlusion. The formation of a trough was more pronounced when bodies were presented on top of the occluder pattern. Furthermore, the second peak did not align with response onset shifts but maintained latency differences between regions, occurring earlier in the mid-STS. This suggests that the second response peak in the mid-STS is unlikely to arise from recurrent processing within the region or feedback from anterior STS. If generated by top-down feedback, one would expect it to appear earlier in anterior STS or at a similar time in the two regions and potentially exhibit better body selectivity. However, classification accuracies around the second peak, when training a classifier on responses to the unoccluded stimuli and testing on the ones under occlusion, never surpassed early response accuracies.

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Poster

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Title: The novel encoding schemes of dynamic natural stimuli in IT cortex revealed by spatial-temporal network

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Abstract: Under natural conditions, the visual stimuli that we receive are continuous and dynamic scenes. However, the stimuli used to study the primate visual system, especially the ventral visual pathway, are predominantly static images. This limitation restricts our understanding of the neural mechanisms underlying natural visual processing. To bridge this gap, we analyze neural response data from human and macaque IT with video stimuli. The human data is sourced from an open fMRI dataset, which collected BOLD signals from 25 subjects while viewing the video clips from the movie “The Grand Budapest Hotel” (Visconti, et al., 2020). Additionally, responses were collected from three macaque subjects freely viewing the same clips during fMRI scans. Features were extracted using three different neural networks—AlexNet, Visual Transformer (ViT), and Spatial-Temporal Visual Transformers (TimeSformer)—to construct encoding models for each voxel and predict their responses with cross-validation methods. The results revealed that models built with TimeSformer features accounted for an average of 20.37% and 18.40% more variance in human subjects, 31.8% and 19.8% more variance in monkey subjects, compared to AlexNet and ViT, respectively. This enhanced performance was exclusive to video clip inputs; static image inputs yielded comparable prediction performance across models. This improvement may partially stem from TimeSformer's ability to capture the high correlation structure observed when subjects view continuous dynamic scenes. For instance, regions representing scenes and bodies show high positive correlation during video viewing but exhibit small or even negative correlation with static images. TimeSformer's capacity to capture such correlations, which are not as detectable with models trained on static images, underscores its superiority in modeling visual system when processing dynamic stimuli. Also, the difference about the correlation structure between viewing video and static images suggests the novel mechanism of IT cortex processing the dynamic visual inputs for both human and macaque subjects.

Disclosures: W. Jin: None. **H. li:** None. **P. Bao:** None.

Poster

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China Postdoctoral Science Foundation Grant 2023M740125, 2022T150021, 2021M700004

Title: Characterizing the food-specific areas in the primate inferotemporal cortex

Authors: *B. GONG[1], L. YIPENG[1], W. LI[1], X. LIU[1], Z. ZISHUO[1], P. BAO[1,2,3];
[1]Peking Univ., beijing, China; [2]IDG/McGovern Institute for Brain Research, Beijing, China; [3]School of Psychological and Cognitive Sciences, Beijing, China

Abstract: Recent studies have identified a new category-specific area, known as the food area, through the analysis of 7T fMRI data from subjects viewing the Natural Scenes Dataset (NSD). While three groups have utilized different analysis methods and obtained similar results, their conclusions predominantly rely on this single dataset. To access the generalizability of the food area findings, we expanded this research to include both human subjects and macaques with fMRI and single-unit recording with different kinds of stimuli. Initially, we employed fMRI to measure human subjects' brain responses to two types of food stimuli: Chinese foods (e.g., Baozi) and Western foods (e.g., Pizza), contrasted against non-food stimuli in a blocked design. This approach identified specific subregions that are preferentially activated by food images with identified regions showing substantial overlap with the food areas determined using the NSD dataset. Subsequently, we scanned three macaques with fMRI using two stimulus sets, each containing both food and non-food images. One set comprised natural stimuli from the NSD, while the other consisted of isolated objects. Despite their differing low-level properties, both sets consistently indicated the similar food network in the IT cortex, featuring three distinct patches arranged from posterior to anterior.Moreover, by employing electrophysiological techniques, we recorded the single unit responses of the middle food area in macaques to the same stimuli used in the macaque fMRI experiment. Generally, about 63% neurons showing high food selectivity (d'>0.5) at least in one data set with 52% of the neurons demonstrating high selectivity for natural food stimuli and 23% showing high selectivity for single food items. These findings emphasize the robustness of the food area's selectivity across different species and stimulus complexities, underscoring its potential role in categorical food recognition within the IT cortex.

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Poster

PSTR171: Higher Visual Areas

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Topic: D.06. Vision

Title: A species-conserved integrative computation on neuromodulation

Authors: *A. M. BRIGANDE, A. A. DISNEY;
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Abstract: Neuromodulators such as dopamine and serotonin dynamically modify neuronal excitability and synaptic strength to wield powerful control over the brain and behavior. Although neuromodulators are often studied individually or pairwise, the joint action of multiple neuromodulators influences input-output mapping, the 'state' in which neural processing takes place. At least five subcortical modulatory systems - cholinergic, dopaminergic, histaminergic, noradrenergic, and serotonergic - innervate the cortex, where these inputs must be integrated. Despite anatomical and functional evidence that local circuits - and even single neurons - respond to multiple neuromodulators, how the cortex integrates neuromodulation remains poorly understood. Each neuromodulator can bind multiple receptors with opposing cellular effects, enabling divergent effects from the same neuromodulator and convergent effects from different neuromodulators. To investigate the extent to which individual neurons could compute integrated responses to multiple neuromodulators, we evaluated patterns of receptor transcript co-expression for acetylcholine, dopamine, histamine, noradrenaline, and serotonin. For mouse and human cortex, we analyzed publicly available single-cell transcriptomic data (Allen Institute) using a hypothesis-driven cell typing pipeline. We found that neurons formed clusters based on co-expression of modulatory receptor transcripts, suggesting that different groups of neurons perform distinct integrative computations. In rare cases, an integrative computation was unique to a traditional transcriptomic cell type. Across cortical regions and species, layer 5/6 near-projecting excitatory neurons co-express transcripts for Gq-coupled serotonin 2C receptors, Gs-coupled histamine H2 receptors, and Gi-coupled acetylcholine m2 receptors. However, there are nuances. For Gq-coupled acetylcholine receptors, we noted a potential mechanistic substitution: m1 in mouse and m3 in human. Interestingly, we found similar, but not identical, receptor transcript co-expression in layer 5/6 near-projecting neurons from human, chimpanzee, gorilla, macaque, and marmoset middle temporal gyrus (Allen Institute). Due to methodological differences - SMART-Seq v4 (Takara) for human and mouse cortex versus 10x Chromium v3 (10x Genomics) for primate middle temporal gyrus - it is difficult to reconcile the differences we see. These data suggest that there may be 'canonical' computations on neuromodulation - that is, similarities at the algorithmic level despite substitutions at the receptor level - across cell types, cortical regions, and species.

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Poster

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NSF CAREER 2143077
David and Lucile Packard Foundation

Title: Bridging the gap between synthetic and preferred stimuli in deep networks and macaque visual cortex

Authors: *A. MONTANARO[1], C. R. PONCE[2];
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Abstract: To understand a visual recognition system such as the brain or an artificial deep network, we must know the function of its individual units. Neurons in the brain are tuned to visual features present in the natural world, while in convolutional neural networks (CNNs), units learn patterns present in their training data sets. For example, in the first convolutional layer of CNNs such as AlexNet, units learn Gabor-like patterns and color patches. We know this is true because we can visualize them directly, as filters that act on pixel space. However, it is not clear how to interpret units in deeper hierarchical layers, a set that includes visual neurons in the primate brain. One path is to use a closed-loop image optimization (via a generative model) to find the best stimulus for each unit. In this study, we used this method to infer the visual patterns that best activate units in the first convolutional layer of various CNNs. We recorded the activation of each unit and compared it to the theoretical upper bound, represented by the L2-norm of the filter itself. Initially, we found that optimized images evoked higher activations than the filters themselves. However, by increasing the contrast of the filters to a level comparable to that of the optimized images, we found that the high-contrast filters then caused higher activations than the optimized images. This suggested that image optimization worked by approximating the filter shape and by optimizing nuisance variables such as contrast and luminance. To determine if these results applied to units in deeper layers, we implemented a series of simple transformations to natural images (e.g., rotation, contrast, scaling, and more) to increase the activation of these hidden units. We found that this was successful. Finally, to test this approach in monkey neurons from areas V1, V4, and posterior inferotemporal cortex (PIT), we first used the same closed-loop image optimization to approximate the best activating features for individual neurons and neuronal microclusters. Then, we used the same simple image transformations to optimize natural images to resemble the synthetic images, increasing neuronal firing rate successfully. We conclude that image optimization improves multiple

features and mechanisms, including identification of key excitatory features, improving contrast, size, and surround clutter.

Disclosures: A. Montanaro: None. **C.R. Ponce:** None.

Poster

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Title: Integrating iEEG and fMRI to capture dynamics of visual computation during natural scenes viewing

Authors: *Z. QADIR[1], H. HUANG[1], M. MONTOYA[1], M. JENSEN[2], G. OJEDA VALENCIA[1], K. J. MILLER[3], G. A. WORRELL[4], T. NASELARIS[5], K. N. KAY[6], D. HERMES[1];
[1]Physiol. and Biomed. Engin., Mayo Clin., Rochester, MN; [2]Neurosci., Mayo Clin., Rochester, MN; [3]Neurosurg., Mayo Clin., Rochester, MN; [4]Neurol., Mayo Clin., Rochester, MN; [5]Neurosci., Univ. of Minnesota, Minneapolis, MN; [6]Radiology, Univ. of Minnesota, Twin Cities, St Paul, MN

Abstract: Visual processing evolves rapidly over time. The fMRI BOLD signal is thought to capture the aggregate of the neuronal activity over time. Intracranial-EEG (iEEG) measurements, on the other hand, can capture visual processing at the millisecond timescale. Previous studies have integrated these measurements, showing strong correlations between the fMRI BOLD responses and the iEEG broadband signal. We use a multimodal framework combining iEEG and fMRI, to test how these correlations evolve over time across different visual areas. We recorded iEEG data in 8 patients who had electrodes implanted for clinical epilepsy monitoring in early visual areas as well as along the dorsal, ventral and lateral-occipital (LO) regions . Each patient was shown a subset of 1000 stimuli from the NSD-fMRI dataset. Electrodes with significant broadband (70-170 Hz) power increases with respect to baseline were considered for further analysis. From the NSD-fMRI dataset, we obtained average fMRI beta-weights for the 1000 stimuli that were repeated thrice across the 8 subjects. Next, for each iEEG electrode we computed a Pearson correlation map with all the fMRI vertices, across the 1000 stimuli, giving us a time by vertices correlation matrix. This provided us with a brain-wide temporally evolving map of how similar the modalities respond to the same images. In every subject, we observed

significant correlation of the iEEG broadband responses with that of fMRI beta-weights: broadband responses of iEEG electrodes correlated strongly with fMRI responses in the area corresponding to the location of the electrode. Moreover, these correlations evolved over time, first increasing, spreading throughout the visual pathways until ~ 300 ms. While the stimuli were presented for 800 ms, the two signals correlated only for a duration of ~500ms. Interestingly, the broadband signal measured in ventral temporal areas additionally correlated with fMRI signal changes in IPS areas in the dorsal stream. These temporally resolved correlation maps show the dynamic nature of the multimodal integration. Overall, we propose that our multimodal framework enables us to compute functional connectivity at high spatiotemporal resolution reflecting the rich dynamics of interaction across different brain regions.

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Poster

PSTR171: Higher Visual Areas

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Program #/Poster #: PSTR171.11/H15

Topic: D.06. Vision

Title: Identifying the neuronal selectivity landscape in Macaque area V4; a deep learning and inception loop approach.

Authors: *N. KARANTZAS[1], K. FRANKE[2], K. RAMAKRISHNAN[3], K. F. WILLEKE[4], P. ELUMALAI[5], P. FAHEY[6], K. RESTIVO[7], S. S. PATEL[1], A. S. ECKER[8], F. H. SINZ[9], A. S. TOLIAS[1];
[1]Ophthalmology, Stanford Univ., Stanford, CA; [2]Stanford Sch. of Med., Stanford, CA; [3]Neurosci., Baylor Col. of Med., Houston, TX; [4]Cognitive Neurol., Stanford Univ., Stanford, CA; [5]Univ. of Gottingen, Gottingen, Germany; [6]Dept. of Ophthalmology, Stanford Univ., Stanford, CA; [7]Baylor Col. of Med., Houston, TX; [8]Ctr. For Integrative Neurosci., Tuebingen, Germany; [9]Inst. of Computer Sci., Univ. of Gottingen, Gottingen, Germany

Abstract: Area V4 in the primate visual system is primarily involved in processing visual information related to object features such as shape, color, and texture, playing a crucial role in object detection and discrimination. Most research to date has characterized the selectivity of V4 neurons to individual stimulus features (i.e., color or texture) and, therefore, a systematic understanding of the selectivity landscape of V4 neurons across features is missing. Here, we addressed this important problem in the macaque as a model system. We performed large-scale laminar probe recordings in fixating macaques (~400 neurons from n=2 animals) while showing natural images exhibiting complex shape, color, and texture compositions to the monkey. Based on this data, we trained convolutional neural network (CNN) models, which accurately predicted the firing rate of single V4 neurons to hold-out test images, allowing us to perform a thorough in-silico analysis of stimulus selectivity, jointly for multiple object features at the same time. For the in-silico analysis, we used natural-scene-inspired rendered scenes that provide tight control over distinct stimulus features, while still matching natural scene statistics. Then, we systematically quantified each neuron's selectivity to different features and their combinations by predicting the firing rate to manipulated scenes with the trained CNN model. We are currently confirming model predictions in-vivo by performing closed-loop experiments, as described recently (Willeke et al. 2023). Our results will reveal how V4 neurons tile the landscape of object feature selectivity, thereby significantly contributing to a deeper understanding of the neuronal correlates of object recognition.

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Poster

PSTR171: Higher Visual Areas

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR171.12/H16

Topic: D.06. Vision

Title: Explaining neural selectivity for natural images in IT (inferotemporal) cortex

Authors: *D. GAMBLE[1], C. E. CONNOR[2];
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Abstract: IT (inferotemporal) cortex comprises the final processing stages in the ventral/object pathway in primate visual cortex. Neurons in IT are known to be selective for complex 2D and 3D geometry in abstract stimuli. IT neurons are frequently studied with random sets of natural (photographic) stimuli. Responses to these natural stimuli are highly selective, but the basis for that selectivity is unknown. In this study, we seek to explain IT neural selectivity for natural images in terms of tuning for 2D and 3D geometry. To do this, we use a genetic algorithm to evolve photorealistic images of complex 3D objects. These stimuli are presented in linear array

IT recording experiments in monkeys performing a fixation task. Neural responses across related groups of IT neural signals are used as the fitness metric to drive stimulus evolution across 8-10 generations of 100 stimuli each (divided into two independently evolving lineages). We also record the responses of these same neurons to a set of 150 natural and virtual reality target stimuli. We have observed strong responses and clear selectivity across both the genetic algorithm stimuli and the natural/naturalistic target stimuli. Our aim is to elucidate the geometric tuning of individual IT neurons by analyzing their patterns of responses to genetic algorithm stimuli and then show how that tuning determines responses to the natural target stimuli. We expect the results to show for the first time the specific object information conveyed by IT responses to natural stimuli. Analysis of object information at the population level will provide a comprehensive picture of how natural stimuli are represented in the final stages of visual processing.

Disclosures: D. Gamble: None. **C.E. Connor:** None.

Poster

PSTR171: Higher Visual Areas

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR171.13/H17

Topic: D.06. Vision

Title: Shape processing algorithms in V4 derived from neural network models

Authors: R. SRINATH[1], A. CHEN[2], ***C. CONNOR**[2];
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Abstract: Studies of neural coding in intermediate and higher level stages of the ventral/object pathway of primate visual cortex have revealed that neurons represent 2D and 3D shape in terms of object fragments, their geometric properties, and their object-relative spatial configuration. However, almost nothing is known about the neural algorithms that generate this information from lower level inputs. Here, we used analysis of visual neural network processing to derive hypotheses about these algorithms in area V4, an intermediate stage where individual neurons encode orientation, curvature, and object-relative positions of 2D contour fragments, 3D surface fragments, and 3D medial axis fragments. Our aim is to test these algorithms with experiments in V4, V2, and V1, to discover how this geometric information about object fragments is generated from the 2D Gabor-like filter signals that tile visual space in area V1. We have analyzed both AlexNet, a visual network trained on the ImageNet database, and neuromimetic vision network works trained to reproduce response patterns of individual V4 neurons across large numbers of abstract stimuli evolved in a response-driven genetic algorithm experiment. Previous work has shown that V4 responses are most closely modeled by layer 3 of such convolutional vision networks. We analyzed how V4-like layer 3 response patterns depend on differential inputs from convolutional neurons in layer 2 (conv2) modulated through, max pooling, rectification, and connection weight patterns to the layer 3 neuron. In turn, we analyzed how the responses of these

conv2 neurons depended on differential inputs from layer 1 2D Gabor-like filters (conv1) modulated through max pooling, rectification, and connection weight patterns to conv2. One hypothesis emerging from these analyses is that V4 neurons that encode 3D shape fragments should be driven almost entirely by Gabor-like signals for achromatic contrast and low spatial frequencies. In contrast, V4 neurons selective for 2D shape should be driven more strongly by signals for chromatic contrast. Our initial tests of this hypothesis contrast the sensitivity of 3D- vs. 2D-responsive neurons in V4 to achromatic vs. chromatic contrast and low vs. high spatial frequencies.

Disclosures: R. Srinath: None. **A. Chen:** None. **C. Connor:** None.

Poster

PSTR171: Higher Visual Areas

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Program #/Poster #: PSTR171.14/H18

Topic: D.06. Vision

Support: NIH Grant R01MH11847

Title: Reduced object discrimination in autism is associated with structural properties of the Lateral Occipital Complex

Authors: *T. KOLODNY[1,2], X. FAN[5,2], K. M. WOODARD[2], A. TASEVAC[3], W. R. GANZ[2], H. REA[3], E. C. KURTZ-NELSON[6,3], S. J. WEBB[4,7], S. O. MURRAY[2];
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Abstract: There is evidence for atypical visual perception in autism spectrum disorder (ASD), especially in perception of complex stimuli such as faces. However, it is unclear whether face perception differences are related to the social content, or to the processing of complex visual stimuli more broadly. To investigate the latter possibility, we tested object discrimination in autistic and non-autistic young adults, using a combination of psychophysics and neuroimaging. We present preliminary results from 24 autistic and 20 non-autistic participants, who completed an object discrimination task as well as anatomical and functional MRI scans. The objects were novel complex shapes that required processing of fine spatial details to successfully determine whether two small objects presented near the fovea were the same or different. Discrimination performance (d') showed substantial individual differences, and was overall significantly lower in the autistic group compared to the non-autistic group ($t(42)=3.7$, $p<.001$).
To investigate possible neural substrates underlying individual differences in object processing, we examined associations between task performance and structural properties of object-selective cortical areas. A functional localizer, contrasting intact vs. scrambled real-life objects, was used to define object-selective regions within the occipital lateral and inferior temporal cortex in each

individual participant. Cortical thickness within these regions was extracted from high-resolution T1-weighted images using a surface-based pipeline in Freesurfer. We found that among the autistic participants, cortical thickness in object-selective areas in the left fusiform gyrus was strongly inversely correlated with object discrimination performance ($r=-.49$, $p<.05$). Exploratory whole-brain analyses confirmed this finding and revealed further areas within the lateral occipital complex (LOC) where cortical thickness is larger in autistics than in not-autistics, and is inversely correlated with task performance.
Overall, our results show reduced object discrimination ability in autism and indicate that individual differences within the autistic group are related to structural features of object-selective cortical areas. This association was not observed in non-autistic participants, suggesting that the neural mechanisms mediating object perception might differ between groups. Importantly, these results demonstrate that differences in visual perception in autism are not limited to the social domain.

Disclosures: T. Kolodny: None. **X. Fan:** None. **K.M. Woodard:** None. **A. Tasevac:** None. **S.J. Webb:** None. **S.O. Murray:** None.

Poster

PSTR171: Higher Visual Areas

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Program #/Poster #: PSTR171.15/H19

Topic: D.06. Vision

Support: NIH Grant RF1DA055666

Title: Cortical state dynamics and visual representations in the inferotemporal cortex

Authors: *C. MCGRORY[1], J. K. HESSE[2], F. LANFRANCHI[3], S. REGUYAL[4], D. Y. TSAO[5], T. A. ENGEL[4];
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Abstract: Spontaneous fluctuations in cortical state are prevalent dynamics in the early visual cortex of non-human primates, which manifest as periods of vigorous (On) and faint (Off) spiking among all neurons in a local region of the retinotopic map. In visual areas V1 and V4, the On-Off dynamics are modulated during selective attention and impact perceptual sensitivity, which suggest their role in visual perception. However, it remains unknown whether cortical state dynamics exist in higher visual areas, and if so, how they affect encoding of visual information. We recorded spiking activity with Neuropixels probes from face-selective patches in the inferotemporal cortex (IT) of monkeys performing various fixation tasks, while viewing a diverse set of stimuli. Synchronous On-Off fluctuations were apparent in spike rasters of single-trial population activity across all visual tasks. These fluctuations dominated spiking activity on

single trials and were comparable in magnitude to stimulus-driven changes in spike rates. We quantified the firing-rate modulation and timescales of On-Off dynamics in the IT cortex using a hidden Markov Model that segments spike data into On and Off episodes. We found that the timescales of On-Off switching in IT were nearly invariant across all visual tasks, indicating that these fluctuations do not directly depend on the viewed stimulus and behavioral context. We next investigated the effect of On-Off fluctuations on stimulus representations in IT. We trained linear decoders to predict the static image seen by a monkey from spiking activity during On and Off phases separately. Stimuli could be decoded with high accuracy during both On and Off phases, despite the Off phases having more than two-fold lower firing rates. Decoders trained on data from one phase could also decode stimuli during the other phase with only a slight degradation in performance. Moreover, On-Off fluctuations and stimulus-driven changes in firing rates were represented in separate principal components, indicating that On-Off fluctuations and visual information are confined to largely separate neural subspaces. This separation was stark enough that removing from the spike data the first principal component that was aligned with the On-Off dynamics did not significantly degrade our ability to decode the stimulus image. Our work reveals On-Off dynamics in IT and suggests they are separate from visual representations, raising a question about what function do these dynamics play in visual processing.

Disclosures: C. McGrory: None. **J.K. Hesse:** None. **F. Lanfranchi:** None. **S. Reguyal:** None. **D.Y. Tsao:** None. **T.A. Engel:** None.

Poster

PSTR171: Higher Visual Areas

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Program #/Poster #: PSTR171.16/H20

Topic: D.06. Vision

Support: NIH Grant RF1DA055666

Title: Impact of Spontaneous Fluctuations in Local Cortical State on Visual Stimulus Encoding in Area V4

Authors: *S. REGUYAL, C. MCGRORY, T. A. ENGEL;
Princeton Neurosci. Inst., Princeton, NJ

Abstract: Spontaneous fluctuations in local cortical excitability are prevalent dynamics in non-human primate visual cortex. These fluctuations manifest as periods of vigorous (On) and faint (Off) spiking activity within cortical columns. Previous studies show that they are modulated by global arousal and selective attention, suggesting a possible role in visual information processing. However, how switches between On and Off phases in population spiking activity affect visual stimulus encoding has not been studied. We analyzed a publicly available dataset of spiking activity in single columns of macaque area V4 during a spatial attention task, studying the effects of On-Off dynamics on visual information encoding in single neuron firing rates,

neural population responses, and correlated neural variability. We used a Hidden Markov Model to segment On and Off episodes in spiking activity on single trials. On the single-neuron level, we found that many neurons that were tuned in the On phase slightly changed or lost their tuning in the Off phase. On the population level, we trained linear stimulus decoders and also identified stimulus representations using principal component analysis on averaged firing rates during On and Off phases separately. Cortical neural populations encoded stimulus information during both On and Off phases. However, during Off phases, less information was available and stimulus representations in the population state space showed signs of rotation and scaling. Finally, since On-Off dynamics are a major source of correlated variability in neural responses, we studied how this co-variability impacts the visual stimulus encoding, and found that it may have less impact on encoded information than noise correlations from other sources. To interpret these results, we built models of neural population responses for different scenarios of how stimulus encoding intersects with On-Off dynamics. In sum, V4 neurons encoded visual information throughout On-Off fluctuations although stimulus representations shifted between On and Off phases. These results reveal how cortical state dynamics affect visual information encoding in V4, pointing to a possible strategy for how attentional modulation of local cortical state may enhance visual representations.

Disclosures: S. Reguyal: None. **C. McGrory:** None. **T.A. Engel:** None.

Poster

PSTR172: Cerebellum: Sensorimotorand Learning

Location: MCP Hall A

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Program #/Poster #: PSTR172.01/H21

Topic: E.02. Cerebellum

Support: Scientific and Technological Research Council of Turkey (TUBITAK) Grant 215S699

Title: Chemoarchitecture of the human inferior olive

Authors: *G. SENGUL[1], Ö. KANAT[2], E. CANDAR[3], I. DEMIRCUBUK[3], F. ÇETIN[4]; [1]Ege Univ, Sch. Med., Izmir, Turkey; [2]Physiol., Istanbul Arel University, Sch. of Med., Istanbul, Turkey; [3]Ege Univ., Izmir, ; [4]Physiol., Istanbul Medeniyet University, Sch. of Med., Istanbul, Turkey

Abstract: Located in the ventrolateral part of the brainstem, the inferior olive is a key structure in the hindbrain, essential for motor learning and coordination through its provision of climbing fibers. In our study, we utilized a variety of immunohistochemical markers to explore the chemoarchitecture of the inferior olive in both human and mouse models. Using resources from The Allen Institute for Brain Science (Lein et al., 2007; http://www.brain-map.org/) and the Anatomic Gene Expression Atlas (AGEA) tool, we examined gene expression specific to the inferior olive. We prepared serial sections of the human brainstem using a cryostat at 45 µm

thickness, followed by staining and examination under light microscopy. Our observations included immunoreactivities for substance P (NK1), tyrosine hydroxylase, neuropeptide Y, calbindin, calretinin, parvalbumin, calcitonin-gene-related peptide, choline acetyltransferase, cocaine- and amphetamine-related peptide (CART), GABA, glutamate, glycine, galanin, nitric oxide synthase, and VIP in the inferior olivary complex of both species. These results indicate a largely identical neurochemical organization, with minor differences across the specific subnuclei (principal, medial, and dorsal) of the inferior olive.

Disclosures: G. Sengul: None. **Ö. Kanat:** None. **E. Candar:** None. **I. Demircubuk:** None. **F. Çetin:** None.

Poster

PSTR172: Cerebellum: Sensorimotorand Learning

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR172.02/H22

Topic: E.02. Cerebellum

Support: JSPS 23K14677
JSPS 24H00064
JST-CREST JPMJCR23P3

Title: Involvement of the lateral cerebellum in the detection of rhythmic deviations as revealed by primate optogenetics

Authors: *M. KAMEDA, M. TANAKA;
Sch. of Med., Hokkaido Univ., Sapporo, Japan

Abstract: When we are feeling the rhythm, we can immediately detect its disturbance. This requires accurate timing prediction of periodic sensory event. Previous studies in our laboratory have shown that neurons in the dentate nucleus (DN) of the cerebellum exhibit periodic activity in monkeys attempting to detect the omission of regularly presented repetitive visual stimuli (the missing oddball paradigm). The neuronal activity correlated with reaction time to the stimulus omission and inactivation of the DN delayed the response, suggesting that these neurons may represent periodic timing prediction (Ohmae et al., 2013). However, it is also possible that these neurons simply regulate movement timing as the cerebellum is involved in motor control. To address this, we asked animals to judge slight changes in rhythm and examined the relationship between their detectability and neuronal activity in the DN. In the modified missing oddball task, a slightly longer interstimulus interval was inserted in a series of repetitive visual stimuli at 400 ms. Monkeys received a liquid reward if they responded with hand movement to either a stimulus delay (Hit) or a subsequent stimulus omission (Miss). Logistic regression of the relationship between delay length and hit rate showed that the delays that resulted in 50% hit rate were 93 ± 9 ms (20-25% of 400 ms) and 134 ± 20 ms (30-35%) for the two monkeys. During recording sessions, we presented a delay of 120-140 ms and compared neuronal activities

between hit and miss trials. Neuronal activity in the DN immediately before the delayed stimulus was greater in hit trials than in miss trials (paired t-test, $p < 0.01$, $n = 16$), but neuronal activity before one previous stimulus was comparable ($p = 0.53$). Furthermore, when the animals incorrectly responded to a regular stimulus (False alarm), the activity before the stimulus was significantly greater than that before the stimulus omission (paired t-test, $p = 0.02$, $n = 10$). These results indicate that the magnitude of neuronal activity in the DN predicts the detectability of rhythmic deviations. To investigate the causal role, the activity of DN neurons was optogenetically manipulated. An AAV9 vector expressing ChR2 under the Purkinje cell-specific L7 promoter was injected into the Clus lobules of the cerebellum. We confirmed that laser stimulation (473 nm, 14-30 mW) of Purkinje cell terminals effectively suppressed the activity of DN neurons. Moreover, the hit rate decreased when the DN was illuminated immediately before the delayed stimulus. These results suggest that the periodic neuronal activity in the DN represents sensory prediction and determines the detectability of rhythmic deviations.

Disclosures: M. Kameda: None. **M. Tanaka:** None.

Poster

PSTR172: Cerebellum: Sensorimotorand Learning

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Program #/Poster #: PSTR172.03/H23

Topic: E.02. Cerebellum

Support: NIH R35-NS116854

Title: Short-term synaptic plasticity enables detection of novel sensory input by crus I/II Purkinje cells in awake mice

Authors: *M. RAMAKRISHNA HOLLA[1], S. BROWN[1], I. M. RAMAN[2];
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Abstract: Cerebellar Purkinje (Pkj) neurons receive synaptic inputs with different short-term plasticity (STP) profiles. Recording from Pkj cells in mouse cerebellar slices *in vitro*, we previously reported that EPSCs of parallel fiber (PF) show strong synaptic facilitation, while IPSCs from molecular layer interneurons (MLIs), activated through feedforward inhibition, remain stable. Consequently, during trains of PF stimulation, E/I ratios favor inhibition at train onset but drop thereafter. *In vivo*, recordings of crus I/II Pkj cells in awake head-fixed mice during air puffs applied to the whisker pad show well-timed, short-latency, brief (2-4 ms) suppressions of Pkj simple spikes (SS), sufficient to drive cerebellar output. We recently found that when 5-puff trains are applied with different intervals (200, 100, 50, 25 ms), SS suppression remains present across stimuli with longer intervals (200, 100 ms) but greatly decreases with shorter intervals (50, 25 ms). Thus, the distinct STP of convergent synaptic inputs permit a computation suited to respond strongly to the onset of sensory events, whereas responses to rapidly repeating stimuli are filtered. Here, we tested whether introducing a novel sensory event

into a train of identical stimuli could engage feed-forward inhibitory pathways that might be sufficient to restore SS suppression. *In vitro*, after facilitating one set of PFs by a stimulus train, activating a distinct set of PFs indeed shifted the E/I ratio to re-favor inhibition of Pkj cells. *In vivo*, trains of 3 puffs (25 ms intervals) were applied to the contralateral ("contra") whiskers and a 4th puff either to the ipsilateral ("ipsi"), the contra, or both sets of whiskers. Of 57 Pkj cells with SS suppressions, 39% responded only to ipsi, 24% only to contra, and 37% to both puffs. In these ipsi/contra Pkj cells, suppression was reduced with trains of contra puffs (ratio of suppressed rate, 4th to 1st puff: 0.68). When either the novel ipsi puff or the pair of puffs was introduced, however, suppression remained high (ratio: 0.94; 1.17). Target neurons in the cerebellar nuclei (CbN) showed corresponding increases in firing. These data reveal that a subset of Pkj cells receive convergent ipsi and contra tactile inputs through distinct microcircuits. As a result, these Pkj cells synchronously suppress their SSs to novel sensory inputs, even when that input arises on a background of repetitive stimuli to which the population has adapted. Thus, specializations of STP superimposed on convergent feed-forward microcircuits allow these Pkj cells to behave as novel event detectors, rather than processors of specific sensory features, and to drive CbN cells accordingly.

Disclosures: M. Ramakrishna Holla: None. **S. Brown:** None. **I.M. Raman:** None.

Poster

PSTR172: Cerebellum: Sensorimotorand Learning

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Program #/Poster #: PSTR172.04/H24

Topic: E.02. Cerebellum

Support: NIH R35-NS116854

Title: In vivo high-speed voltage imaging of action potentials of cerebellar molecular layer interneurons reveals synchronous spiking to sensory input in awake mice

Authors: *S. BROWN[1], M. LAND[3], S. YANG[4], A. MCDONALD[5], F. ST-PIERRE[6], I. M. RAMAN[2];
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Abstract: The cerebellum contains many classes of neurons that fire action potentials spontaneously at high rates (10 - 100 spikes/sec), enabling them to encode information within their spike rate or through well-timed spiking in a population. Multiple lines of evidence suggest that Purkinje cells, the projection neurons of the cerebellar cortex, encode distinct information in the rate and timing of their simple spikes, depending on the behavioral task and cerebellar region studied. With single unit electrophysiological recordings from awake mice, we previously found that crus I Purkinje cells respond to whisker deflections with brief (2-4 ms) suppressions of simple spiking that effectively drive well-timed spiking in neurons of the cerebellar nuclei via

rapid disinhibition. The resulting cerebellar output is sufficient to augment movement. Since the suppression has a fixed latency across neurons, we have inferred that it is synchronous across the population. Here, we developed a low magnification, 1-photon microscope for high speed (1-2 kHz) voltage imaging of action potentials in awake behaving mice. Since molecular layer interneurons (MLIs) of the cerebellar cortex are the primary source of synaptic inhibition to Purkinje cells, they may provide concerted inhibition that results in the observed suppression of simple spikes. To test this idea, groups of 10-30 MLIs expressing a novel genetically encoded voltage indicator were imaged using targeted illumination. In awake resting mice, crus I MLIs fired narrow action potentials (1-2 ms) at 30-50 spikes/sec. With the application of air puffs to the whisker pad, MLIs consistently fired synchronous, short-latency (<10 ms) spikes across the imaged population. On a large fraction of trials, synchrony (4-ms window) among MLIs exceeded 50% in the imaged group. The air puff also evoked whisker movements, during which spike rates increased in MLIs, without ms-scale temporal coherence, i.e., asynchronously. These results offer direct measurements of single-trial, ms-scale spike synchrony in the cerebellar cortex. They also provide further evidence of the notion that synchronous inhibition in the cerebellar cortex encodes the onset of a sensory event while motor input is encoded by a rate code. Therefore, although rate coding is prevalent throughout the brain, spike synchrony between neurons may be a common mode of information transmission in the cerebellum.

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Poster

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Topic: E.02. Cerebellum

Support: NIH R35-NS116854

Title: Projections of whisker-related sensory trigeminocerebellar and motor pontocerebellar mossy fibers to crusI/II of the cerebellar cortex

Authors: *T. ROSSI, I. M. RAMAN;
Neurobio., Northwestern Univ., Evanston, IL

Abstract: Both sensory signals and motor commands are conveyed to the cerebellum by mossy fibers (MFs) originating in many brain regions, and action potentials of Purkinje cells carry information about both sensation and movement. For example, recording in vivo from crus I/II Purkinje cells, work from our lab has reported well-timed inhibition in response to tactile whisker input, and broadly timed excitation in association with whisks, raising the question of the extent to which, and levels at which, sensory and motor streams converge to permit computations relevant to sensorimotor integration. Previous studies from several labs have

shown that granule cells receive convergent information from multiple sensory modalities. Here, we investigate the anatomical overlap and/or segregation of sensory-related MFs carrying sensory signals from the whiskers, and motor-related MFs transmitting signals from motor cortex via the pons in whisk-related regions of the cerebellar cortex. Sensory and motor MFs were identified with viral tracing in mice. Trigeminocerebellar MFs were labeled by injection of AAV1.hSyn.hChR2(H134R)-eYFP or AAV9.hSyn.ChETA-eYFP in the principal trigeminal nucleus (Pr5), which relays tactile sensory input from the whiskers. To label pontine MFs that transmit whisking motor commands to the cerebellum, mice received a double injection of the transsynaptic virus AAV1.hSyn.Cre.WPRE.hGH into motor cortex, and AAV5.hSyn.FLEX-rc[ChrimsonR-tdTomato] into the pontine nuclei (Pn), resulting in labeling of "motor" pontocerebellar MFs. Preliminary results show that trigeminocerebellar MFs terminate in lobules crus I/II and the paraflocculus, primarily ipsilateral to the injected Pr5. Motor pontocerebellar MFs were found in almost all cerebellar lobules with a majority contralateral to the injected Pn. While trigeminocerebellar and pontocerebellar terminals in the granule cell layer of crus I/II lie in close proximity, making convergence at the level of some granule cells appear possible. At the same time, regions of segregation were clearly evident, consistent with the idea that crus I/II Purkinje cells may receive at least some information from separate populations of "sensory" and "motor"-related granule cells. Such a segregation could account for the different spiking patterns observed in whisking related Purkinje cells, in which sensory input and motor commands appear to feed into different microcircuits that engage different amounts of feedforward inhibition.

Disclosures: T. Rossi: None. **I.M. Raman:** None.

Poster

PSTR172: Cerebellum: Sensorimotorand Learning

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Topic: E.02. Cerebellum

Support: JST CREST/JPMJCR22P5
JSPS KAKENHI/JP20H04286
JSPS KAKENHI/JP18KK0286

Title: Memory length of the goldfish predictive optokinetic response

Authors: T. YAMANAKA[1], *R. BAKER[4], Y. HIRATA[5,2,3];
[1]Robotic Sci. and Technol., Chubu Univ., Kasugai, Japan; [2]Chubu Univ., Ctr. for Math. Sci. and Artificial Intelligence, Japan; [3]Chubu Univ., Academy of Emerging Sciences, Japan; [4]New York Univ. Langone Med. Ctr., New York, NY; [5]Artificial Intelligence and Robotics, Chubu Univ. Col. of Engin., Aichi, Japan

Abstract: The vestibulo-ocular reflex (VOR) and the optokinetic response (OKR) are reflexive eye movements that collaboratively stabilize the visual field during head motion. The VOR

counter-rotates the eyes to match head motion speed while the OKR rotates the eyes to track visual field motion. Previous research has shown that goldfish acquire a predictive (p)OKR after prolonged exposure to a temporally periodic visual stimulus (Marsh and Baker, 1997; Miki et al., 2018, 2020). The pOKR is primarily characterized by two predictive features: ① a decrease in eye velocity before the end of each visual stimulus period (eye velocity reduction), and ② a continuation of OKR-like periodic eye velocity in dark following the end of the visual stimulus (eye velocity oscillation in dark). The cerebellum and the velocity storage mechanism (VSM) are shared by the VOR and the OKR and have been found necessary for the acquisition of pOKR (Miki et al., 2018, 2020). It has also been demonstrated that the predictive feature ① can be recalled by a vestibular stimulus even after the initial eye velocity oscillations in dark have subsided (Yamanaka et al., 2023). However, the duration of acquired pOKR memory including its recallability after cessation of visual training remains unknown. To address this issue, goldfish (N = 3) were exposed for 3 hours to a periodic visual stimulus which rotated at a constant velocity (20 or 40 deg/s) for 8 s, followed by an 8-s pause (1 cycle = 16 s). After pOKR acquisition, eye velocity oscillation in dark was observed until it subsided. Subsequently, at random intervals, a visual stimulus with twice the period of training (extended visual stimulus) was presented for only one cycle in an otherwise dark environment. Results showed that eye velocity reduction (predictive feature ①) persisted even after 1 hour, while eye velocity oscillation in dark (predictive feature ②) did not reappear. By contrast, when a constant velocity (20 deg/s) vestibular stimulus was provided for 1 min instead of the extended visual stimulus, the predictive feature ② returned. Retention of pOKR memory for longer than 1 hour is similar to that observed in VOR gain adaptation induced by visual-vestibular interaction paradigms for 2-3 hours (Kuki et al.,2004; Soga et al., 2020). A finding that suggests involvement of the same memory formation mechanism. Furthermore, eye velocity oscillation in dark was not triggered by a short visual stimulus, but by a longer vestibular stimulus. This suggests that a significant amount of velocity information needs to be stored within the VSM to empower the oscillation in dark.

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Poster

PSTR172: Cerebellum: Sensorimotorand Learning

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Program #/Poster #: PSTR172.07/H27

Topic: E.02. Cerebellum

Support: 1R37NS128416

Title: Towards a real-time neural decoder for the cerebellum

Authors: *M. HEYDARI[1], K. A. CAI[2], M. FAKHARIAN[3], A. SHOUP[3], P. HAGE[4], R. SHADMEHR[5];
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Abstract: The input to the cerebellum via the mossy fibers represents a snapshot of activity in the entire nervous system including the cerebral cortex, brainstem, and spinal cord. Thus, if one could decode the activities of mossy fibers, one would have a mechanism with which to predict the state of the subject, including their future goals as well as their current motor commands. Here, we focused on mossy fibers that projected to the oculomotor vermis and used mathematical models to attempt to decode the activity and predict the state of the subject. We used silicon probes to record spiking activities from hundreds of mossy fibers in 4 marmoset monkeys from lobule VI and VII of the vermis of the cerebellum. Mossy fibers were identified via their "m" shaped spike waveforms that they produce at the glomerulus, exhibiting a negative after-wave associated with the postsynaptic response of the granule layer neurons. In a typical recording, we isolated 5-30 simultaneously recorded mossy fibers. We found that when a visual cue was presented, the location of that stimulus was encoded in retinal coordinates in the firing rates of a subset of mossy fibers. This encoding resembled that of the neurons in the superior colliculus, exhibiting a preferred location with respect to the fovea. When the subject made a saccade, another subset of mossy fibers responded with an activity pattern resembling those of the burst generators, encoding the motor commands in muscle coordinates of the eyes. Thus, the mossy fibers reported to the cerebellum both the goal that the subject had selected, and the motor commands that were being produced to accomplish that goal. We next built a forward model by fitting the data to a linear system consisting of a state update equation and an output equation. These equations transformed the measured displacement of the eyes and predicted mossy fiber activity. We then inverted these equations to predict the state of the eyes from the measured mossy fiber activities. This approach provided two advantages: first, we could build a model that included all mossy fibers, even ones recorded non-simultaneously. Second, the resulting model had interpretable matrices, rather than an uninterpretable neural network, that mapped mossy fiber activity to the state of the eye. As an alternative approach, we directly built an inverse model using a neural network that mapped mossy fiber activity to eye movements. In summary, in a visually guided saccade task, the mossy fibers reported to the cerebellum both the immediate goal of the subject, and the current motor commands being generated to accomplish that goal. Real-time decoding of this input provided the ability to predict the state of the subject.

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Poster

PSTR172: Cerebellum: Sensorimotorand Learning

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR172.08/H28

Topic: E.02. Cerebellum

Support: National Science Foundation (1825489 and 1825931)
National Institutes of Health (R01-HD040289)

Title: Leveraging Visual Feedback Control to Improve 3D Reaching Performance in Cerebellar Ataxia

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Abstract: Damage to the cerebellum can cause ataxia, a condition associated with impaired movement coordination. Prior research indicates that people with ataxia have only modest deficits in visuomotor feedback control (lower gain and greater time delay), allowing them to respond appropriately to visual perturbations.
Building upon this understanding, our study explores the potential of leveraging visual feedback control to improve movement performance in people with ataxia. Our prior work demonstrated that by modifying visual feedback, specifically by introducing an artificial acceleration-dependent term to the perceived hand position, we could reduce dysmetria in a constrained single-joint elbow movement among people with ataxia. However, it is known that ataxia is substantially more pronounced in unconstrained multi-joint movements. Here, we extend our investigation to unconstrained 3D arm movements. We studied 18 individuals with cerebellar ataxia and 16 age-matched controls performing reaching movements in a virtual reality environment, where we manipulated the visual feedback displayed to participants. Participants reached to targets with veridical feedback of a hand cursor or with feedback altered by the addition of an acceleration-dependent gain. Under positive gain conditions, the hand cursor was displayed leading (i.e., ahead of) the actual hand position during acceleration, and lagging the actual hand position during deceleration. The opposite relationship was displayed under negative gain conditions.
We found that altering visual feedback can manipulate dysmetria in unconstrained 3D reaching movements. There was a significant correlation between the artificial acceleration gain and the dysmetria along the reaching direction, with positive gains leading to more overshooting and negative gains resulting in more undershooting, evident in both control and ataxia groups. Notably, this altered visual feedback did not significantly impact other movement parameters such as max deviation, peak velocity, total reaching time, acceleration time, or deceleration time. Our findings indicate that altering self-movement feedback can enhance movement control performance for individuals with ataxia, even for unconstrained 3D reaching. As augmented reality technology becomes more widely accessible, these findings may inform the development of novel wearable interventions aimed at improving everyday movement performance for this population.

Disclosures: D. Cao: None. **K. Oh:** None. **N.J. Cowan:** None. **A.J. Bastian:** None.

Poster

PSTR172: Cerebellum: Sensorimotorand Learning

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Topic: E.02. Cerebellum

Support: NIH-K12 HD001399
Raynor Cerebellum Project

Title: The impact of maternal immune activation on the cerebellar susceptibility to neonatal hypoxia and long-term locomotor learning

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Abstract: Preterm labor is commonly preceded by *in-utero* exposure of the preterm cerebellum to maternal immune activation (MIA). Neonatal exposure to hypoxia (Hx) comes next due to lung immaturity and respiratory distress syndrome. An association has been established between MIA and Hx with long-term neurodevelopmental deficits, including motor incoordination and cerebellar ataxias. However, the mechanisms driving the impact of MIA and Hx on cerebellar injury is yet to be defined. Through the utilization of C57BL/6 mice (N=10-15/group), divided into 4 groups based on their in-utero exposure to MIA and neonatal Hx: Normoxia (Saline-Nx), MIA+Nx (LPS-Nx), Hypoxia-only (Saline-Hx), and MIA+Hx (DH), we assessed the long-term locomotor behavioral outcomes through a cerebellum-specific Erasmus Ladder paradigm. We then investigated the effects of the insults on cerebellum by utilizing histology, transcriptomics, immunohistochemistry, Seahorse bioenergetics and electron microscopy (EM) in neonatal period (P11) and adulthood (P30). LPS-Nx, Sal-Hx, and DH resulted in cerebellar-specific functional deficits in adulthood. However, DH mice exhibited more profound deficits in both adaptive locomotor and behavioral learning compared to the other groups. In addition, DH cerebellum demonstrated long-term neuropathological, transcriptomic (metabolism-related), and bioenergetic alterations. DH cerebellum demonstrated loss of PC contour and dark cytosolic inclusions in the EM which persisted in adulthood with decreased dendritic complexity. In contrast, the majority of Hx or MIA-only induced alterations resolved by adulthood. EM and Seahorse analysis of granule cells (GCs) and PCs displayed increasing mitochondrial injury in the DH group, whereas the injury occurring in single insult groups improved by adulthood. Combination of MIA with Hx results in behavioral, cellular, and molecular cerebellar alterations that persist until adulthood. Importantly, MIA shifts the cerebellum to non-mitochondrial metabolism rendering the neurons more susceptible to subsequent Hx-induced injury.

Disclosures: G. Simonti: None. **C. Byrd:** None. **G. Sanidas:** None. **J. Ghaemmaghami:** None. **N. Wolff:** None. **I. Koutroulis:** None. **V. Gallo:** None. **P. Kratimenos:** None.

Poster

PSTR172: Cerebellum: Sensorimotorand Learning

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Topic: E.02. Cerebellum

Support: NIH Grant 90084097

Title: The cerebellar Nodulus/Uvula integrates vestibular and proprioceptive inputs in a context independent manner during active and passive self-motion

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Abstract: Maintaining an accurate representation of our spatial orientation and self-motion is essential for stable gaze and posture as we navigate through the world. This sense of equilibrium arises from the integration of multimodal sensory and motor information, including vestibular, proprioceptive, and motor command signals. Where and how this integration occurs in the brain is an important area of investigation.
The Nodulus/Uvula (NU) is a major vestibular processing area that is the only region of the cerebellum that receives direct input from primary vestibular afferents. Prior work has focused on the integration of canal and otolith vestibular inputs in the NU to compute a representation of head orientation and motion relative to gravity. However, the NU also receives proprioceptive input, and lesions of this area produce severe impairments to both head and trunk postural control. Thus, we hypothesized the NU contributes to computing a representation of body movement relative to gravity during natural self-motion.
To test this possibility and understand how the NU encodes active self-motion, we recorded high-density extracellular activity from NU Purkinje cells in rhesus monkeys. We first examined the integration of vestibular and neck proprioceptive inputs during passive translations. We found that neck proprioceptive input shapes Purkinje cell responses to vestibular stimulation, which is essential for generating appropriate motor responses to stabilize the body. We then tested how NU Purkinje cells respond to active voluntary head translations. Surprisingly, we did not see gating of responses during active head movements. Furthermore, when we unexpectedly blocked active head movements such that an efference copy was sent but no motion occurred, we found Purkinje cell activity was not modulated by motor commands. Finally, we applied passive motion stimuli concurrently during active head movements and found that NU Purkinje cells robustly encoded the total head motion, consistent with providing a veridical representation of both active and passive self-motion.
Taken together, these findings demonstrate that the NU transforms streams of sensory feedback to faithfully represent head and body motion relative to gravity independent of the behavioral context.

Disclosures: R.L. Mildren: None. **K.E. Cullen:** None.

Poster

PSTR172: Cerebellum: Sensorimotorand Learning

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Topic: E.02. Cerebellum

Support: Life Science Research Foundation
F31-NS113395
NS114430

Title: Exploring the role of the cerebellar nucleoolivary neurons in the control of voluntary movement

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Abstract: Cerebellar plasticity resembles a supervised learning rule. In many parts of the cerebellum, inferior olivary neurons (IO) report sensory prediction errors that occur during motor errors or sensory perturbations. Yet how IO computes discrepancies between predicted and experienced conditions is unknown. The clearest hypotheses suggest the computation involves comparisons between inhibitory inputs from the cerebellum via nucleoolivary neurons and excitatory sensory afferents. While nucleoolivary inhibition plays a role in extinguishing learned associations and are hypothesized to provide sensory predictions to the IO, almost nothing is known about their activity patterns during motor behaviors.
We aimed to test the role of nucleoolivary neurons in reaching movements. We first developed an opto-tagging approach that allows for nucleoolivary identification in vivo using high-density recording, as well as optogenetic manipulation in behaving mice performing a single pellet reach task. In Vgat-Cre mice, we used an intersectional approach to express ChR2 in IO-projecting interposed neurons (AAVretro-flpo and AAV-DIO-ConFon-ChR2 injected into IO and interposed, respectively) or AAVretro-DIO-GtACR2, injected into IO. We identified 52 units that were responsive to light stimulation and examined their activity during motor performance. Using hierarchical clustering on the neuronal activity patterns during reach, we identified four general response patterns. Interestingly, we found that ~35% of nucleoolivary neurons showed prominent and selective firing rate increases upon successful retrieval of the food pellet. Consistent with this pattern of activity, we found that the probability of complex spikes in Purkinje neurons was higher in trials where the mouse failed to retrieve the pellet. These observations are not crisply in alignment with a sensory prediction model of nucleoolivary firing, but follow-up experiments will continue to probe these response profiles to distinguish sensory predictions from outcome reports, along with assessing the functional role of these neurons in sculpting behavior.

Disclosures: A. Nashef: None. **D. Calame:** None. **A.L. Person:** None.

Poster

PSTR172: Cerebellum: Sensorimotorand Learning

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Topic: E.02. Cerebellum

Support: JSPS 24H00064
JST-CREST JPMJCR23P3

Title: Cerebellar learning underlies neural entrainment to rhythmic visual stimuli in monkeys

Authors: *M. TANAKA, K.-I. OKADA;
Physiol., Hokkaido Univ., Sapporo, Japan

Abstract: The cerebellum is involved in both rhythmic movement and rhythmic perception. We have previously shown that neurons in the cerebellar dentate nucleus gradually synchronize their activity to periodically presented repetitive visual stimulus (RVS) in the absence of movement. Since the dentate nucleus receives GABAergic projections from Purkinje cells (PCs) in the cerebellar cortex and the interaction between simple spikes (SSs) and complex spikes (CSs) in PC is central to cerebellar learning, we examined PC activity to understand how rhythmic neuronal activity is generated. Animals were trained to respond to the omission or color change of isochronically presented RVS, depending on color of the fixation point on each trial. Detection of stimulus omissions required temporal prediction, whereas detection of color change did not. We have so far analyzed the periodic activity of 83 well-isolated PCs recorded from the crus lobules in three monkeys. Neurons were classified into 3 groups based on the time course of SS and CS activities during stimulus repetitions at 400 ms intervals. Cluster #1 (40%, N = 33) showed a SS peak around 300 ms after each stimulus and exhibited a transient CS activity for RVS but not for stimulus omission. Cluster #2 (25%, N = 21) showed an early SS peak at 100 ms and often exhibited predictive CS around the time of RVS and omission. Cluster #3 (35%, N = 29) showed a clear SS peak around the time of RVS, but no clear CS response to RVS. Clusters #1 & 2 gradually decreased their baseline SS firing during stimulus repetition, likely because their CSs tended to occur at specific times as the repetition progressed. In all clusters, the magnitude of periodic SS activity was greatly reduced in the color change condition compared to the omission condition, suggesting that neuronal activity reflects temporal prediction. Importantly, CS activity in clusters #1 & 2 also decreased during color detection, indicating that CS generation is highly context-dependent. As expected, CS-triggered averaging of SS activity showed a transient pause in SS activity in all PCs. Clusters #1 & 2 exhibited two additional decreases in SS activity, one just before the CS and one after the stimulus cycle(s), suggesting that SS activity may control CS probability and that CS may induce time-specific learning in SS activity. Since the difference in SS activity between omission and color change conditions could be explained by the difference in CS activity and the relationship between spikes, the entrained SS activity to rhythm might be shaped by cerebellar learning through the context-dependent occurrence of CSs.

Disclosures: M. Tanaka: None. **K. Okada:** None.

Poster

PSTR172: Cerebellum: Sensorimotorand Learning

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Topic: E.02. Cerebellum

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Simons Foundation 717106
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FCT PD/BD/128291/2017

Title: Locomotor learning under climbing fiber control

Authors: A. I. GONÇALVES[1], A. GEMINIANI[1], H. G. MARQUES[1], F. COSTANTINO COSTABILE[1], T. N. SILVA[1], M. KRUSE[1], ***M. R. CAREY**[2];
[1]Neurosci., Champalimaud Fndn., Lisboa, Portugal; [2]Champalimaud Fndn., Lisboa, Portugal

Abstract: The cerebellum plays a key role in motor learning, adapting movements in response to perturbations or errors. We recently demonstrated that mice exhibit locomotor adaptation over several minutes of exposure to a split-belt treadmill that controls the speed under each side of the body independently (Darmohray et al., 2019). In mice, as in humans, split-belt adaptation is cerebellum-dependent and reflects learned changes in interlimb symmetry. The cerebellar circuit mechanisms underlying this form of motor adaptation are unknown. For simple tasks like classical eyeblink conditioning, climbing fibers originating in the inferior olive and projecting to Purkinje cells in the cerebellar cortex provide neural instructive signals sufficient to drive learning. For complex whole-body behaviors like locomotion, however, it is not known what role climbing fibers play. Here, we used in vivo calcium imaging, as well as optogenetics combined with real-time tracking of limb kinematics, to test the hypothesis that climbing fiber modulation can induce learned changes in gait symmetry. We find that unilateral optogenetic climbing fiber inhibition or activation, precisely locked to specific phases of the locomotor cycle, is sufficient to drive bidirectional, learned changes in interlimb symmetry, depending on the phase of the locomotor cycle in which it occurs. Moreover, in vivo calcium imaging from cerebellar Purkinje cells reveals functionally distinct ensembles of neurons encoding different stages of the gait cycle, along with signals related to body acceleration and locomotor asymmetries, which could be used to drive adaptation under physiological conditions. Taken together, these results reveal powerful climbing fiber control of learned changes in interlimb coordination, opening the door to potential neurotherapeutics for gait asymmetries.

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Poster

PSTR172: Cerebellum: Sensorimotorand Learning

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Topic: E.02. Cerebellum

Support: JSPS KAKENHI Grant Number JP22J23214
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Title: Cerebellar spiking network model as a reinforcement learning machine

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Abstract: The cerebellum has been considered to perform error-based supervised learning via long-term depression, driven by climbing fibers, at synapses between parallel fibers and Purkinje cells. Recent anatomical and physiological studies have demonstrated multiple synaptic plasticity mechanisms other than the long-term depression at parallel fiber synapses within the cerebellum. These distributed plasticity mechanisms could enhance learning capabilities of the cerebellum. We have proposed that such distributed plasticity mechanisms allow the cerebellar circuit to perform reinforcement learning rather than conventional supervised learning. However, its detailed spike-based implementation is still missing. In this research, we implemented a cerebellar spiking network as an actor-critic model, based on the known anatomical properties of the cerebellum. In our implementation, Purkinje cells act as the actor, and calculate the policy to let neurons in the deep cerebellar nuclei emit appropriate actions, whereas stellate cells act as the critic, and calculate the state-value that represents the values of the present state. Basket cells assist Purkinje cells in selecting actions. Parallel fibers convey state information to both Purkinje cells and molecular layer interneurons, while climbing fibers deliver negative rewards. Additionally, long-term synaptic plasticity at both parallel fiber-Purkinje cell and parallel fiber-stellate cell synapses are implemented to update internal parameters. To evaluate the learning capability of our model, we conducted the linear track task to evaluate whether the agent could calculate a state value, and the mountain car task that is a simple learning benchmark. We confirmed that our model successfully solved them. Furthermore, we conducted simulation of the delay eyeblink conditioning, which is a standard cerebellum-dependent motor learning task, to examine whether the cerebellar reinforcement learning could provide consistent results with neurophysiological behaviors. These results support the concept of cerebellar reinforcement learning rather than supervised learning. Further studies on the synergistic roles of multiple synaptic plasticity mechanisms within the cerebellum will be necessary to gain our understanding of cerebellar computation and learning.

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Poster

PSTR172: Cerebellum: Sensorimotorand Learning

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Topic: E.02. Cerebellum

Support: MRC NRF-2018R1A5A2025964

Title: The role of presynaptic plasticity at parallel fiber-to-Purkinje cell synapse on optokinetic reflex

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Abstract: Long term depression (LTD) at parallel fiber-to-Purkinje cell (PF-PC) synapses is essential for driving a proper optokinetic response (OKR). There have been numerous studies about the relation between synaptic LTD and intrinsic PC LTD after cerebellar motor learning. Moreover, it has been suggested that memory is acquired in the cerebellar cortex and is transferred to the vestibular nucleus for the memory consolidation phase. However, the synaptic mechanism occurred at PF-PC has not been completely identified yet. Until now, Purkinje cells have long been the major focus for the cerebellar motor learning, as their depressed activity is the main driving force for OKR gain-UP. On the other hand, although they also participate in the cerebellar learning circuit, the role of PFs, which are the axons of granule cells (GC), has remained obscure. In addition, defining the site of presynaptic plasticity occurrence is also necessary. To be specific, GC soma may act as a primary stage for signal modification, or it is also possible that PFs uniquely integrate and modify the inputs. Moreover, it also remains unidentified that how presynaptic activity affects postsynaptic activity, in this case, how GC activity affects PC activity. Therefore, in this study, we take advantage of patch clamp recording to unravel the synaptic mechanism at PF-PC synapse after OKR training and GC manipulation to demonstrate the effect of GC activity on behavioral result. Likewise, we observe distinct changes in training-dependent synaptic plasticity at three time points (right after, 1hour, and 24 hours) and the significant role of GCs during OKR for proper expression of learning. This study validates the function of GC transmission during the cerebellar motor learning and clarifies the direction of the training-dependent synaptic plasticity.

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

Poster

PSTR172: Cerebellum: Sensorimotorand Learning

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NINDS #R01 NS085136
NINDS #R01 NS088257

Title: Reduced cerebellar rhythm by climbing fiber denervation contributes to motor rhythm deficits in ataxia pathophysiology

Authors: C.-C. LIN[1], K. FANG[2], I. BALBO[3], A. KUMAR[3], P. L. FAUST[4], E. D. LOUIS[5], S.-H. KUO[3], ***M.-K. PAN**[2];
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Abstract: Cerebellar ataxia arises from a variety of genetic and non-genetic disorders, yet these disorders exhibit the same clinical features of ataxia: involuntary movements that lose precision and motor rhythm. In this work, we investigated the underlying common pathophysiology for motor rhythm loss. By investigating human cerebellar pathology in patients with spinocerebellar ataxia (SCA) type 1,2, 6, and multiple system atrophy, we found two shared pathophysiology: Purkinje cell (PC) degeneration and climbing fiber (CF) to PC denervation. To model the consequence of the loss of CF-PC synaptic activity, we optogenetically or pharmacologically silenced CF-to-PC synaptic activities in wild-type mice, which showed motor dysfunction in balanced beam, video-based gait analysis, and reaching movements as typical readouts for loss of motor precision in ataxia. Notably, motor rhythm loss was also identified by a step-phase analysis of gait and kinematic tracking using a pressure-sensing force plate. The motor rhythm loss is linked to the loss of cerebellar oscillations with the same frequency range at 5-20 Hz. The SCA1 mouse model also revealed the same motor precision and rhythm deficits. Next, we investigated the roles of PC loss by injecting cre-dependent viral constructs carrying diphtheria toxin into *PCP-2* mice, which lesions more than half of the PCs in the motor cerebellum. While the mice developed motor precision deficits, their motor rhythms and cerebellar oscillations were preserved. This finding strongly suggests that motor precision and motor rhythm are regulated by two overlapping mechanisms. The CF dysfunction causes cerebellar rhythm loss and motor deficits in both precision and rhythm, while the PC loss only causes motor imprecision. We also investigated whether patients with cerebellar ataxia also developed similar cerebellar rhythm dysfunctions. Patients with genetic or non-genetic cerebellar ataxia all developed cerebellar rhythm loss that correlated to their clinical severity. Notably, patients with exclusive inferior olive pathology also developed ataxia and severe cerebellar rhythm loss. Finally, we tested a

therapeutic strategy to augment CF function with chemogenetics in the SCA1 mouse model. We found this approach not only resulted in enhanced cerebellar rhythm but also improved ataxia-like motor deficits. Our results indicate that CF-PC synaptic denervation could be a common pathophysiology that leads to cerebellar rhythm loss and, thus, motor rhythm deficits in ataxia. CF-based therapy, even under the pathological background of significant PC loss, can rescue the motor rhythm and improve motor function.

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Poster

PSTR173: Basal Ganglia: Physiology and Plasticity

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MOST Grant 110-2320-B-A49A-532-MY3 (F.-C.L.)

Title: Nolz1 gene expression in dopaminergic neurons of the midbrain regulates motor learning

Authors: S.-Y. CHEN, *F.-C. LIU;
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Abstract: Dopamine neurons in the substantia nigra pars compacta (SNc) project their axons to the dorsal striatum to form the mesostriatal pathway, which is critical for movement and motor learning. Nolz1 (Znf503/Zfp503), a zinc finger-containing transcriptional regulator, is expressed in developing midbrain dopamine neurons. Nolz1 immunoreactivity is not detectable in developing SNc dopamine neurons until postnatal day 4 (P4) and was persistently expressed in P14 and adult SNc dopamine neurons. Here, we investigated the Nolz1 function in developing SNc dopamine neurons. In the open-field locomotion assay, the total travel distance and time in the horizontal and vertical planes of Dat-Cre;Nolz1fl/fl;EGFP conditional knockout (cKO) mice were not different from that of control Dat-Cre;Nolz1fl/+;EGFP mice. In the accelerated speed rotarod learning task, mice were subjected to a 3-day training protocol with 4 trials each day. Nolz1 cKO mice exhibited a marked increase in the performance of accelerated speed rotarod learning, as the latency to fall was increased as early as the first day of training. Nolz1 cKO and control mice exhibited similar performance levels in the constant speed rotarod task. These results suggest that Nolz1 deletion in dopamine neurons does not affect sensorimotor function, but it could improve motor learning. The density of TH-immunoreactive and EGFP-labeled dopaminergic fibers in the striatum of P14 cKO mice appeared to be not different from that of control mice. We further examined the physiological activity of SNc dopamine neurons in Nolz1 cKO mice. We current clamped the patched dopamine neurons with 1 sec injection of

hyperpolarizing currents to hyperpolarize the cell membrance potential initially to −120 mV. There was a significant decrease in Ih sag in SNc cKO dopamine neurons compared to SNc control dopamine neurons. A trend of decreases in the frequency of pacemaker activity was observed in SNc cKO dopamine neurons compared to controls. No changes in rebound delay, AHP, action potential amplitude, slope of the pacemaker activity, and cell soma size were found between SNc cKO and control dopamine neurons. Therefore, the deletion of Nolz1 induces a mild but significant change in the electrophysiological activity of SNc dopamine neurons. The findings that Nolz1 knockout in dopamine neurons enhanced motor learning are intriguing, because most gene knockout mice exhibit impairment rather than enhancement in neurological functions. Our study of Nolz1 cKO mice helps decipher the genetic basis of dopamine-mediated motor learning in mesostriatal circuits and provides insight into dopamine-related neurological and neuropsychiatric diseases.

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Poster

PSTR173: Basal Ganglia: Physiology and Plasticity

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Program #/Poster #: PSTR173.02/H38

Topic: E.03. Basal Ganglia

Title: Differential K_v4 channel function in projection-defined dopamine VTA neurons

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Abstract: Fast-inactivating K_v4 channels are key regulators of pacemaking frequency in dopamine (DA) midbrain neurons. They also contribute to post-inhibitory rebound excitability. While previous studies have characterized K_v4 channel subunit expression, biophysical properties and cellular functions in identified substantia nigra (SN) and ventral tegmental area (VTA) DA neurons, the contribution of K_v4 in axonal-projection defined DA VTA subpopulations is still unknown.To investigate the biophysical K_v4 properties in projection-defined VTA DA populations, we combined axonal retrograde tracing with nucleated outside-out patch clamp recordings of labelled VTA DA neurons. The steady-state activation curve of K_v4 channels was significantly shifted to more depolarized membrane potentials in VTA DA neurons projecting to the medial shell of nucleus accumbens ($V_{50\text{-}A}$ mNAc: -1.4 ± 6.0 mV; n=14, N=3) compared to those projecting to lateral shell ($V_{50\text{-}A}$ lNAc: -11.7 ± 3.5 mV; n=15, N=3) or to core projecting neurons ($V_{50\text{-}A}$ cNAc: -14.8 ± 1.7 mV; n=15, N=3). Similar differences in voltage range were observed for the steady-state inactivation ranges with mNAc VTA DA neurons again most negative ($V_{50\text{-}I}$ mNAc: -88.6 ± 2.4 mV; n=15, N=3) compared to lateral shell projecting ($V_{50\text{-}I}$ lNAc: -72.2 ± 2.3 mV; n=15, N=3) and core projecting VTA DA neurons ($V_{50\text{-}I}$ cNAc: -80.1 ± 7.8 mV; n=15, N=3). Taken together, these differences in K_v4 gating properties suggested

functional differences regarding K_v4-mediated pacemaker control. To test these predictions, we recorded the spontaneous activity of retrogradely traced VTA DA neurons before and after wash-in of the specific K_v4-channel inhibitor AmmTX3 (1 µM). Consistent with the differences in biophysical properties, K_v4 inhibition increased the mean firing frequency in lNAc VTA DA neurons by about 60% (1.61-fold increase; n=27, N=3), while it had no effect on discharge rates in mNAc VTA DA neurons (0.98-fold; n=16, N=2).These results demonstrate a differential K_v4 channel function for projection-defined VTA DA subpopulations. Like in SN DA neurons, K_v4 channels control pacemaker frequency in lNAc VTA cells. In contrast, in mNAc VTA DA neurons K_v4 channels appear to operate only in the subthreshold voltage range. Using the new biophysical K_v4 data set, we are currently refining our subtype-specific computational DA VTA models to predict the functional implications of K_v4 diversity for *in vivo* like states. Also, to test the model's predictions experimentally in the intact brain, we are currently establishing recordings of projection-defined VTA DA neurons in freely moving mice.

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Poster

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Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR173.03/H39

Topic: E.03. Basal Ganglia

Title: In vivo awake Neuropixels recordings of putative dopamine neurons in the substantia nigra reveal distinct firing patterns related to movement

Authors: *Y. LU, P. VOGEL, J. ROEPER;
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Abstract: Dopamine (DA) neurons in the substantia nigra (SN) are associated with a wide range of key brain functions such as cognition, motor control and reward processing. However, dysfunctions of the nigrostriatal DA system contribute to movement disorders such as Parkinson disease. To investigate the role of SN DA neurons in motor control, we conducted *in vivo* extracellular high-density Neuropixels recordings of SN neurons in awake, head-fixed mice engaged in self-paced running on a treadmill. We recorded putative SN DA neuron activity during both onset and cessation of self-paced locomotion, alongside simultaneous movement tracking using a camera and a position sensor. In total, we recorded the activity of ~3000 units across the span of the probe (30 sessions, ~100 units/session, N=11). Among these, 48% (n=1500) responded to movement initiation by significantly increasing (n=882, +6.0 ± 6.3 Hz) or decreasing (n=618, −3.8 ± 4.4 Hz) their firing rates in a relevant time window (-2 to -1s vs. 0 to +1s). A similar proportion of neurons (42%) responded to movement stop by significantly increasing (n=579, +4.8 ± 6.3 Hz) or decreasing (n=717, −5.4 ± 6.0 Hz) their firing rates. Moreover, 64% of all units (n=1988) exhibited firing rate changes to both running start and stop,

suggesting their involvement in both processes. As a first attempt, we identified a subset of 251 neurons (8%) as plausible candidates for DA SN neurons based on their mean firing rates (≤ 10Hz) and spike waveform shapes (spike-half-width ≥ 200 μs). Recording tracks in the SN were identified by DiI together with post-hoc TH staining. Forty-four percent (n=110) of these putative DA SN neurons exhibited robust responses to spontaneous running start. Twenty-six percent (n=65, +3.0 ± 3.5 Hz) significantly increased and 18% (n=45, −1.4 ± 1.2 Hz) decreased their mean firing rates. In addition, 34% (n=85) of all putative DA SN neurons responded with significant activity changes to the cessation of running. About half of them decreased (n=41, −2.9 ± 5.8 Hz), while the other half increased their firing rates (n=44, +1.5 ± 1.0 Hz) upon running stop. Furthermore, 56% (n=141) putative DA SN neurons responded to both movement start and stop. Overall, the initial results of our head-fixed study are in accordance with previous studies using freely moving mice in demonstrating distinctive firing patterns in relation to the self-paced movements among DA SN neurons (da Silva et al., 2018). The functional relevance of these firing patterns has not been fully resolved. They might serve as START/STOP signals for ongoing motor action and/or as reinforcement for future actions. As a next step, we aim to improve the identification of DA SN neurons.

Disclosures: Y. Lu: None. **P. Vogel:** None. **J. Roeper:** None.

Poster

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Topic: E.03. Basal Ganglia

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Title: Sex-specific effects of aerobic exercise on motor skill learning and basal ganglia neural properties

Authors: *V. J. LEWITUS[1], L. RUSS[1], C. B. SCOTT[2], G. SHAUTIDZE[2], O. KRUSZEWSKI[2], Z. COLON[2], M. CROOM[3], R. C. EVANS[2];
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Abstract: Aerobic exercise is known to have beneficial effects on motor skill learning, making it a low-cost therapy against the effects of Parkinson's disease and normal aging-related motor deficits. Exercise promotes changes in multiple areas of the brain, including major regions affected by Parkinson's disease such as the substantia nigra pars compacta (SNc), the dorsal striatum, and the pedunculopontine nucleus (PPN). These effects include upregulating the release of brain-derived neurotrophic factor and dopamine in the dorsal striatum and inducing neurotransmitter switching in the PPN. Estrogen, which can pass through the blood-brain barrier,

is also known to have neuronal effects in the basal ganglia. Estrogen is also found to boost the beneficial effects of exercise on cardiovascular health. However, there is a gap in our knowledge about how these two factors (estrogen and exercise) interact to affect basal ganglia cellular physiology and motor skill learning. To address these knowledge gaps, we used behavioral techniques, *ex vivo* whole-cell patch clamp recording, and confocal imaging in male and female mice to determine: (1) the effect of voluntary aerobic exercise on motor skill learning on an accelerating rotarod task, and (2) changes in synaptic, intrinsic, and morphological properties of three key neuron populations in extended basal ganglia circuitry: PPN cholinergic neurons, SNc dopaminergic neurons, and dorsal striatal cholinergic interneurons. We find that one week of voluntary exercise enhances motor skill acquisition in female, but not male mice. We also find neuronal properties such as intrinsic excitability, firing rate, rebound activity, and response to synaptic input are differentially altered by exercise in male and female mice. These findings show that exercise affects motor learning and basal ganglia neural characteristics in a sex-specific way.

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Poster

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Topic: E.03. Basal Ganglia

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Title: Reciprocal firing rate changes of dopamine substantia nigra neurons associated with self-paced movement initiation and termination

Authors: *D. SCHENKEL[1,2], P. VOGEL[2], M. KUHN[3], N. HAMMER[2], S. BETZ[2], G. SCHNEIDER[3], J. ROEPER[2];
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Abstract: Dopamine substantia nigra (DA SN) neurons are important for voluntary movement and their degeneration in Parkinson Disease leads to motor impairments. We performed chronic multi-electrode recordings of pharmacologically (i.e. >50% inhibition by systemic D2R agonist quinpirole) identified DA SN neurons in awake freely-moving, adult (8-16 weeks) male C57Bl/6N mice, while simultaneously tracking their movements in open field. Our data set of n=59 (N=16) putative DA SN neurons was in accordance with the results by Da Silva et al (2018): About 30% of DA SN neurons (n=17/59) transiently increased their firing rates (baseline to max: +5.3 ± 4.8Hz, mean ± SD) in a time window (-500 - 0 ms) before initiation of self-paced movements. A similar fraction (n=18/59) of DA SN neurons transiently decreased their firing

rate (baseline to min: -3.6 ± 1.2Hz, mean ± SD) shortly after initiation of self-paced movement (time window 0 - 500ms). Reconstruction of recording sites showed that DA neurons with transient rate reductions were predominantly found in the medial SN (n=11/22). In contrast, the DA neurons with transient rate increases were more prominent in central SN (n=13/30). As we have previously shown that anatomical position of DA cell bodies across the SN and axonal projection targets were associated (Farassat et al 2019), we aimed to record from projections-defined DA SN subpopulations. Based on our recently published protocol (Hammer et al 2024), we carried out AA9-based retrograde axonal tracing in 7 adult DAT-Cre mice (3 male, 4 female, aged 8 - 20 weeks) to selectively express the inhibitory DREADD hM4D in either dorso-lateral striatum (DLS) or dorso-medial striatum (DMS) projecting DA SN neurons. For identification of projection-specific DA SN neurons, we systemically applied the agonist DCZ (100µg/kg). DA SN neurons reducing their mean firing rate >40% within 30min post-application were considered positive for the respective striatal projections (DLS or DMS). So far, we identified n = 8 (N = 3) DLS-projecting and n = 15 (N = 4) DMS-projecting DA neurons recorded in the medial SN. We found that half of DLS-projecting neurons (n=4/8) and a similar proportion of DMS-projecting neurons (n = 8/15) transiently increased their firing rate prior to movement initiation (DLS: +162,27 ± 81,81% mean ± SD / DMS: +104,67 ± 108,67% mean ± SD). In contrast, only a minority of chemotagged DA SN neurons transiently decreased their firing rate shortly after movement initiation (DMS: 1/15, DLS: 1/8). Therefore, we are currently exploring the contribution of DA neurons in the medial SN with axonal projection to the ventral striatum.

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Poster

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Program #/Poster #: PSTR173.06/I2

Topic: E.03. Basal Ganglia

Support: NIH R01 MH-109471
NIH P30 ES-025128

Title: Medium spiny neuron excitability is greater during early development in female and male rats

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Abstract: The striatal brain regions are key for important processes like premotor function, habituation, and motivated behaviors. When these regions are disrupted, disorders such as Parkinson’s Disease and drug addiction can result. The medium spiny neuron (MSN) is a key neuron type that serves as the output neuron of the striatal brain regions. Though the MSN is

present in striatal regions such as the caudate putamen (CP) and the nucleus accumbens core (AcbC), it is unclear whether the physiological properties of the MSN differ between each of those regions across sex and developmental period. Very few studies of the striatum differentiate between region, sex and developmental period even though there are known sex and developmental differences in relevant functions. Thus, we tested the hypothesis that MSN electrophysiological properties differ by striatal brain region, sex, and developmental period. Meta-analysis included four previously collected datasets, each capturing whole-cell patch clamp recordings in tissue from adult Sprague-Dawley female and male rats from the CP and AcbC during the pre- and post-pubertal developmental periods. Excitability was assessed via a battery of biophysical attributes describing the input-output properties of the MSN. We found that MSN excitability differed by all three variable groups, though developmental period exerted the most influence. For example, rheobase, the amount of current needed to elicit an action potential, differed significantly in the CP between prepubertal and adult rats. Overall, we quantified a robust age-linked difference in neuronal excitability wherein prepubertal rat MSNs were more excitable than those of adult rats with select modulations by region and sex. These findings indicate that MSN electrophysiological properties, particularly those implicated in excitability, exhibit complex regional, developmental, and sex-based specificity not well accounted for by existing models.

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Poster

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Topic: E.03. Basal Ganglia

Support: R01 NS041280
R01 NS121174
ASAP 020600

Title: Glutamatergic Subthalamic Innervation and Patterning of Substantia Nigra Dopamine Neuron Subtypes

Authors: *A. HUNTER[1], J. F. ATHERTON[2], M. DATUNASHVILI[3], M. D. BEVAN[2];
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Abstract: Objective:
The aim of this project was to determine whether the subthalamic nucleus (STN) differentially innervates substantia nigra dopaminergic (SN DAergic) neuron subtypes.
Methods:
AAV-DJ hSyn FLEX-mGFP-2A-Synaptophysin-mRuby was injected into the STN of adult

Pitx2-Cre mice to label axons and putative synapses. 3 weeks later mice were perfuse-fixed and sections through the SN were immunolabeled for PSD-95 and tyrosine hydroxylase (TH). Putative contacts onto SN pars compacta (SNc) and pars reticulata (SNr) DA neurons were visualized using confocal microscopy and counted using stereological analysis.
Injections of AAV-DJ hSyn-Con/Foff-hChR2(H134R)-eYFP-WPRE and AAV-8-EF1a-fDIO-TdTomato-WPRE centered on the STN and SN, respectively of Pitx2-Cre or Vglut2-Cre, DAT2A-Flpo mice were employed for optogenetic stimulation of STN inputs and cell-attached and/or whole-cell patch clamp recording of identified SN DAergic neurons in ex vivo brain slices, respectively.
Results:
STN neurons labeled 3.2-5.3 million synaptic terminals in the SN, with ~10% in the SNc and ~90% in the SNr. Approximately 13% of all STN inputs were to TH+ immunoreactive DA neurons, with ~95% of those putative contacts apposed to distal dendrites.
Relative to baseline activity, 20 Hz stimulation of STN inputs for 250 ms elevated firing by 0.85, 0-1.6 z-scores. STN-recipient DA neurons were located throughout the SN including its ventral and dorsal tiers.
Conclusions:
Distal glutamatergic STN inputs modestly pattern the activity of both susceptible and resilient DA subtypes throughout the SN.

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Poster

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Program #/Poster #: PSTR173.08/I4

Topic: E.03. Basal Ganglia

Support: NIH Grant R01 NS119520

Title: Building an anatomically detailed subthalamic nucleus neuron model through optimization

Authors: *H. CHEN[1], M. NOOR[1], C. S. BINGHAM[2], C. C. MCINTYRE[1];
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Abstract: Deep brain stimulation (DBS) targeting the subthalamic nucleus (STN) is an effective therapy for Parkinson's disease; however, the precise mechanisms underlying the therapy remain unclear. Detailed multi-compartment computational models of STN neurons are often used to study how DBS electric fields modulate the neurons. However, existing STN neuron models possess certain limitations in their biophysical accuracy. Therefore, our study aimed to enhance a detailed STN neuron model originally created by Gillies & Willshaw [2006]. Our design objectives included incorporating an axon into the neuron structure, updating ion channel

distributions based on the experimental literature, and refining biophysical parameters to mirror established electrophysiological characteristics of rodent STN neurons. We found that the addition of an axon to the STN neuron model yielded significant alterations in firing behavior. Therefore, we employed a genetic algorithm to fine-tune the biophysical parameters of the model. The optimization process involved systematically adjusting parameters to achieve a close match with experimental observations of STN neuron electrophysiology. The optimized model exhibited spontaneous firing, action potential shape, hyperpolarization response, and frequency-current curve that aligned well with experimental recordings. We also assessed the general compatibility of the updated biophysics by applying them to multiple distinct STN neuron morphologies derived from 3D anatomical reconstructions. Although morphologies impacted firing behavior to some extent, the updated biophysics reliably preserved key electrophysiological features, highlighting the model's versatility. It also indicates the further need of examining the relationship between morphological features and electrophysiological characteristics. In conclusion, our updated STN neuron model offers an improved representation of STN neuron characteristics. We propose that this tool will be relevant for use in the analysis of STN firing behavior during intrinsic synaptic influences, or extrinsic electrical stimulation from intracellular or extracellular sources. The addition of the axon and the model's versatility also allows the creation of a population of STN neurons with various morphologies which opens up the possibility of capturing essential aspects of real-world neuron population behavior that were previously unaccounted for. The optimization algorithm and cost function designed for this project could also pave the way for broader applications in computational neuroscience.

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Poster

PSTR173: Basal Ganglia: Physiology and Plasticity

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Topic: E.03. Basal Ganglia

Support: R01MH125835

Title: Revealing cell-type specific striatum-wide representations of stimuli motivational salience, relative value and location during value-based action selection with multi-fiber arrays

Authors: *Z. ZHANG[1], Y. DING[1], M. HOWE[2], M.-A. T. VU[3], D. A. BOAS[4], T. M. OTCHY[5]; [1]Boston Univ., Boston, MA; [2]Boston Univ., Needham, MA; [3]Psychological & Brain Sci., Boston Univ., Boston, MA; [4]Biomed. Engin., Boston Univ., Boston, MA; [5]Harvard Univ. Press, Boston, MA

Abstract: Adaptive behavior requires animals to evaluate and localize stimuli to direct actions accurately. The striatum is a crucial structure regulating these processes, as it can integrate and filter converging external sensory and internal value inputs to regulate appropriate action strategies. Striatum-dependent action control is facilitated by the concurrent activation of the direct and indirect pathway spiny projection neurons. Past studies have suggested that different striatal subregions make distinct, simultaneous contributions to stimulus representation and action. However, due to technical limitations in simultaneously recording cell-type specific neural activity across the striatum, it remains unclear how dSPN and iSPN signals in various striatal regions support different aspects of stimulus evaluation, localization, and approach. We developed a novel multi-optical-fiber array photometry approach to record neural activity at over 70 locations simultaneously across the entire 3-D volume of the striatum. We applied this technology to measure striatum-wide population calcium signals in head-fixed mice from dSPNs and iSPNs during tasks requiring evaluation, localization, and movements toward or away from visual cues. To isolate representations of cue motivational saliency, relative value and spatial location, mice were initially presented with either a positive Cs+ cue (water droplet), a negative Cs+ cue (airpuff) or a Cs- cue(neutral), presented at different locations in their visual field. To establish dynamics related to the execution of corresponding actions, mice were then trained to execute appropriate responses such as approaching, avoiding or neglecting the cue. Preliminary results indicate that representations of stimulus location, relative value, and actions vary across cell types and striatum space. Calcium signals in the anterior ventral striatum(aVS) and posterior dorsal medial striatum(pDMS) were sensitive to the motivational salience or relative value of the stimuli. Stimulus encoding was similar between dSPNs and iSPNs in the aVS, but representations were divergent in the pDMS. Moreover, stimulus location was represented in the aDMS and pDMS but not the aVS. Furthermore, SPN calcium signals in the pDMS had the highest correlations with the changes in directional velocity. The overlapping representations of motivational saliency or value, stimulus location, and locomotion changes suggest that the pDMS may be a crucial region to integrate cue information to generate learned, cell-type specific activity patterns that influence proper orienting during value-based action selection.

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Poster

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Topic: E.03. Basal Ganglia

Support: H2020 ERC 755745

Title: Eye movement and reward signals in optogenetically identified cortico-striatal neurons

Authors: *A. LIXENBERG[1], Y. HENSHKE[2], T. KREISEL[1], I. GOSHEN[3], E. LOTTEM[2], M. JOSHUA[4];
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Abstract: A common perspective on processing in the basal ganglia suggests that corticostriatal inputs interact with dopaminergic reward signals to drive reward-dependent behaviors such as decision-making or enhancing movement vigor. Underlying this view is an assumption that cortical inputs do not contain the reward-related information needed to guide movements. Alternatively, reward signals from the cortex could already be present in the cortical inputs to the basal ganglia. To test the content of the signal conveyed from the cortex to the basal ganglia, we combined optogenetics and electrophysiological recordings in primates alongside a reward-dependent eye movement tasks. We injected AAV-retro-ChR2 into the eye movement areas of the striatum. We then identified projection neurons from the frontal eye field to the striatum based on their short latency (< 5 ms) response to light stimulation. We then recorded the activity of these identified projection neurons (N = 110), and other neurons that did not respond to the light (N = 405) but were often recorded with the same optetrode, in tasks that included reward size manipulations and reward-based decisions. We found that corticostriatal inputs to the basal ganglia already contain reward-related signals and decision-making variables. In fact, reward expectation signals were stronger in the corticostriatal projection neurons, as more neurons with larger reward expectation signals were identified in this group. The eye movement signals were also partially dissociated in these pathways. The overall activity of the projection neuron decreases firing rate around movement, whereas the population average of unidentified neurons increased rate around the movement. Neuron-by-neuron analysis indicated that this difference was due to a subpopulation of projection neurons that strongly decreased activity around movement. Our findings challenge the view of the basal ganglia as the center in which reward information is linked to contextual or movement information from the cortex. In addition, our results support recent findings (Kim et al., 2022) proposing that reduction rather than elevation of the inputs to the basal ganglia is the signal that triggers movement.

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Poster

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Title: The basal ganglia are progressively less feature-selective and more behavioral-selective

Authors: *J. M. J. FABRE[1], A. J. PETERS[2], M. CARANDINI[1], K. D. HARRIS[1];
[1]Univ. Col. London, London, United Kingdom; [2]Oxford, Oxford, United Kingdom

Abstract: Introduction. The basal ganglia are critical for linking sensory input with motor actions; however, it remains uncertain to what extent activity in these regions is related to sensory versus motor events.
Methods. To address this question, we used Neuropixels probes to record in the striatum, globus pallidus external (GPe), and substantia nigra pars reticulata (SNr) in head-fixed mice. A first cohort of mice were naive and were shown a wide range of visual stimuli. A second cohort of mice were trained to move a wheel in response to any of three visual stimuli ("go-go-go" task). A third cohort of mice were trained to move a wheel in response to two visual stimuli, and to hold the wheel still for a third stimulus ("go-go-nogo" task). To disentangle stimulus responses from movement responses, we also recorded while mice were passively viewing the same stimuli, and we excluded any trials with movements for our analysis.
Results. In naive mice we found visually responsive neurons in specific subregions of the striatum, GPe, and SNr that matched data from the Allen Connectivity Atlas. While the striatum responded selectively to visual features, the GPe and SNr had much more generalized stimulus responses. After training on visuomotor tasks, the fraction of visually responsive neurons in all regions increased. The striatum remained selective for visual features across all stimuli, while GPe and SNr responded selectively to go vs no-go-stimuli, but did not have distinguishable responses across different go-stimuli in either the go-go-go or go-go-nogo tasks.
Conclusions. These findings indicate that the basal ganglia, and especially the striatum, encode the properties of visual stimuli even if they are not associated with behaviors. In addition, these responses increase after visuomotor training, such that the striatum encodes stimulus-specific and movement-association information, while the GPe and SNr encode only the movement association of a stimulus.

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Poster

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Program #/Poster #: PSTR173.12/I8

Topic: E.03. Basal Ganglia

Title: The Impact of Phase-Targeted Stimulation on Motor Cortex Excitability and Functional Connectivity

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Abstract: In Parkinson's disease (PD), there is an abnormal increase in coupling between the oscillations of the motor cortex and basal ganglia in both beta range and beta-gamma phase-amplitude coupling. These abnormal synchronizations are associated with the severity of motor symptoms. On the other hand, there are changes in the levels of synaptic plasticity in both the motor cortex and basal ganglia in patients with PD. This may contribute to the development of abnormal synchronization in the motor network that underly motor symptoms in PD. However, the connection between abnormal synchronicity and neuronal plasticity is not fully understood. Here, we applied stimulation pulses triggered by specific phases of the beta oscillations (phase-targeted stimulation; PTS) of the motor cortex of five PD patients during deep brain stimulation (DBS) lead placement surgery while a temporary grid of electrodes was implanted over the motor cortex. In each subject, bipolar biphasic direct electrical stimulation was delivered to the motor cortex using a grid of electrodes, and the response was recorded in electrocorticography (ECoG) format. As a measure for functional connectivity and an indirect probe of neuroplasticity, we measured the cortico-cortical evoked potential (CCEP) and tested whether this measure differed depending on the phase direction targeted by stimulation. Our findings indicate that when the stimulation is synchronized with the peak of the beta phase, it leads to an increase in the early and late components of the response evoked by stimulation. However, when the opposite phase (trough) is stimulated, it shows a tendency to reduce the early and late components of the evoked response. To probe motor cortex excitability change after PTS, we analyzed the CCEP before and after peak or trough stimulation. Our findings show how pathologically elevated beta oscillation and beta-gamma coupling could alter the neuronal plasticity in the motor cortex and indicate that the response to stimulation in the human motor system is dependent on the beta phase. Furthermore, they suggest that motor cortex phase-dependent stimulation can help to correct abnormal complex oscillations and reduce the severity of motor symptoms by normalizing the function of the motor network. If oscillation-associated loss of neuroplasticity contributes to irreversible changes in motor control, targeting specific oscillation phases could maintain or enhance motor network plasticity and alter the course of motor symptom progression.

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Poster

PSTR173: Basal Ganglia: Physiology and Plasticity

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Topic: E.03. Basal Ganglia

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Title: Striatal Lateral Inhibition Modulates Expression of Levodopa-Induced Dyskinesia

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Abstract: The basal ganglia coordinate motor control and action selection. In the striatum, the input nucleus of the basal ganglia, D1- and D2-expressing medium spiny neurons (MSNs) have been conceived as movement promoting and suppressing, respectively. More recent evidence suggests a more nuanced relationship between the pathways, in which both MSN populations are activated during movement. One possible explanation for this apparent discrepancy is that the two populations have coordinated activity: an ensemble of D1-MSNs facilitate the planned behavior while a linked ensemble of D2-MSNs suppress competing (unplanned) behaviors. A potential coordinating mechanism is collateral inhibitory synapses between MSNs. To better understand the function of lateral inhibition in motor control, we have studied a movement disorder in which multiple, normally competing actions are selected simultaneously, levodopa-induced dyskinesia (LID). In LID, patients with Parkinson's disease develop abnormal involuntary movements in response to dopamine replacement therapy. While some aspects of LID induction and expression remain unclear, convergent evidence suggests that increased firing of D1-MSNs may be a central mechanism. One possible explanation for this hyperactivity is a loss of lateral inhibitory control of D1-MSNs. To understand how striatal lateral inhibition might shape LID, we used *ex vivo* electrophysiology and chemogenetics. We find that D2-D1 and D1-D1 oIPSC amplitudes are reduced in parkinsonian animals. In parkinsonian animals chronically treated with levodopa, D1-D1 oIPSC amplitudes remain reduced while D2-D1 oIPSC amplitudes are restored to control levels. These connections are further modulated by acute dopamine signaling. Lastly, local chemogenetic inhibition of striatal connections originating from D2-MSNs shapes dyskinesia expression. Together, these findings help elucidate the role of lateral inhibition in basal ganglia function and dysfunction, thus expanding the framework on the role of striatal microcircuitry in action selection.

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Poster

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CTSI TL1

Title: Modulation of Striatal Membrane Potential by Movement

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Abstract: As the input nucleus of the basal ganglia, the striatum has been heavily implicated in the generation and selection of motor commands. Despite this, we know very little about how movement modulates the *in vivo* synaptic dynamics of striatal neurons. Here, we conduct whole cell membrane potential (V_m) recordings of identified D1 and D2 striatal projection neurons (SPNs) in head-fixed mice under three conditions – 1) ketamine/xylazine anesthesia, 2) awake and head-fixed on a stationary platform, and 3) awake and head-fixed on a mobile disk that allows for locomotion. We compare recordings in anterior dorsolateral striatum (aDS) and posterior striatum (pDS), to quantify differences in V_m dynamics along the anterior-posterior axis of the striatum. Under anesthesia, spontaneous V_m activity is characterized by prominent UP and DOWN states, at a frequency of 1 – 2 Hz, in both D1 and D2 SPNs. In awake animals on a stationary platform, during quiescence, we observe subthreshold slow-wave, large-amplitude V_m fluctuations (+8.1 mV +/- 1.2 mV) at 3 – 8 Hz. These fluctuations are quantitatively different from UP/DOWN states. During periods of movement, such as whisking or grooming, striatal V_m significantly depolarizes (+3.7 mV +/- 0.6 mV), and oscillatory structure is replaced by small-amplitude V_m fluctuations, with high spectral power above 20 Hz. Interestingly, in awake animals on a mobile disk, there is a notable absence of oscillatory activity regardless of movement. Although locomotor activity is robustly represented in striatal V_m amplitude (+5.8 mV +/- 0.4 mV), differences in standard deviation and spectral power of V_m activity are non-significant between bouts of quiescence and locomotion. The remarkable absence of oscillatory structure in the V_m of animals on a mobile disk suggests that context, and the corresponding degree of possible movement, influences baseline striatal V_m dynamics. Furthermore, we noted differences in motor representations between aDS and pDS. Recent studies demonstrate topographical distribution of cortical inputs along the anterior-posterior axis of the striatum, which points to functional specialization between sub-regions. For example, aDS is rich in motor inputs, whereas pDS is primarily innervated by sensory regions. In line with this, we observe greater modulation of aDS neurons by movement (+4.1 mV +/- 0.4 mV), compared to pDS neurons (+2.4 mV +/- 0.5 mV). Additionally, in awake animals on a stationary platform, cross-correlation analyses reveal greater synchrony between spontaneous V_m dynamics and movement in aDS neurons, compared to pDS. In all, this work points to dynamic modulation of striatal V_m by movement, context and region.

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Poster

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Title: Beta frequency activity reflects sensorimotor processing in the human subthalamic nucleus

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Abstract: The role of the subthalamic nucleus (STN) in sensorimotor circuits remains unclear, as does its significance within Parkinson's disease (PD) pathophysiology. In particular, how sensory information is processed in STN is understudied even though STN responds to tactile and proprioceptive stimuli, and patients with PD have impaired sensory processing. Here, we recorded intracranially from the dorsolateral STN and sensorimotor cortex from patients with PD undergoing deep brain stimulation (DBS) surgery to investigate the response of STN to simple vibratory stimuli (100 Hz and 200 Hz) of the wrist and to hand opening-closing. Both STN and sensory cortex displayed a robust response in the beta band (13-35 Hz) after vibratory onset. This response occured in cortex prior to STN. The event-related average response appeared to arise primarily from signal phase alignment (over trials) rather than changes in signal amplitude. This can be interpreted as a frequency-specific phase reset which has been observed to occur in other brain regions in response to auditory and visual stimuli. Similarly, single-unit activity in the STN, aligned to vibratory stimuli onset, showed spike-LFP phase locking in the beta frequencies without significant changes in firing rate itself. Patients also performed hand opening-closing tasks both with and without simultaneous vibration. We observed STN beta responses on directional contacts similar to those from vibratory stimuli alone. However, movement related beta responses were weaker than those from vibratory stimuli. Neural responses were further attenuated during simultaneous movement and vibration. Our findings suggest that STN and cortical beta synchronization relates to both motor intention and sensory signals such as vibrations. This sheds light on the role of the beta band in sensorimotor circuits, which, despite not being understood, is being used as a control signal in closed-loop DBS. Our findings also indicate that multiple signals may interfere with each other, possibly due to sensory gating mechanisms. Together, these observations may help explain previous findings of beta band's unreliable connection to parkinsonian symptoms such as rigidity.

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Poster

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Trinity University Department of Biology and Neuroscience funds

Title: The Laterality and Strength of Excitatory and Inhibitory Synapses From PPN Neurons Onto SNc Dopaminergic Neurons

Authors: *N. FERNANDEZ[1], H. ARIAS[1], A. BALDWIN[1], M. NEELALA[1], M. RODRIGUEZ[1], D. PENNY[1], I. KHETARPAL[1], L. MUZYKA[3], G. M. BEAUDOIN, III[2]; [1]Neurosci., [2]Biol., Trinity Univ., San Antonio, TX; [3]Dept. of Neurosurg., Dell Med. Sch. at The Univ. of Texas at Austin, Austin, TX

Abstract: The nigrostriatal dopaminergic pathway is involved in motivation, reward, and movement planning. Inputs to this region are critical for regulating changes in firing rate of dopaminergic neurons, a major teaching signal throughout target structures. However, these inputs have multiple targets, so now we are dissecting the microcircuit between these long range inputs and dopaminergic neurons.

One critical input to the substantia nigra pars compacta (SNc) dopaminergic neurons is the pedunculopontine tegmental nucleus (PPN). Interestingly, the PPN sends both ipsilateral and contralateral projections, unlike other brain structures that generally confine their innervations to either ipsilateral or contralateral. The current study is dissecting the nature of these two projections in combination with the neurotransmitter phenotype of the neurons of the PPN. The PPN has cholinergic, GABAergic, and glutamatergic neurons, but how these neurons contribute to these two projections is unclear. We use electrophysiological and confocal microscopic methods to investigate these connections. These projections have not been previously studied due to a lack of tools.

Using a combination of anterograde and retrograde labeling of PPN to the SNc, we have confirmed that the PPN indeed innervates both ipsilateral and contralateral SNc. Additionally, the density of the input is higher on the ipsilateral side than the contralateral side. We show that there are single neurons in the PPN that innervate both sides, and SNc neurons that receive bilateral input from PPN.

We are also using optogenetics to characterize the neurotransmitter identity of the PPN inputs to SNc both functionally and structurally. We use unilateral stereotaxic surgery to deliver a virus expressing a depolarizing light-gated ion channel, Channelrhodopsin (ChR2), which is tagged with either a yellow or red fluorescent protein. The virus labels glutamatergic and GABAergic neurons in the PPN. Finally, the PPN innervation of the SNc is further confirmed using imaging by immunostaining with markers for dopamine neurons, GABAergic synapses, and glutamatergic synapses.

Current findings suggest that GABAergic PPN neurons only innervate the ipsilateral SNc. However, glutamatergic PPN neurons innervate both ipsilateral and contralateral SNc, with the stronger glutamatergic innervations being found ipsilaterally to SNc DA neurons. This research is clarifying the precise neural network and synaptic-level processes regulating the nigrostriatal dopaminergic pathway.

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Poster

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Topic: E.03. Basal Ganglia

Support: Sorensen U01NS132267

Title: Exploring the morphological diversity of mouse basal ganglia in a multiple modality context

Authors: *M. MALLORY[1], S. A. SORENSEN[1], R. DALLEY[2], N. W. GOUWENS[2], A. BUDZILLO[3], B. R. LEE[4], T. JARSKY[5], B. E. KALMBACH[6], S. WALLING-BELL[2], Q. WANG[2], A. A. BHANDIWAD[2], S. YAO[7], Y. WANG[8], H.-C. KUO[2], O. GLIKO[2], U. SÜMBÜL[1];
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Abstract: Despite recent advances in understanding the complexities of the transcriptomic cell type landscape in mouse basal ganglia (BG), little is known about the morphological diversity of the transcriptomically-described neurons within these brain regions. We investigated the morphological properties of neurons in Caudoputamen (CP), Nucleus Accumbens (ACB), and Substantia Nigra (SN) through an integrative multi-modal approach. Previous studies have shown that linking whole neuron reconstructions (e.g., from whole brain fluorescent Micro-Optical Sectioning Tomography images) and Patch-seq data through local morphology can facilitate joint analysis of morphology, electrophysiology, gene expression, and long-range projections, and offer additional insights into the role of multimodal cell types in brain circuits (Sorensen et al., 2024). Initial efforts to classify BG transcriptomic subclasses or types based on dendritic properties from Patch-seq data required the development of new morphological features. Looking at whole neuron morphology (WNM) data sets (Peng et al., 2021), we noticed clear differences in local axonal phenotypes between D1- and D2-types that were defined based on their projections. To determine if local dendrite and/or axonal morphologies of WNMs could be used to predict these long-range projections, we assessed morphologically-based projection probabilities to target regions. Five distinct subdomains of CP have recently been delineated in the Allen Common Coordinate Framework (CCFv3) based on integrating multi-modal datasets. Interestingly, by registering reconstructed neurons into CCFv3, we observed MSN morphological diversity that is aligned with these sub-domains of CP. Further exploration of

these multimodal datasets will likely lead to additional insights into the functional roles of BG through the cell type-specific subnetworks.

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Poster

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Topic: E.03. Basal Ganglia

Title: Cholinergic modulation of striatal plasticity in motor learning

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Abstract: During learning of a motor skill there is a reorganization of activity in the striatum of spiny projection neurons (SPNs) from the dorsomedial striatum (DMS), during the early phase, to the dorsolateral striatum (DLS) in expert animals (Yin et al 2009). While previous work has focused on SPNs and their glutamatergic or dopaminergic inputs, striatal cholinergic interneurons (CINs) can have a critical regulatory role contributing to striatal plasticity during acquisition of a skill. CINs are the major source of acetylcholine (ACh) in the striatum and may provide a regionally biased modulation of SPNs in the DMS and the DLS. Furthermore, CINs also directly modulate release of dopamine in the striatum by activating presynaptic nAChRs on dopaminergic axons, providing another potential mechanism of local regulating of SPN activity in the striatum. In this study, we examined whether CINs play a role in modifying local circuits in the striatum that enables the plasticity in the striatum during motor training. We combined a single pellet reaching task (SPRT) with pose estimation to study motor learning in mice. Mice demonstrated forearm reaching trajectories that became more stereotypical (with less variance) as they became experts in the task and had a higher success rate at obtaining the pellet. Deeper analysis of mice with low success rates in the later training stage which would be termed as non-learners, found that they also demonstrated motor skill maturation regardless of the success at obtaining the pellet. Analysis of the error types during the reach demonstrated that mice performed movements that changed in category type over the course of training demonstrating that outcome measures in the task do not correlate with motor refinement. Using fiber photometry, we simultaneously monitored ACh release with the $GRAB_{ACh}$ sensor and activity of SPNs with GCaMP8 to determine the neural correlates of learning in the task in mice in each of the categories. Finally, by measuring the effect of chemogenetic inhibition of CINs on the

development of reaching trajectories in mice, we determined how learning was disrupted in the task. Together, these results provide insights into the contribution of local striatal cholinergic signaling in the development of motor skill learning.

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Poster

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Topic: E.03. Basal Ganglia

Support: CHDI

Title: Selective vulnerability of subthalamic nucleus neuron subtypes in the Q175 mouse model of Huntington's disease

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Abstract: Huntington's disease (HD) is a progressive neurodegenerative disorder that results from an expanded CAG repeat in the gene encoding huntingtin (Htt) protein. The subthalamic nucleus (STN) is an element of cortico-basal ganglia-thalamo-cortical circuitry. In the initial stages of HD, action suppression is impaired, resembling the effects of STN lesioning or inactivation. The Q175 knock-in mouse model of HD, which expresses full length human mutant htt with ~179 CAG repeats, is associated with abnormalities in cortical and striatal neurons together with neuronal degeneration and behavioral abnormalities. Intrinsic activity of STN neurons from 6-month-old Q175 het mice is diminished compared to wild type littermates (WT), due to NMDA receptor activation, mitochondrial oxidant stress, H_2O_2 generation, and K_{ATP} channels activation. Historically STN neurons have largely been regarded as being homogenous, however this pathology of STN neurons is heterogeneous, with some neurons seemingly unaffected. Here we further characterize STN abnormalities in the Q175 mouse model, with the goal of elucidating the factors that underlie this heterogeneity. A sub-population of STN neurons express the Ca^{2+} binding protein parvalbumin (PV). As the Ca^{2+} binding capacity conferred by PV could be protective in Q175, we made loose-seal cell-attached recordings in the STN of PV-cre x Q175 mice injected with AAV9-hSyn-DIO-eGFP. In WT mice there was no difference in the firing of PV+ and neighboring PV- neurons (PV+ = 9.23 [3.55-17.62] Hz, PV- = 10.24 [0-12.11] Hz), however in Q175 the PV- neurons showed greater disruption of autonomous firing than PV+ neurons (PV+: 5.00 [0-10.26] Hz, PV- = 0.13 [0-4.42] Hz). Next, we assessed STN neurons based on their afferent and efferent connections. Firing disruption of STN neurons retrogradely labelled from the substantia nigra was similar to the general population (WT: 10.0 [5.6-17.8] Hz; Q175: 6.1 [0-12.5] Hz), showing pathology in the direct/hyperdirect pathway. STN neurons that were driven by optogenetic stimulation of M1 cortical neurons showed

profound firing disruption (WT: 10.57 [3.45-23.30] Hz; Q175: 0.49 [0-5.31] Hz) whereas those driven from dorso-medial prefrontal cortex were aphenotypic (WT: 11.10 [8.19-13.31] Hz; Q175: 8.77 [5.07-15.12] Hz). These data show that phenotypic heterogeneity reflects an underlying heterogeneity in the STN, with pathology restricted to neurons that participate in specific circuits within the basal ganglia.

Disclosures: J.F. Atherton: None. **M.D. Bevan:** None.

Poster

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Title: Lack of m^6A mRNA methylation blunts neuronal responses to environmental challenges, impairs learning, and reveals the opposing and cooperative roles of striatal D1 and D2 neurons

Authors: *Z. SHI[1], K. WEN[1], W. FU[1], N. H. SAMMUDIN[1], X. ZHUANG[2];
[1]Neurobio., Univ. of Chicago, CHICAGO, IL; [2]Neurobio., Univ. of Chicago Dept. of Neurobio., Chicago, IL

Abstract: Adaptation to environmental challenges involves changes at the behavioral, circuit, cellular, synaptic and molecular levels. Changes in gene expression in neurons are essential in this regard. N6-methyladenosine (m^6A) modification of mRNAs affects almost every phase of eukaryotic mRNA metabolism, especially in the brain, where precise spatial and temporal control of protein synthesis is essential for neuronal function and plasticity. The functional significance of m^6A mRNA methylation is exerted by three groups of proteins: “writers” (methyltransferases) that install, “erasers” (demethylases) that remove, and “readers” that are RNA binding proteins that recognize m6A and determine the cellular fate of the modified RNA. That m^6A level drastically increases in the brain by adulthood further suggests its unique role in the adult brain, but it’s not well studied. We take advantage of the striatum which only contains two major cell types: dopamine (DA) D1 and D2 receptor-expressing medium spiny neurons (MSNs) with well-documented opposing functions in motor control, simplifies the molecular studies and allows comparison of behavioral phenotypes.Using transgenic mouse models with selective deletion of the methyltransferases gene *Mettl14* in D1 and D2 MSNs, we examined the impacts of m^6A on D1 and D2 MSN activities and behaviors in different behavioral paradigms that may involve D1 and D2 MSNs differently. Using fiber photometry Ca^{2+} imaging, we simultaneously recorded cell-type specific activities and behaviors in freely moving mice. *Mettl14* deletion in both MSN types blunted the cellular responses to cocaine but led to opposite behavioral phenotypes. Furthermore, *Mettl14* deletion in D1 MSNs impaired motor learning and

blunted changes in neuronal activity during motor learning, whereas *Mettl14* deletion in D2 MSNs blunted changes in neuronal activity during haloperidol-induced catalepsy and abolished sensitization of catalepsy. Notably, D2 firing was positively correlated with movement speed, but haloperidol increased D2 firing and inhibited movement. *Mettl14* deletion blunted both types of modulation. Our discovery demonstrated the role of a specific gene, by maintaining the normal neuronal activities, in regulating learning and environmental adaptation in a cell type specific manner. This study represents a good example in which knocking out the same gene in D1-MSNs vs D2-MSNs resulted in opposite behavioral phenotypes, and clearly demonstrated D1 MSN-dependent learning vs D2 MSN-dependent learning.

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Support: NIH R01MH122142
NIH R01DA043361

Title: Ythdf1 mediates the essential role of m^{6}A mRNA methylation in translational control in the adult brain and learning

Authors: Z. SHI[1], X. RUAN[2], X. ZHANG[2], *X. ZHUANG[1];
[1]Neurobio., Univ. of Chicago, CHICAGO, IL; [2]Human Genet., Univ. of Chicago, CHICAGO, IL

Abstract: Dynamic regulation of protein synthesis in neurons is essential for memory formation and environmental adaptation. While gene expression regulation at the transcriptional level has been extensively studied, post-transcriptional regulation of *de novo* protein synthesis offers better spatial and temporal control. The discovery of reversible m^{6}A mRNA methylation provides a new layer of post-transcriptional mRNA regulation, which potentially offers an ideal mechanism for spatial and temporal control of translation, allowing synaptic plasticity regulation and dendritic remodeling by targeting many transcripts at once. In cells, m^{6}A deficiency affects many proteins and cellular functions, but the exact mechanisms by which these effects manifested in adult brain function remain elusive. An important question is whether the functional consequences of m^{6}A are mediated by a specific reader protein in the adult brain, or, as a recent model suggests, the reader proteins YTHDF1, 2 and 3 have redundant functions. Our previous work with YTHDF1 constitutive knockout mice revealed its important role in promoting protein synthesis in neurons, in synaptic plasticity, and in learning. However, it is not clear if the impaired learning we observed in the YTHDF1 constitutive knockout mice has cell-type specificity effects.By using cell-type specific deletion of *Ythdf1* in dopamine D1 receptor

expressing or D2 receptor expressing neurons, as well as in dopamine neurons, we showed that *Ythdf1* deletion resembles impairment caused by deletion of the writer protein *Mettl14* in a cell type specific manner, suggesting that YTHDF1 is the main mediator of the functional consequences of m^6A mRNA methylation in the striatum. This contrasts the previous claims of redundancy among YTHDF proteins. At the molecular level, boosting dopamine release by cocaine drastically increased YTHDF1 binding to many mRNA targets in the striatum, especially those encoding structural proteins, suggesting the initiation of long-term neuronal and/or synaptic structural changes. Importantly, in the *Ythdf1* knockout, striatal neurons have a much higher baseline *de novo* protein synthesis rate. While striatal neurons in control mice responded to elevated cAMP by increasing *de novo* protein synthesis rate, striatal neurons in *Ythdf1* knockout mice didn't.In summary, our study reveals the essential role of m^6A mRNA methylation and its reader protein YTHDF1 in regulating translational control in the striatum, offering insights into molecular mechanisms underlying learning and adaptive behaviors.

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Topic: E.03. Basal Ganglia

Support: Arizona Alzheimer's Consortium

Title: Choline and Myo-Inositol in the Basal Ganglia: Spectroscopic Insights into Their Role in Motor Function and Connectivity with the SMA

Authors: *E. OFORI[1], M. ORTEGA[2], A. B. DOHERTY[2], B. BARTELLE[2], M. WICKLUND[3]; [1]Arizona State Univ., PHOENIX, AZ; [2]Arizona State Univ., Tempe, AZ; [3]Neurol., Mayo Clin. Arizona, Phoenix, AZ

Abstract: Choline, a vital nutrient, plays a significant role in neurophysiological processes. This study explores its correlation with motor output variability and abilities by examining myo-inositol levels in the basal ganglia and their functional connectivity to the Supplementary Motor Area (SMA). Using advanced spectroscopic techniques, we quantified myo-inositol and choline concentrations in the basal ganglia of 30 participants. We employed motor task functional magnetic resonance imaging (fMRI) to assess connectivity patterns between the basal ganglia and the SMA. Motor abilities were evaluated through various motor tasks. Our results indicate a positive correlation between choline levels and motor function stability, and a significant link between higher myo-inositol levels and decreased motor abilities. These findings suggest a bidirectional interaction of choline and myo-inositol concentrations in the basal ganglia, along with their connectivity to the SMA, are crucial for motor performance, offering new insights into the biochemical underpinnings of motor control.

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Poster

PSTR173: Basal Ganglia: Physiology and Plasticity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR173.23/I19

Topic: E.03. Basal Ganglia

Support: R00NS112417
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Title: SNr and GPe inhibition of anatomically and genetically defined PPN subpopulations

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Abstract: The pedunculopontine nucleus (PPN) is a midbrain structure heavily interconnected with the basal ganglia (BG) and dopamine systems. PPN cholinergic neurons degenerate in Parkinson's Disease and PPN neurons show reduced activity in Parkinson's model animals. Two inhibitory BG structures, the substantia nigra *reticulata* (SNr) and the globus pallidus *externa* (GPe) have been identified as inputs to the PPN via rabies-tracing experiments, but the extent of their functional inhibition of PPN subpopulations is not well understood. Evaluating the connections between these BG nuclei and the PPN in the healthy brain will lay the groundwork for understanding how PPN inhibition may be enhanced in PD. To address this need, I used whole-cell patch clamp electrophysiology paired with optogenetic stimulation of GPe or SNr axons to characterize their functional inhibition of PPN subpopulations. Because the PPN contains cholinergic, glutamatergic, and GABAergic neurons and is anatomically divided into rostral and caudal regions, I assessed the synaptic inputs from the GPe and the SNr on both anatomically and genetically-defined subpopulations. I found that the GPe mildly, but preferentially inhibits caudal glutamatergic and GABAergic neurons. This inhibitory connection undergoes significant short-term synaptic depression in glutamatergic neurons and only weakly suppresses their action potential firing. By contrast, the SNr strongly inhibits each cell type in both the rostral and the caudal PPN. The SNr input to PPN glutamatergic neurons was particularly strong and was observed in 100% of the neurons recorded. This synaptic connection did not undergo significant short-term synaptic depression and was able to strongly inhibit action potential firing. To evaluate the functional impact of differential inhibition from the SNr and GPe to the PPN subpopulations, we performed *in vivo* optogenetics to stimulate the SNr or GPe axons over the PPN while mice were tracked during open field and real time place preference. We found opposing outcomes in the total distance traveled and preference behaviors when stimulating the SNr or GPe axons. This characterization of BG inhibition of the PPN is an important first step in understanding how molecularly-defined subpopulations in the rostral and

caudal PPN differentially transmit information from the BG to modulate motor output in the healthy and Parkinsonian state.

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Poster

PSTR173: Basal Ganglia: Physiology and Plasticity

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Title: Neurophysiological comparison of the STN and GPi during an action conflict task

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Abstract: *Introduction*: Treatment of Parkinson's disease (PD) often includes the placement of a deep brain stimulator (DBS) at one of two targets, the subthalamic nucleus (STN) or the global pallidus internus (GPi). The placement decision is made by a multidisciplinary team including neurosurgeons, psychologists, and neurologists. There is an ongoing discussion among researchers regarding the merits of each target. The objective of this abstract is to compare the two targets' neural activity. Within the basal ganglia circuit, the STN receives input from the frontal cortex via the hyperdirect pathway which continues to the thalamus via the GPi. Connections between the STN and GPi are excitatory, and there is strong evidence that STN neurons affect neuronal activity in the GPi. Through this pathway, cortical regions associated with executive functions may directly influence both STN and GPi activity. Using a conflict task, therein imposing a cognitive demand, theta band oscillations were measured in each target region and used to infer action control function. Based on previous work it was hypothesized that the theta band modulations found in the STN would be present in the GPi as well. *Methods*: Data was collected from 20 participants with PD who would receive a stimulator at one of the two targets (STN N=10, GPi N=10). Data was collected intraoperatively while the Simon task was performed. The Simon task requires a left or right directional response to a feature (color) of a spatially lateralized stimulus. When the action signaled by the location and color of the stimulus are non-corresponding, this induces conflict and the inappropriate action impulse must be suppressed. Local field potentials were used to analyze neurological data. *Results*: The two populations did not have significantly different behavioral outcomes, however, their neurophysiology differed. It was found that the STN ($F(1, 280)= 17.02$, $p<.001$, $m_{Cs} =.65$, $m_{NC}=.73$)) had conflict related task modulation in the theta that was not present in the GPi

(Fs<.57, ps>.45). *Conclusion*: With the GPi's downstream connection to the STN it can be inferred that the conflict processing has been completed and the signal received from the GPi conveys only motor intent. These findings indicate the STN possesses a unique function in conflict modulation, one not propagated onto the GPi.

Disclosures: J.L. Bowersock: None. **T. Stewart:** None. **A. Zemmar:** None. **J.S. Neimat:** None. **N.C. Van Wouwe:** None.

Poster

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Title: Prefrontal mechanisms underlying evaluation of action cost when deciding to act with another agent

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Abstract: Cooperative behavior in form of joint action (JA) is pervasive in nature, enabling to achieve goals unattainable by single agents. Despite its potential benefits, JA entail behavioral costs, due to inter-individual motor coordination necessary to accomplish the task. This cost must be considered when deciding to act alone or with another agent to estimate the utility of social interactions. We studied the neural underpinning of value-based decision-making, on a pair of macaque trained to act individually (SOLO) or jointly (JA), and free to choose between two different action types (SOLO vs JA), to obtain variables amount of liquid rewards, associated to each action. In the task, monkeys were required to guide through an isometric joystick a cursor toward one of two targets, which cued the action modality (SOLO/JA) and the amount of obtainable reward. Single-unit activity (n=366) was recorded from dorsolateral prefrontal cortex (area 9) simultaneously from the two-interacting monkey. Behavioral data showed that within a wide range of reward amount differences (Δ) associated to the two options, both animals showed an utter preference toward the SOLO action, despite the highest amount obtainable if acting together. However, by doubling the reward unit from .15 to .30 ml, thus increasing the Δ between the offers, both animals opted for the JA, since this guaranteed higher payoff. This suggests that when choosing between different action modes, the economic choice is subdued not only to the payoff value, but also to the joint-action cost. This cost, paid by each

monkey by adapting its own action kinematics to that of the partner, resulted also in a decreased success rate, with concomitant reduction of movement vigor. Our findings indicate that macaques are able to accurately estimate these costs to decide whether to act alone or jointly with others.
At neural level in both animals about 70% of prefrontal cells were modulated during the choice phase with a majority them showing a firing rate increase, relative to control time (CT), thus encoding the differential value of the two options. We also found neurons highly selective for the choice of JA, i.e., activated only when animals opted to act jointly with its partner. Interestingly, among the neurons (about 60% in both animals) activated during action planning and execution most of them (>70%) were suppressed relative to CT. Furthermore, the social nature of the task highlighted a neural representation in area 9 of the prediction of other's choices. Overall our findings support the role of prefrontal area 9 in the good-to-action transformation, when acting alone or in a social context.

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Poster

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Title: Functional cell-types in frontal cortex measured across multiple tasks

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Abstract: The frontal cortical neurons show diverse activity profiles that reflect sensation, action, and cognition during decision-making, but the underlying neural circuit for this complex selectivity remains unclear. We previously found that stimulus, choice, and action were coded nonrandomly by largely distinct neuronal populations in mouse frontal cortex (Yang et al Nat Neurosci 2022). These "functional cell-types" display distinct patterns of temporal response profiles and are consistently identified in the frontal cortex of different mice. However, it remains unknown whether each functional cell-type is dedicated to a specific type of information encoding or interchangeable across different tasks.

Using an autonomous home-cage system (Hao et al eLife 2021), we established a continual learning paradigm in which mice sequentially learned to perform delayed directional licking tasks instructed by distinct sensory stimuli, i.e. different tasks. We combined this behavior paradigm with longitudinal two-photon calcium imaging to track the same neuronal populations in the anterior lateral motor cortex (ALM) across multiple tasks.
We quantified individual ALM neurons' contributions to different functional encodings in each task. Within each task, the encoding of stimulus, choice, and action were selectively represented in three largely separated populations of ALM neurons, consistent with previous electrophysiology measurements (Yang et al Nat Neurosci 2022). Across different tasks, the majority of ALM neurons maintained their contributions to one type of functional encoding, e.g. neurons encoding sensory stimulus in one task were less likely to encode choice or action in other tasks and vice versa. Across different tasks, we observed a reorganization of choice-selective neurons across ALM population. Each task recruited a random subset of choice-selective neurons, but this recruitment primarily occurred within the choice-encoding population and rarely from the stimulus- or action-encoding populations defined in other tasks.
Our results suggest that ALM neurons constitute segregated and largely dedicated functional cell-types, which are mostly preserved across different tasks. The dedicated cell types may reflect fixed underlying subnetworks within frontal cortex.

Disclosures: J. Kim: None. **N. Li:** None.

Poster

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Topic: E.04. Voluntary Movements

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Title: Dissociation of efference copy and afferent feedback signals in somatosensory cortex

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Abstract: Motor brain regions not only send efferent signals to descending spinal pathways, but also a copy of those signals to other brain areas to inform sensory expectations. This enables discriminating self-generated from externally-generated movements, and allows for more timely estimation of body state (proprioception). One area that likely participates in the integration between sensory (afferent) feedback and efference copy signals is area 2 of somatosensory cortex, a brain area that processes proprioceptive information. While we know area 2 receives information from the motor cortex, we don't understand how these signals interact. Here, we

investigated the interaction of efferent and afferent signals in area 2, using neural decoding- and encoding-based approaches.
Our decoding analyses discovered two separable signals within the area 2 population, occurring at distinct time shifts relative to movement, and which resided on orthogonal neural axes-this supports simultaneous, demixed representations of efference and afference. Evidenced by our encoding analyses, the orthogonal neural representations are enabled by a mixed encoding of two signals in the majority of area 2 neurons, instead of there being distinct populations encoding each signal.
To further explore the nature of these representations, we compared these signals during active movements and passive perturbations. We found the pre-movement signal to be absent during passive perturbations, confirming our interpretation of it being related to efference copy. Unique from previous results, we discovered feedback signals for voluntary movements arrived slower than those from external perturbations. A simple model with suppression of afferent feedback during active movements recapitulates this finding.
Overall, our findings characterize separate representations of efferent/afferent signals within area 2, find a novel time-delay between afferent feedback in active and passive movements, and demonstrate a broadly applicable approach to dissect efferent and afferent signals within neural population data.

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Poster

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Title: The effect of behavioral state on motor cortex optogenetic stimulation outcomes

Authors: *R. BULLINS, J. D. COHEN, N. ELKIN, K. LU, A. W. HANTMAN;
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Abstract: Skilled motor behaviors are essential to performing daily activities. Complex patterns of neural activity in the primary motor cortex (M1) tiles to skilled motor behavior. Considerable work has been done to characterize the neural dynamics and circuitry of skilled behavior leveraging optogenetic neural manipulation techniques. However, the extent to which different behavioral states affect neural manipulation outcomes, particularly during performance of a skilled motor behavior, remain unknown. Here, we tested whether optogenetic perturbations applied to M1 at different behavioral timepoints elicit a single deterministic movement outcome

or distinct movement outcomes.
We trained mice to perform a dexterous reach-to-grab task wherein naïve mice were trained to reach for a food pellet upon presentation of an auditory cue. Mice achieved high performance on this task within two to three weeks of daily training. Next, we imposed full-field optogenetic stimulation to excitatory motor cortical neurons (VGLUT1/Ai32) during different behavioral timepoints (at rest, at auditory go cue, or at different phases of the reach-to-grasp behavior). We found that optogenetic stimulation of the motor cortex at different behavioral timepoints elicited differential movement effects. For example, stimulation applied at cue evoked a large displacement of the upper limb, whereas stimulation applied at rest did not evoke a movement. Interestingly, stimulation administered at cue also elicited a quicker movement response (~<100 ms to cue onset) compared to control reach-to-grab trials reaction time (~>200 ms to cue onset). These results support the hypothesis that the timing of optogenetic stimulation, set by the current behavioral state, affects the movement outcome. Future work using large-scale in-vivo electrophysiology will focus on how M1 neural state at time of stimulation affects movement outcome.

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Poster

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Title: Composition of continuous behavior from distinct skills evident in motor cortex

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[1]Columbia Univ., New York, NY; [2]Stanford Neurosciences, New York, NY; [3]Neurosci., Columbia Univ., New York, NY; [4]Columbia University/HHMI, NEW YORK, NY

Abstract: Motor cortex (M1) neural responses have been proposed to mirror either muscle activity or movement kinematics. An alternative is that most aspects of M1 activity reflect computations instantiated by population dynamics. If so, there may exist multiple such computations that can be deployed flexibly, allowing an outwardly continuous stream of behavior to be composed from a repertoire of previously learned skills. Under this ‘flexible repertoire hypothesis’, the complexity of M1 activity should primarily reflect the number and nature of skills used within a given task, rather than the complexity of motor output per se. The

hypothesis makes additional predictions if one considers the common network strategy of separating different computations into different neural subspaces. If this strategy is used compositionally, the active subspace should change with the internal computation, and overall neural dimensionality should grow quite large.
We tested these predictions using a force-tracking task that is challenging to perform and might benefit from control-policy flexibility. Monkeys controlled a cursor with the goal of continuously intercepting a scrolling dot path. Cursor height was proportional to force, generated isometrically by pressing on an immovable handle in a single direction. Different dot paths involved different frequencies and features. Behavior was consistent with different feedback-control strategies being used at different times. Yet biomechanically the task was simple: essentially all task-relevant variables - visual and physical - mirrored force or its derivative. Muscle activity also mirrored force. We used Neuropixels Probe recordings to record from arm-related M1, and analyzed populations of 1179 and 814 neurons from two monkeys.
Individual M1 responses rarely resembled force. While force could be accurately decoded, force explained only ~10% of population response variance. Neural dimensionality grew across conditions, in part because activity occupied different neural dimensions for different conditions. Unsupervised dimensionality reduction uncovered a set of distinct subspaces, roughly corresponding to low, medium and high frequencies, that were reused compositionally within and across conditions. Similarity of the dimensions used across two conditions correlated with similarity of behaviorally inferred control policies. These results indicate that the dominant features of M1 activity reflect internal computations that relate to specific skills or control policies. This repertoire is then used compositionally to generate an even greater range of behaviors.

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Poster

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Topic: E.04. Voluntary Movements

Title: Rapid timescale engagement and disengagement of the motor cortex in the control of movement

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Abstract: Movements require contributions from different sets of brain regions depending on the demands of the behavioral context in which they are performed. Some reflexive movements require only genetically specified circuits in the spinal cord. Decerebrate animals, in which the

brainstem and spinal cord are isolated from the forebrain, retain the ability to spontaneously locomote, vocalize, and ingest food and water. These animals exhibit poor dexterity, however, and lack the flexibility to adapt basic movement patterns to achieve abstract behavioral goals. These 'higher' functions of the motor system require contributions from additional motor centers distributed across the brain, including motor and sensory cortical areas. Much of our understanding of the context-specific contributions of cortical regions to the control of movement has come from studies relating lesions and other chronic perturbations to subsequent impairments in function. Because of this, less is known about how cortical regions are dynamically engaged in, and disengaged from, the control of movement during behavior, and what features of behavior cause these switches to occur. To address these questions, we developed a set of head-fixed behavioral tasks in which the motor cortex becomes engaged in, and disengages from, the control of movement dynamically on sub-second timescales. The timing of cortical engagement and disengagement could be reliably manipulated based on task contingencies. Using a combination of high-speed video, silicon probe electrophysiology, and optogenetics, we demonstrate an abrupt and predictable switch from engaged to disengaged states when sensory outcomes of actions lost behavioral relevance. These results provide key conceptual insights into when and why the motor cortex becomes engaged in and disengaged from the control of movement and provides an experimentally predictable example of state switching in the mammalian motor system, opening the door for future studies aimed at delineating the neural mechanisms through which state switches are regulated in the brain.

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Poster

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Topic: E.04. Voluntary Movements

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Title: Sensory responses evoked in motor cortex of mice engaged in a whisking to touch task

Authors: *F. FREITAG, L.-G. HARDER, J. K. W. DE VRIES, M. E. LARKUM, R. N. SACHDEV;
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Abstract: The function of the motor cortex in decision making, preparing for movement and in executing movements is an active area of research. Whether individual neurons in cortex are active in one or more behavioral contexts, whether preparation to move affects responses to sensory input are still open questions. In this study, we trained mice to perform a whisking to

touch task, or to simply lick in response to an air puff. The trials were intermixed randomly between whisking to touch, air-puff alone, and with some unexpected air-puffs delivered while mice were actively engaged in whisking to touch. We used behavioral tracking of whiskers, and face, with high-speed cameras and multichannel probe recordings of M1 in head-fixed mice. Analysis of the data revealed strong and widespread encoding of the air-puff stimulus in the motor cortex across all layers with stronger responses to the air puff in L5, whereas active whisking to touch, and the expected touch against the contact sensor, elicited only minimal changes in the firing rate of recorded neurons. Additionally, specific neurons were found to be active only in response to the unexpected sensory stimulus. These are putative positive prediction error neurons in the predictive processing framework. Even though the task on each trial was chosen randomly, mice prepared to whisk to touch, by subtly shifting their posture, and preparing their whiskers and jaw. This active preparation to move, could be one element in the air-puff evoked response in our recordings. Surprisingly, neurons in motor cortex barely increased their firing rate during whisking and even though they responded to passive stimuli to whiskers, they did not respond much to active touch of a whisker against a sensor. This study highlights the multimodal nature of motor cortical function and shows the potential for online readout of motor planning and sensory responses in motor cortex in situations of high uncertainty.

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Poster

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Title: Evidence for distinct internal states influencing motor cortex

Authors: *S. BORGOGNON[1], A. L. SMOULDER[2], H. MIYATA[2], J. WEN[2], A. CHANDRASEKARAN[1], M. A. SMITH[2], S. M. CHASE[2], A. P. BATISTA[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Neural activity in the cerebral cortex is typically associated with sensory, motor, and cognitive processing, but it is also known to be influenced by internal state processes such as attention, reward anticipation, and arousal. For instance, neural population activity from visual and prefrontal cortical areas slowly drift together in a manner indicative of arousal-like signals (Cowley et al., 2020). Even the motor cortex is not immune from the influences of internal states. We recently reported the presence of a signal encoding the value of a reward the monkey expected to receive. Here, we are asking if reward modulations relate to these previously documented cortical arousal-like signals. If both arise from the same mechanism, we expect indissociable signals; if different mechanisms, they should be separable. To test between these two alternatives, we trained two adult male rhesus monkeys to perform a skilled arm-reaching task in which we cued the reward that would be given upon trial success. We recorded motor cortical activity using high-density electrode arrays and measured pupil size as the animal performed the task. We first identified a "reward axis" and a "slow drift axis" in neural activity during movement preparation, where each axis was a linear combination of firing rates that best captured reward and arousal-like signal variance, respectively. To test our hypothesis, we first computed the angle between these two axes and assessed how well each axis captured the other's variance. We found that the reward axis and the slow drift axis were nearly orthogonal and accordingly, captured little variance of the other signal. We also found that the direction of the reward axis was consistent over time. Similarly, the slow drift axis was consistent across the reward conditions. Finally, we asked if reward effects were mediated by arousal. If true, we expect similar correlations of both slow drift and reward axis projections with pupil size. Instead, we found that the slow drift axis was better correlated to pupil size than was the reward axis. Altogether, these results suggest that the motor cortex processes reward- and arousal-related signals separately. This suggests that these signals may arise from different subcortical neuromodulatory drive systems.

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Poster

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Title: Spatio-temporal components of joint action are encoded by local field potentials in the primate frontal cortex

Authors: *S. GRASSO[1], L. SACHELI[2], E. QUARTA[3,1], L. ZAPPAROLI[2], E. PAULESU[2], A. BATTAGLIA MAYER[1];
[1]Sapienza Univ. of Rome, Rome, Italy; [2]Univ. of Milano-Bicocca, Milan, Italy; [3]Italian Inst. of Technol., Rome, Italy

Abstract: Even though social interactions are central to our daily activities, little is known about neuronal mechanisms underlying motor control during joint actions (JA). Previous work has revealed that macaques, as humans, leverage on a "we-representation", suggesting phylogenetically preserved neural processes. Moreover, our previous study showed a class of neurons in macaque premotor cortex preferentially encoding JA. Here we investigate whether LFPs also represent shared behavioral dynamics during JA. To address this issue, we explored the extent to which spatio-temporal components of joint action are encoded by LFP in PMd. Neural signals were recorded simultaneously from PMd of two macaques, performing a pre-cued visuomotor task, by exerting a force on a isometric joystick to guide visual cursors on a screen, either individually (Solo) or jointly. Results showed that both monkeys adopted a more linear trajectory during JA compared to the Solo condition. At variance, divergent movement patterns in terms of reaction times and speed profiles between monkeys were found, in particular one subject exhibited motor adaptation reducing kinematic differences with its partner. These patterns improved the efficiency of motor coordination by reducing inter-cursor distance (ICD). To assess if ICD was a consequence of dyadic coordination or mere overlap in movement strategies between individuals, we simulated the JA condition by randomly pairing Solo trials of both monkeys, which confirmed that ICD emerged from shared motor dynamics. Together, these results suggest that inter-individual coordination is facilitated by reducing gaps between individual kinematics signatures. Conversely, the divergent movement patterns were reflected in subject-specific modulation of Movement Related Potential (1-15 Hz), with larger amplitude during the joint action condition relative to solipsistic action. Interestingly, during joint behavior LFP amplitude correlated with the ICD, where larger amplitude signaled higher degree of coordination. Furthermore, these signals emerged during the planning phase of dyadic interactions. Our results provide further evidence on the role of premotor cortex during inter-individual motor coordination when acting jointly with others.

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Poster

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Title: Recordings from macaque motor cortex using 1024 channel SiNAPS probes

Authors: *S. BAKER[1], A. BAKER[1], S. GONZÁLEZ TORRECILLA[2], V. SILVEIRA GONCALVES M TENORIO[3], G. N. ANGOTZI[4], G. ORBAN[5], M. VINCENZI[6], L. BERDONDINI[7];
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Abstract: Acquiring electrophysiological data from large numbers of single neurons in the macaque brain remains challenging. Chronic implants such as the Utah array have reasonable channel count (~100), but sample from only a small (and fixed) region of cortex at a fixed depth. Acutely inserted electrodes can sample from a wider region by making penetrations at different sites each day; however, the need to penetrate through the dura within a recording chamber places severe limits on electrode configuration. We have recently produced a custom probe using a modified SiNAPS technology integrating additional multiplexing circuits to reduce the number of outputs and designed to access both superficial and deep parts of the macaque motor cortex. The probes are 10 mm in length, 155 µm wide and 50 µm thick. An active area of 7.75 mm x 0.097 mm contains a 4 x 256 grid of 1024 electrodes (14 µm x 14 µm in size) with 30 µm interelectrode (center-to-center) pitch. This is sufficient to reach down the anterior bank of the central sulcus, which contains many corticospinal cells making direct connections to motoneurons in the spinal cord. Critically, the probe is mounted on a narrow printed circuit board, allowing the assembly to fit within the confined space of a recording chamber. Penetration through the dura of such thin probes is typically not possible unaided; we find that it can be eased by making a small pilot hole using a 30G needle. Meticulous care of the dura has been essential to success, involving daily removal of tissue growth under an operating microscope, and use of the anti-mitotic compound 5-fluorouracil to suppress hypertrophy and vascularization. We have recorded from two SiNAPS probes simultaneously (total of 2048 electrode channels) within primary motor cortex (M1), or with one probe in M1 and the other in pre-motor cortex. We have found that these long, thin probes are also very effective in recording from the supplementary motor area, where they can access cells within the mesial wall of the cortex. In all areas, corticospinal cells are identified by their antidromic responses to stimulation of chronically implanted electrodes in the pyramidal tract at the medulla. Cortical recordings using SiNAPS probes have been made simultaneously with recordings from neurons within the brainstem reticular formation, using stainless steel electrodes with 32 contacts (U probes, Plexon Inc). We will illustrate the high quality recordings which can be obtained using these techniques, and highlight the practical steps necessary for success.

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intellectual property rights/patent holder, excluding diversified mutual funds); Owner of shares in Corticale Srl.. **G. Orban:** None. **M. Vincenzi:** None. **L. Berdondini:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owner of shares in Corticale Srl..

Poster

PSTR174: Cortical Planning and Execution: Neurophysiology

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR174.11/I31

Topic: E.04. Voluntary Movements

Support: NINDS 1R01NS129576-01A1
NSERC-PDF

Title: Motor cortical dynamics reveal rhythmic and transient dimensions during voluntary gait modification in the mouse.

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Abstract: Locomotion in complex environments requires continual adjustment of muscle activity across steps. With the basic locomotor pattern produced by spinal networks, another pattern by cortex must be added in response to environmental challenges, like when stepping over an obstacle. In primates, cats, and rodents, motor cortex is necessary for precise changes of muscle activity to traverse obstacles, acting on top of the ongoing locomotor pattern. Single neuron recordings in cats have identified changes in motor cortical firing rates during obstacle traversal. What remains unclear is how the coordinated, population-level patterns in motor cortex are related to specific aspects of behavior, and how transient patterns related to gait modification interact with an ongoing locomotor rhythm. Here, we use three-dimensional motion tracking and cortical neural ensemble recordings during unrestrained locomotion in mice. Animals were trained to trot on a linear treadmill with obstacles attached to the belt that were ~0.9 cm in height. Steps were segmented based on forelimb swing and stance kinematics, and sequences of steps relative to obstacles were identified. In comparison to locomotion over a flat surface, obstacle traversal required the mouse to make a voluntary gait modification and increase paw height. Cortical activity was recorded using silicon probes chronically implanted in the forelimb motor area. On the step over the obstacle, mice alternated between two movement strategies, with the forelimb contralateral to the neural probe either traversing the obstacle before the ipsilateral limb (lead condition), or after the ipsilateral limb (trail condition). Neuron firing rates aligned to step sequences showed that some cells were responsive to both the stepping rhythm and obstacle, whereas other cells were only responsive to the stepping rhythm, obstacle traversal, or the step preceding the obstacle. Because individual neuron responses were complex and heterogeneous, we next leveraged population analyses. From step sequences, we identified neural dimensions in which activity is rhythmic and others with large transients just before the

obstacle. Unexpectedly, the transient dimensions exhibited limb-independent activity synchronized with the leading forelimb, regardless of whether the limb was ipsilateral or contralateral to the neurons. Subsequent experiments are aimed at identifying how inputs from other brain areas, like the cerebellum and posterior parietal cortex, drive activity in these dimensions.

Disclosures: E. Kirk: None. **K. Hope:** None. **B. Sauerbrei:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: NIH Grant NS111148

Title: Neural coding of force and stiffness during a multi-directional ballistic release task

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Abstract: In our previous work, we found that the activity of motor cortical neurons was tuned to stiffness as monkeys performed a ballistic release task in a single movement direction. Here we report results when this task was performed in different directions.
A rhesus macaque was trained to do a multi-directional ballistic release task which required precise regulation of both force and displacement across eight directions. The monkey was implanted with two UTAH arrays in M1 to record neural activity. In addition, 16 muscles of the left arm were implanted with bi-polar epimysial EMG electrodes to record muscle activity. The task was designed to encourage the predictive tuning of arm end-point stiffness. The monkey grasped a locked handle and exerted a constant specified force until the handle was released after a random duration. This resulted in a rapid, ballistic movement. Subsequently, the monkey was required to arrest the handle within a target position range without corrective movements. To facilitate performance and allow monkey to preset its stiffness, we presented the same required force and target range five times in blocks. Nine blocks of different force/displacement combinations were carried out in each of eight movement directions.
We found that the behavior of the ballistic movements was well described by a 2^{nd} order mechanical system in which the force-displacement relationship is well defined by mass, damping, and stiffness parameters. A linear regression model between neural activity and the parameters of the task showed that the firing rates were strongly correlated with direction of movement ($r > 0.8$) and exerted force ($r > 0.6$). Stiffness could be decoded from the population

of recorded motor cortical activity reasonably well in individual directions (average actual to predicted stiffness correlation, r = 0.79). However, when the data were pooled across all directions, stiffness could not be decoded. This suggests that direction and stiffness encoding interact in the firing rate model of individual neurons. Regression of the combined muscle activity during the pre-movement period was related to both force ($R^2 > 0.7$) and stiffness ($R^2 > 0.45$) irrespective of the movement direction. Co-activation of different antagonist muscle pairs for fingers, wrist, elbow, and shoulder were quantified using a co-activation index (CAI). The CAI of the anterior and posterior deltoid muscles was found to be well related to stiffness regardless of direction. This indicates that the strategy of presetting stiffness was carried out by muscle co-activation. The preparatory stiffness was encoded by motor cortical activity in a direction-dependent manner.

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Poster

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Title: Involvement of the primate dorsomedial frontal cortex in the control of the voluntary respiration

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Abstract: Voluntary control of breathing is a fundamental brain function that is related to speech and singing. Many studies have reported the mechanism of autonomous breathing regulated in the brainstem. However, it is still unclear how voluntary breathing is controlled because a procedure for generating voluntary breathing in model animals is lacking. In this study, we succeeded in making voluntary breathing in macaque monkeys by using an inspiration-timing task. In this task, the monkey's breathing was monitored by the measure of nasal flow or nasal air temperature which we recently established (Kunimatsu et al., 2022). The monkey was required to make an inspiration within an early time window (100 - 1500 ms) or within a late time window (1500 - 4000 ms) to obtain a liquid reward. In a control condition, the monkey obtained the reward at any time when making an inspiration after the onset of a visual cue. These three conditions were instructed by the color of the visual cue (yellow, white, or blue). We

observed that monkeys changed their inspiration timing depending on the given instruction compared with the control condition. This result indicates that the monkeys were able to voluntarily control their breath timing. Next, we examined the neural mechanism underlying the voluntary control of breathing. It is well-known that the dorsomedial frontal cortex, including the supplementary motor area (SMA) and the pre-supplementary motor area (pre-SMA), is involved in the voluntary control of body movement (Matsuzaka et al., 1992), although whether these regions mediate the voluntary control of breathing remains unknown. To investigate this, we recorded the activity of single neurons in the SMA and pre-SMA of two monkeys during the inspiration-timing task. Of 451 recorded SMA and pre-SMA neurons, 150 (33%) showed an increase in activity after the onset of the cue (visual-related) and/or before the onset of the inspiration (motor-related). Notably, many of the motor-related neurons were recorded from the SMA, while many of the visual-related neurons were from the pre-SMA. These results suggest that the SMA contributes to the generation of voluntary breathing and the pre-SMA represents the rule or motor plan. This functional segregation is consistent with a previous study focusing on the voluntary control of arm movement. Voluntary breathing and arm movement seem to be controlled by common neuronal mechanisms at least at the frontal cortex.

Disclosures: R. Yi: None. **T. Takei:** None. **M. Matsumoto:** None. **J. Kunimatsu:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: THE SYDNEY AND J.L. HUFFINES INSTITUTE FOR SPORTS MEDICINE AND HUMAN PERFORMANCE

Title: Facilitatory influence of the supplementary motor complex on the primary motor cortex reduced after motor chunking

Authors: *H. KIM[1,2], Y. LEI[3], A. T. HUYNH[3], J. J. BUCHANAN[3], J. A. BERNARD[4], J. C. BROWN[1,2], D. L. WRIGHT[3];
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Abstract: Numerous dual-coil paired-pulse transcranial magnetic stimulation (ppTMS) studies revealed that the supplementary motor complex (SMC) exerts a facilitatory influence on the primary motor cortex (M1), thus increasing the peak-to-peak amplitude of motor-evoked potentials (MEPs) at M1 (Arai et al., 2011, 2012; Green et al., 2018; Rurak et al., 2021; Neige et al., 2023; Bevacqua et al., 2024). To our knowledge, however, there have been no studies that investigate the changes in SMC-M1 connectivity following motor sequence learning, especially

in the context of skill acquisition that involves the emergence of motor chunks. The purpose of the present study was to examine the effect of learning a novel motor sequence on the connectivity of SMC and M1. 51 right-handed undergraduate students (mean age: 20.39 ± 2.20; 38 females) participated in this study. The individuals were randomly assigned to one of the three sequential learning conditions: (1) implicit sequence learning (n = 17), (2) explicit sequence learning (n = 17), or (3) random sequence condition (n = 17). Electromyography signals were sampled from the right first dorsal interosseous (FDI) muscle, and the mean peak-to-peak MEPs of the FDI muscle were analyzed to quantify the excitability of cortical motor neurons. The stimulation intensity administered at the left M1 was 110% of the resting motor threshold (rMT), and the intensity administered at SMC (i.e., 4 cm anterior to Cz in the 10-20 system) was 140% of rMT. The coil orientation for conditioning SMC was 270 degrees to the midline of the brain (Arai et al., 2012), whereas the coil orientation for M1 stimulation was 45 degrees to the midline. The inter-stimulus interval between conditioning and test stimuli was 7 ms (Rurak et al., 2021). All participants experienced two separate administrations of the dual-coil SMC-M1 ppTMS before and after the skill acquisition period: (1) baseline stimulation and (2) post-training stimulation. The findings of the current study confirmed the facilitatory influence of SMC on M1 (22.28% larger MEPs) in accordance with the observation of the previous studies. In addition, our findings revealed that the facilitatory influence of SMC on M1 diminished following explicit sequence learning only during which motor chunking was revealed (p = .0001). The reduced impact of SMC on M1 might be attributed to the role of the basal ganglia (more specifically, putamen), which is the only region that has inhibitory connectivity among SMC-M1 pathways, in motor chunking (cf. Wymbs et al., 2012). Future studies are required to dissociate unique contributions from explicit learning strategies and motor chunking per se to the reduced influence of SMC on M1.

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Poster

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Topic: E.04. Voluntary Movements

Support: VA CDA IRX004558A

Title: Decoupling of inhibition during replay events in micro-offline periods during sensorimotor learning

Authors: *S. ARROYO[1,2], S. BARATI[3,2], K. GANGULY[3,2];
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Abstract: Animals are adept at associating sensory stimuli with motor actions that lead to reward. Such learning requires that sensory input be bound or linked to a motor program. Strikingly, several studies have shown that when learning a new task, a substantial proportion of learning occurs in brief rest periods that are interspersed with task performance. However, the mechanisms that enable such “micro-offline” gains during learning are poorly understood. Prior work has shown that inhibitory circuits consisting of somatostatin (SST) expressing interneurons, cells that preferentially inhibit the apical dendrites of neighboring pyramidal neurons, may play an important role in facilitating and maintaining learning-induced cortical dynamics. To investigate how SST neurons might regulate offline sensorimotor learning, we have recorded from the secondary motor cortex (M2) of mice as they learn an auditory cued lever-push task. We employed acute neural recordings with custom Neuropixels optrodes which allowed for identification of channelrhodopsin-2 (ChR2) expressing SST neurons *post hoc*. Each training session consisted of 5 blocks of 20 trials, interspersed with brief 2.5 minute ‘micro-offline’ periods during which reactivations of task-related cortical activity could be measured. We found that mice rapidly learned the task over 1-4 days, with learning manifesting as within block, across block, and across session gains in rewarded frequency and reaction time. During the task, we observed that SST activity was highly correlated with neighboring untagged neurons (presumably pyramidal neurons). However, during micro-offline rest periods, SST activity was uncoupled from pyramidal activity, with reactivations in untagged networks showing less correlation with SST activity. This finding suggests a mechanism by which offline plasticity might be enhanced in the relative absence of SST-mediated inhibition. To test this hypothesis, in preliminary experiments, we stimulated SST neurons during the duration of the offline period in a separate cohort. In these animals, improvements in task performance were impaired. Together these data suggest a potential mechanism for facilitating micro-offline potentiation of sensorimotor learning in cortical networks and suggest an important role for inhibitory regulation of cortical plasticity during resting wakefulness.

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Poster

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Topic: E.04. Voluntary Movements

Support: NIH Grant R01NS111982

Title: Spatio-temporal patterns across the motor cortical sheet decode kinematics

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Abstract: The classical view of the primary motor cortex (M1) emphasizes its somatotopic organization but fails to account for temporal and spatial dynamics across the motor cortical sheet. Previous research has shown that amplification times of the high-frequency gamma band (200-400 Hz) of the local field potential (LFP) spatially propagate across M1 at the movement onset during a center-out reaching task. The direction of this propagating pattern carries kinematic information about the movement direction. Since high-gamma LFP amplitude is a close proxy for multi-unit activity, these findings suggest that an orderly recruitment sequence of activity across the motor cortical sheet provides behaviorally relevant information. Here, we extend this work by investigating instantaneous spatial patterns of the high-gamma over time, rather than only at the movement onset. We analyze neural data recorded from two rhesus macaques with implanted neural arrays in M1 while they performed a planar center-out task in eight different directions. We compute the spatial gradient of normalized high gamma amplitudes across the array over time using a plane from which we can estimate the gradient direction and magnitude. The gradient direction across the motor cortex dynamically varies throughout a reaching movement and differentiates between different reach directions. Using the time-varying gradient direction, it is possible to decode hand velocity continuously over time using a linear decoder. The decoder performs particularly well at the beginning of movement but then the performance degrades over time suggesting that these dynamic spatial patterns encode movement parameters intermittently. This intermittent hypothesis is supported by a subset of trials from one of the two monkeys which was overtrained to make movements to one target. This behavior led to movement paths that were initially directed to the overtrained target and then switched direction to reach the neighboring instructed target, resulting in bent paths. In these bent path trials, we demonstrate that these patterns carry intermittent information about hand velocity at the beginning of the movement and after the transition point where the hand changes direction. Overall, these results offer a novel perspective on how information about movement initiation and execution is encoded in M1 in the form of spatio-temporal patterns across the cortical sheet.

Disclosures: M. Rybar: None. **W. Liang:** None. **N.G. Hatsopoulos:** Other; N.G.H. serves as a consultant for BlackRock Microsystems, the company that sells the multi-electrode arrays and acquisition system used in this study..

Poster

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Topic: E.04. Voluntary Movements

Support: NIH Grant NINDS K12

Title: Unraveling beta dynamics with a high-density cortical surface array

Authors: *Q. QURESHI[1], P. DAVIS[1], A. VAZ[1], J. HAGGERTY[1], M. KIM[1], K. WINGEL[1], E. HO[2], B. BYUN[2], B. PESARAN[1], B. I. RAPOPORT[3], I. CAJIGAS[1];
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Abstract: Understanding the dynamic patterns of neural activity in the motor cortex during movement is pivotal for advancements in neurological treatment, rehabilitation, and brain-computer interfaces. Exploring neural oscillations such as beta waves, which are linked to movement control, is central to understanding motor function. Recent hypotheses on the spatiotemporal dynamics of beta focus on traveling waves (Zich et al., 2023). However, observing these dynamics has been challenging due to hardware limitations in array size and contact density as well as difficulties in clinical accessibility to the motor cortex. In this study, we seek to characterize beta spatiotemporal dynamics at a unique resolution and scale. Our study employs a high-density 1024-contact cortical surface array in a 2x2cm area to overcome traditional limitations in spatial resolution, providing a never-before-seen view into how these dynamics vary spatially across the motor cortex during and after movement. For an essential tremor patient who underwent thalamic deep brain stimulation (DBS), we placed the electrode array on the top of the hand knob of the motor cortex. During the DBS-OFF condition, the patient mirrored the gestures of rock, paper, and scissors while wearing inertial sensors to simultaneously record hand kinematics. To explore the spatiotemporal dynamics of beta oscillations during the motor task we applied the Hilbert Transform to extract phase components including phase gradients, divergence, and curl of the signal. We observed prominent beta spirals during rest periods, but suppression of beta activity during movement intervals. Relative to rest, beta spirals also reversed their angular direction and radiated outward from a focal point on the array during movement. Our results in this pilot study highlight the spatiotemporal evolution of beta-oscillatory behaviors in the motor cortex. The presence of these spirals may indicate specialized regions within the motor cortex that function as hubs, potentially guiding the distribution and coordination of neural signals across the cortex. The capability to detect and distinguish these features may also offer advantages in neural decoding. Additional studies are necessary to determine the role of such patterns in how the motor cortex processes information and maintains functional connectivity, both locally and in broader brain networks.

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Poster

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Program #/Poster #: PSTR174.18/I38

Topic: E.04. Voluntary Movements

Title: A manifold with oscillatory activity emerges in the macaque premotor cortex in preparation for tapping synchronization with a periodic stimulus

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Abstract: Humans and other animals need to coordinate their actions with predictable structure present in the environment. The corresponding neural substrate is studied by training primates to tap along and if possible synchronize with a periodic stimulus. Single cells in the medial areas of the premotor cortex (MPC) encode the duration and serial order of rhythmic intervals. Properties of the population state trajectory such as its amplitude are also related to the interval of rhythmic tapping (Gámez, Mendoza, Prado, Betancourt, & Merchant, 2019). Such state trajectories are observed because the activities of individual cells are correlated, resulting in lower-dimensional dynamic manifolds over populations of neurons.
We investigated the spiking profile of single cells from MPC and their population dynamics while two monkeys (Macaca mulatta) were in the passive, perceptual stage of a listen-then-synchronize task. The periodic stimuli consisted of discrete visual or auditory signals with a sub-second interval.
Population activity exhibited an oscillatory profile. It had an anticipatory rise time and its peaks were phase-locked to the stimulus. Importantly, the amplitude of each cycle increased over successive beats and reached its maximum at the time when the monkey began tapping. In addition to focusing on the low-dimensional manifold, we investigated the scaling of population dynamics by looking at the full range of dimensionality loadings from principal component analysis.
These results are easier to reconcile with a resonance model of regularity detection rather rather than neural entrainment models which posit that intrinsically periodic activity is aligned with the stimulus during such tasks. Furthermore, we found evidence both for high-dimensional activity with scaling properties and for a low-dimensional oscillatory component emerging over background activity.

Disclosures: D. Dotov: None.

Poster

PSTR174: Cortical Planning and Execution: Neurophysiology

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Topic: E.04. Voluntary Movements

Support: NIBIB U24EB028998
NYS DOH1-C32250GG-3450000

Title: Unveiling the neural basis of behavior-related manifolds in a comprehensive model of primary motor cortex circuits

Authors: *R. BARAVALLE[1], V. BRAGIN[1,2], N. NOVIKOV[1], W. XU[3], E. URDAPILLETA[4], I. C. DUGUID[3], S. DURA-BERNAL[1,5];
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Abstract: Accumulating evidence strongly suggests the existence of low dimensional neural manifolds in the primary motor cortex (M1) associated with motor behavior. Researchers have shown that these low-dimensional representations result from the combined activity of M1 neurons, and are highly consistent across individuals performing the same motor task. However, the specific cell types, cortical layers, and biophysical mechanisms underlying these latent dynamics remain largely unknown. Understanding the relationship between these manifolds and its neural substrate is important to characterize the neural computations underlying behavior. It may also lead to wide-ranging applications, such as developing stable and easy-to-train brain machine interfaces for spinal cord injury. We previously developed a realistic computational model of M1 circuits, which included detailed corticospinal neurons models, responsible for sending motor commands to the spinal cord. We validated the M1 model against in vivo spiking and LFP data and demonstrated it can generate accurate predictions and help to understand brain disease. Moreover, we showed that the M1 dynamics could be represented by low dimensional manifolds that varied according to the experimental condition. Here, we extended the M1 model to incorporate two new interneuron types, and tuned it to reproduce the specific manifolds associated with mouse in vivo recordings during a motor task. For this, we analyzed associations between low dimensional embedding of spiking patterns in M1 and behavioral outcomes in experiments on mice performing a single-target joystick reaching task. During the task, the spiking activity of hundreds of neurons in M1 and ventrolateral thalamus was recorded using Neuropixels probes. We jointly studied spiking patterns and joystick trajectories to identify commonalities in the low-dimensional embeddings, and built a decoder that could predict joystick trajectories from spiking data. To tune the M1 model to reproduce neural manifolds, we explored different approaches, including varying long-range inputs to a specific network instantiation and modifying circuit connectivity via global optimization algorithms. Reproducing experimental behavior-related neural manifolds in large-scale detailed cortical models can serve to 1) link circuit dynamics across scales (membrane voltages, spikes, LFPs, and EEG) to behavior, manipulations, and disease, 2) further constrain the model, and 3) characterize the relation between low-dimensional latent dynamics and the activity of specific cell types. This may lead to better understanding of how the brain circuits generate motor behavior.

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Poster

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Topic: E.04. Voluntary Movements

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Title: Dendritic neurodegeneration precedes age-associated impairment in swallow function and nucleus ambiguus motor neuronal death in Fischer 344 rats

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Abstract: The nucleus ambiguus contains motor neurons (MNs) innervating the muscles of the larynx, pharynx and oesophagus. Many of these motor units these muscles are comprised of are fast fatigueable units - highly vulnerable to neurodegeneration. Loss of the MNs or damage to the nerves innervating the muscles of the larynx, pharynx and oesophagus may impair swallow, vocalization and/or respiratory functions. Of particular importance, human patients with deficits in swallow are at risk of sequelae such as aspiration pneumonia and malnutrition. In aged male Fischer 344-Brown Norway crosses, age-associated loss of nucleus ambiguus MNs contributes to vocalization deficits. Here, we use female and male Fischer 344 (F344) at young (6-months), middle-aged (18-months) and old (24-months) to evaluate dendritic arbors swallow function and nucleus ambiguus MN survival. Swallow function was evaluated under anesthesia by delivering an 0.3 mL bolus of water to the base of the tongue and videoing the number of subsequent swallows. A pressure catheter was inserted into the oesophagus to evaluate the incidence and duration of wallow-associated apneas and the magnitude of the schluckatmung in 30 s following bolus delivery. Following functional evaluations, rats were euthanized, perfused with paraformaldehyde and brainstem tissue cryopreserved, sectioned and stained with Nissl to stereologically evaluate the number of nucleus ambiguus MNs. Unfixed samples were impregnated in golgi-cox solution and processed for pseudo-confocal imaging. At middle-age, we observed dendritic arbor and dendritic spine losses consistent with neurodegeneration. In old age, we observed an ~30% reduction in the number of swallows per delivery of the water bolus and an increased incidence and duration of swallow apnea. Schluckatmung was not affected by age. The number of nucleus ambiguus MNs was reduced by ~20% in old F344 rats, with MN size unaffected. We propose that swallow evaluations are a robust readout of oropharyngeal behaviour, with deficits concomitant with MN death. These findings are similar to age-associated weakness in other skeletal muscle being related to MN death. It is likely that the mechanisms underpinning fast fatigue motor unit MN death in brainstem and spinal pools are similar.

Disclosures: M. Fogarty: None.

Poster

PSTR175: Speech and Oral Movements

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR175.02/J1

Topic: E.04. Voluntary Movements

Support: Supported by CCTS at UIC and the Shirley Ryan AbilityLab

Title: Tongue Yoga: Preliminary efficacy of subject-specific visual-feedback intervention for tongue movement

Authors: A. SCARPELLINI[1], A. CARROLL[1], E. BABBITT[2], J. L. PATTON[3], *H. ESMAILBEIGI[4];
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Abstract: Restoring tongue function is a primary concern after a neurological injury due to the critical role of the tongue in speech, breathing, and swallowing. Effective rehabilitation of tongue function relies on the provision of personalized interventions and the ability to assess their effectiveness. We have developed a quantification method for tongue movement deficit calculation, which involves our standalone wireless intraoral Tongue-Trackpad device, which provides real-time visual feedback. Five participants diagnosed with either dysarthria or apraxia of speech participated in this study across seven sessions. At the start of the first session, participants engaged in a *free-exploration* task where they were directed to continuously touch as much of the explorable area of the Tongue-Trackpad as possible without repeating patterns. Through contrasting participant's movement distributions with average tendencies from individuals who are neurotypical (*healthy model*), participant-specific deficit areas were quantified. A speech-language pathologist tailored 14 specific trajectories to encourage the participant's tongue movement to their deficient areas (*precision therapy*). Participants then attempted to follow a moving target along the trajectories while receiving real-time visual feedback. At the end of the session, the free-exploration task was repeated to quantify changes. On average, participants showed a 4.8±5.2% increase in coverage area across each session and a corresponding deficit area reduction of 2.1±1.1%. However, the retention of these benefits to the next session remained modest. Furthermore, there was a progressive reduction in tracking errors across sessions for all participants. This is promising preliminary evidence of how visual feedback and precision therapy can enhance tongue movement range. Further analysis with more sessions and participants is required to fully determine this intervention's long-term safety, feasibility, efficacy, and effectiveness.

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Poster

PSTR175: Speech and Oral Movements

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Program #/Poster #: PSTR175.03/J2

Topic: E.04. Voluntary Movements

Support: NIH Grant NS110169
NIH Grant HD110951

Title: Characterization of swallow-related sympathetic nerve activity

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Abstract: Sympathetic tone controls vascular tone, and thus the homeostatic maintenance of arterial blood pressure. While the cervical sympathetic nerve (cSN) is known to innervate the upper airways, the activity of the cSN during swallow behavior and any role it might play in swallow dysfunction have not been the subject of many studies. In this study, we characterized cSN activity in rodents to assess its coordination with swallow and breathing. To achieve this aim, we recorded electromyography (EMG) of diaphragm, submental, and laryngeal complex muscles, and neurograms of cervical sympathetic, hypoglossal, and vagus nerves in baseline conditions and during swallow stimulation by injecting 0.1 mL of water into the mouth. Bursts of cSN activity were coincident with both inspiration and swallow. Water swallows triggered higher amplitudes of cSN activity compared to the respective baseline respiratory cycle. Our data suggest that swallow triggers an excitatory vasomotor sympathetic pathway which is involved in the control of upper airway motor activity.

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Poster

PSTR175: Speech and Oral Movements

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Program #/Poster #: PSTR175.04/J3

Topic: E.04. Voluntary Movements

Support: HL160102
HL144801
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HL126523

Title: Silencing the caudal Nucleus of the Solitary Tract and the Postinspiratory Complex to unravel swallow and breathing neurocircuitry

Authors: *A. HUFF[1], L. M. OLIVEIRA, Sr.[2], J. RAMIREZ[1];
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Abstract: The production of swallow and its coordination with breathing is essential for homeostasis in most organisms. The postinspiratory complex (PiCo) is thought to regulate the transition between swallowing and breathing. While the activation of PiCo triggers swallow production and stimulates laryngeal activation in a respiratory phase specific manner, the effects of silencing PiCo neurons is unknown. Additionally, little is known about PiCo's role in swallow generation, which is thought to be controlled by the caudal portion of the Nucleus of the Solitary Tract (cNTS), presumably the swallow pattern generator (SPG). We hypothesize that silencing PiCo specific neurons dysregulates swallow-breathing coordination and PiCo is not necessary for swallow production, rather the cNTS is necessary to trigger a swallow. To test these hypotheses, both male and female ChAT-Cre:Vglut2-FlpO mice are bilaterally injected with a Cre/FlpO-dependent inhibitory DREADD. In a different subset of animals, ChAT-Cre:Vglut2-FlpO mice are bilaterally injected with a Cre/FlpO-dependent ChR2 AAV into PiCo and a FlpO-dependent inhibitory DREADD into the cNTS, specifically targeting the interstitial (SolI) and intermediate (SolIM) solitary nucleus. Twenty-one days after injection, using our freely breathing urethane anesthetized mouse model, swallows were stimulated by 1) injection of water into the oral cavity and/or 2) optogenetic stimulation of PiCo neurons; before and after administration of DREADD activator, clozapine-N-oxide (CNO). Swallow and laryngeal activity were measured via monopolar suction electrodes of the hypoglossal (XII) and vagus (X) nerves as well as bipolar electromyogram (EMG) of the submental, laryngeal complex and costal diaphragm muscles. Post-hoc histological analysis was done to confirm areas of interest were targeted. **We found that breathing was slowed, postinspiration abolished, and swallowing, along with its interaction with breathing, were altered after activation of the inhibitory DREADD, located in PiCo. Silencing the cNTS resulted in an inability to evoke swallows either by water stimulation or optogenetic stimulation.** We confirm that PiCo is important for postinspiration, normal swallow production, swallow-breathing coordination, and relies on direct feedback from the cNTS to trigger swallow production.

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Poster

PSTR175: Speech and Oral Movements

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Program #/Poster #: PSTR175.05/J4

Topic: E.04. Voluntary Movements

Support: JSPS KAKEN 21K16629

Title: Motor and sensory cortical processing of neural oscillatory activities revealed by human swallowing using intracranial electrodes

Authors: *H. HASHIMOTO[1,2], M. HIRATA[2];
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Abstract: Introduction Swallowing is a complex neuromuscular process involving coordinated motor and sensory components. The orchestration of motor output and sensory input is critical for effective swallowing execution. In this study, we investigated the neurophysiological differences between motor and sensory functions using a swallowing task. **Methods** Eight participants with epilepsy, implanted with intracranial electrodes over the orofacial cortex, performed swallowing tasks involving the ingestion of a water bolus at their own pace without external cues. Mouth-opening and swallowing were categorized as motor tasks, while water injection was considered a sensory task. Electrocorticography (ECoG) signals were recorded during the swallowing tasks. Swallowing was concurrently monitored using electroglottography, a neck-attached microphone, and an RGB camera. **Results** Phase-amplitude coupling (PAC) analysis was applied to investigate swallowing-related ECoG signals. Specifically, we examined phase-amplitude coupling between lower frequency bands (alpha and theta) and high gamma (HG) bands (75-150 Hz). Our findings revealed distinct patterns of coupling: an alpha-HG coupling preceding motor-related HG power increases and a theta-HG coupling coinciding with sensory-related HG bursts. Notably, motor-related coupling peaks occurred approximately 0.6-0.7 seconds earlier than HG power peaks.Moreover, motor-related HG activity was modulated by the trough of alpha oscillations, whereas sensory-related HG amplitude was modulated by the peak of theta oscillations. **Conclusion** These contrasting neural dynamics observed during swallowing tasks offer insights into the sensory-motor functions of the human brain. Understanding these processes may contribute to advancing our knowledge of neural mechanisms underlying swallowing and related motor-sensory interactions.

Disclosures: H. Hashimoto: None. **M. Hirata:** None.

Poster

PSTR175: Speech and Oral Movements

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Topic: E.04. Voluntary Movements

Support: ANR grant, ANR-21-CE28-0022
NIDCD R01-DC017439

Title: On-line reflex control for tongue posture stabilization during speech production

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Abstract: Tongue posture can be controlled using on-line sensory feedback for the stabilization of produced speech sounds. Although reflex mechanisms such as stretch reflex have been known to be involved in neural feedback control for the limb system, previous studies have been failed to provide evidence for any short-latency reflex in the human tongue. In our behavioral study using tongue-stretch perturbation, a relatively quick compensatory response (< 130 ms) was found for tongue posture stabilization in vowel production, which might be driven by short-latency reflex mechanisms. To further investigate this idea, this study aims to show neurophysiological evidence for reflex in the tongue during vowel production by the recording of tongue muscle activity. Due to the technical difficulty to combine movement recording with electromyographic (EMG) recording, we also carried out a computer simulation to examine whether the recorded EMG response can reproduce the previously observed articulatory response with the appropriate latency. In the test, we applied tongue-stretch perturbation using a robotic device during the production of the vowel /i/. EMG was recorded from the anterior part of the mouth floor using a uni-polar surface Ag-AgCl electrode. EMG signals from this site reflect in large part the activations of the Genioglossus and Geniohyoid muscles, which are largely involved in the production of /i/. We examined whether EMG signal from the tongue increased in response to tongue-stretch perturbation during the speech task. We also examined whether this response was modulated at resting state, in a voluntary reaction and in a non-speech task respectively. In speech condition, we found an increase of muscle activation in response to tongue-stretch perturbation. The latency was around 50ms. In the resting condition, a response was also induced with a similar latency, but with a reduced amplitude. This response was also induced earlier than the muscle activation induced by voluntarily reaction. A similar response was also seen in non-speech condition, but its peak amplitude was smaller than in the speech condition. All these observations indicate that the observed EMG response was induced by a reflex mechanism and that this reflex is task-dependent. Computer simulations considering muscle dynamics between EMG and force showed that the articulatory response cannot be reproduced by a passive component alone, and that the simulated movement was compatible with the previously observed articulatory response. The results demonstrate the existence and clear involvement of reflex mechanisms in tongue posture control during speech production.

Disclosures: T. Ito: None. **M. Bouguerra:** None. **M. Bourhis:** None. **P. Perrier:** None.

Poster

PSTR175: Speech and Oral Movements

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Topic: E.04. Voluntary Movements

Support: ERC StG 335880
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Evangelisches Studienwerk Villigst

Title: Neural representations of the content and production of human vocalization

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Abstract: Human speech allows to convey information via different execution forms. Thus, on the behavioral level, speech content is independent from production and execution forms. However, it remains unclear whether such a content dimension can be found on the neural level and whether it is possible to dissociate it from the motor dimension. To address this, we recorded magnetoencephalography (MEG) in human subjects that performed two rule-based vocalization tasks. In the first experiment (n=24), content (vowel /u/ or /ʊ/) and production (overt or covert) were instructed separately and in random order. Applying multivariate pattern analysis (MVPA), we were able to decode both content and production independently and several seconds before vocalization behavior. The temporal dynamics of information reflected cue order. In the second experiment (n=30), we used three vowels (/u/, /ü/ and /ʊ/) to assess effects of motor effort. We found that, in line with an abstract neuronal representation, content information indeed generalized across production types. Furthermore, substantial decoding of all vowels, even those with similar motor effort, and little cross-decoding between content and production information suggested content representations to be largely independent from motor effort. In addition, cross-decoding suggested that the content representations are at least two-dimensional, exceeding simple effort dynamics. Together, our results provide insights into the neural dynamics underlying human vocalization and open a new window for noninvasive speech research in health and disease.

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Poster

PSTR175: Speech and Oral Movements

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Topic: E.05. Brain-Machine Interface

Support: R01 NS129703

Title: Neural sub-processes linking speech perception and production

Authors: *A. M. EARLE-RICHARDSON[1,2], S. DURAIVEL[3], G. B. COGAN[4], D. G. SOUTHWELL[5,6,7], M. VESTAL[5,7], G. A. GRANT[5,7];
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Abstract: While typically studied as separate processes, speech perception and production are intrinsically linked in the brain. Evidence for this link comes from conduction aphasia (CA) patients with intact speech comprehension and production but deficits with repetition. The converse set of deficits are found in transcortical aphasic patients, implicating an isolated pathway for speech repetition independent of comprehension. While traditional models propose one pathway as the anatomical locus of this linkage, there is evidence that repetition cannot be attributed to any one neuroanatomical substrate (Buchsbaum, 2011). Furthermore, there is no one-to-one mapping of sound properties in perception to motor properties in production (Liberman, 1967). This suggests that multiple parallel neural sub-processes facilitate the link between speech perception and production (Hickok, 2022). We investigated the sub-processes involved in speech repetition using intracranial recordings for their high spatio-temporal precision. We performed neural recordings from 31 subjects undergoing intracranial monitoring for treatment of epilepsy (mean age = 31, 17 female). Subjects performed a repetition task where they listened to a word that they repeated after a delay, mimed after a delay, or passively listened to. Statistical significance of the high gamma response (HG, 70-150 Hz) for each time point and electrode served as an index of local neural computation (cluster-corrected at $p < 0.05$). We replicated previous results (Cogan et al. 2014, 2017) by showing distinct auditory (163 electrodes), production (553), and sensory-motor (SM - both auditory and production) responses (235). We next investigated the morphology of neural sub-processes by performing an unsupervised decomposition of the temporal profile of HG responses within each electrode category. Results from SM electrodes revealed four distinct components that overlap in time with perception and production: 1) a primarily visual response to the go cue (localized to the occipital and inferior temporal cortex), 2) an early auditory response that also peaked during late production (posterior/superior temporal cortex), 3) a later auditory response that also peaked pre-production (premotor/motor and parietal cortex), and 4) a working memory component showing sustained HG delay activation in the repetition vs. passive listen condition (prefrontal, parietal, and superior temporal cortex). Our results show speech repetition can be broken down into neural sub-components, supporting the hypothesis that the link between speech perception and production is supported by multiple parallel neural sub-processes.

Disclosures: A.M. Earle-Richardson: A. Employment/Salary (full or part-time):; Duke University. **S. Duraivel:** None. **G.B. Cogan:** None. **D.G. Southwell:** None. **M. Vestal:** None. **G.A. Grant:** None.

Poster

PSTR175: Speech and Oral Movements

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Topic: E.04. Voluntary Movements

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Weill Neurohub

Title: Sensorimotor theta oscillation coordinates articulatory movements during speech production

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Abstract: Speech is a defining human behavior. Speakers use a limited number of basic phonetic elements to convey an unlimited range of thoughts. When speaking fluently, our brain generates rapid and precisely timed motor commands that orchestrate the movement of nearly 100 muscles across our speech organs. While previous research has implicated distributed neural representations within the sensorimotor cortex (SMC) in governing these processes, the mechanism by which the underlying neuronal populations achieve precise temporal coordination remains elusive. Using high-density electrocorticography (ECoG) recordings, we identified a prominent 8 Hz theta oscillation within the local field potential of SMC that persisted during both speech and silent intervals. Following speech initiation, we observed a notable increase in theta coherence among sensorimotor speech-responsive sites. This distributed theta-coherent network displayed strong phase-amplitude coupling, with increased high-gamma activity (i.e., population firing) around the theta troughs. Notably, the oscillation maintained a stable frequency despite marked variations in speech rate, suggesting a potential internal origin and not an artifact of speech syllable rate. To explore potential connections between this cortical oscillation and concurrent articulatory movements, we employed a task where participants spoke hundreds of sentences in English. Utilizing a deep learning approach, we inferred articulatory movements from the produced speech acoustics and monitored the Articulatory Kinematic Trajectories (AKTs) for the jaw, lips, and tongue in each participant. Analysis of the AKTs revealed semi-rhythmic motor events characterized by coordinated, pulse-like, changes in AKT velocity, indicative of vocal tract reshaping linked to phoneme production. Strikingly, during fluent speech, these coordinated movements occurred at an average rate of 7-8 pulses/sec and exhibited strong coupling to the ongoing sensorimotor LFP oscillation. Our findings reveal a previously unknown function of theta oscillation in speech motor control, demonstrating its potential role as an internal pacemaker for timed neuronal interactions and coordination. The properties of this neural rhythm within the SMC speech circuits are ideally suited to synchronize excitation across distant neuronal populations, thereby facilitating the coordinated activation of the distributed motor representations essential for fluent speech production.

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Poster

PSTR175: Speech and Oral Movements

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Topic: E.04. Voluntary Movements

Support: AWD-004500

Title: Entrainment of theta oscillations associated with speech inhibition through synchronization of left SMA and IFG

Authors: *K. JOHARI[1], F. TABARI[2];
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Abstract: The dynamic communication between the left supplementary motor area (SMA) and the inferior frontal gyrus (IFG) is crucial for speech planning and execution. The left frontal aslant tract (FAT) is a white matter tract that connects these two regions and subserves speech and language functions. Disruption of FAT has been associated with speech-motor disorders such as stuttering and apraxia of speech. Therefore, it is reasonable to assume that non-invasive neuromodulation techniques may enhance the compromised connectivity between these two regions and improve speech production in neurogenic communicative disorders. In the present study, we examined whether synchronization of the left SMA and IFG through 4 Hz high-definition alternating current stimulation (HD-tACS) could modulate theta activity (4-8 Hz) - a neural marker of motor inhibition- during a speech production task. Twenty-two neurotypical adults, with no speech or language impairments, received three counterbalanced sessions of synchronized (i.e., no phase lag between stimulation waveforms in SMA and IFG), de-synchronized (i.e., 180-degree lag between stimulation waveforms in SMA and IFG) and sham, 4 Hz HD-tACS over the left SMA and IFG. There was a one-week interval between stimulation sessions to minimize task carryover effects. Following each stimulation session, EEG data was collected while participants performed a speech Go/No-Go task. We hypothesized that, relative to sham and desynchronized stimulation, synchronized 4 Hz HD-tACS would lead to a prominent increase in the theta activity associated with speech inhibition during No-Go trials. The results confirmed our hypothesis by demonstrating stronger theta activity over frontal and frontocentral electrodes following synchronized HD-tACS compared to de-synchronized and sham HD-tACS. These findings suggest a more pronounced entrainment of neural markers of motor inhibition following synchronized theta HD-tACS over the FAT. To our knowledge, this is the first study that provided a proof of concept that synchronization of left SMA and IFG can enhance theta activity associated with inhibitory processes of speech production and have translational implications for neurogenic communicative disorders

Disclosures: K. Johari: None. **F. Tabari:** None.

Poster

PSTR175: Speech and Oral Movements

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Title: Dorsal and Ventral LMC: A Tale of Two Laryngeal Motor Cortices

Authors: *N. KSHATRIYA[1,2], G. BATTISTELLA[1], L. O'FLYNN[1,2], R. BELISLE[1], S. A. FRANKFORD[1], K. SIMONYAN[1,2,3];
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Abstract: *Background:* The human laryngeal motor cortex (LMC) is critical for our ability to produce voluntary, learned vocal behaviors. Despite its importance within the voice and speech production network, the location of the LMC within the dorsal portion of ventral primary motor cortex (M1) has been defined relatively recently through a series of brain imaging and electrocorticography studies. Some of these studies reported additional neural activity in the ventral portion of precentral gyrus during production of vocal tasks, forming a notion of the dual LMC representation. However, whether these two regions, often referred to as dorsal and ventral LMC, have indeed functional and structural organization to support laryngeal motor control is unknown. *Methods:* We acquired resting-state fMRI and diffusion-weighted imaging (DWI) at ultra-high-resolution on a 7T MRI scanner in 16 healthy, native-English speakers (53.9±12.5 years; 9 F/7 M). In parallel, we performed a systematic, coordinate-based, activation likelihood estimation (ALE) meta-analysis of neuroimaging literature reporting voice and speech production (n = 40 experiments) to identify the spatial convergence of peak activity in M1 relevant to dorsal and ventral LMC locations. Using the meta-analytical clusters as seed regions, we conducted resting-state functional connectivity and probabilistic diffusion tractography analyses to investigate the connectivity profiles of dorsal and ventral LMC representations. *Results:* The ALE meta-analyses identified bilateral clusters in the dorsal portion of ventral M1 (area 4p) consistent with the dorsal LMC location and a left cluster in the ventral primary somatosensory cortex (area 3b) corresponding to the ventral LMC location. Compared to the ventral cluster, the dorsal clusters had greater connectivity with orofacial motor, premotor, superior parietal, occipital cortices, and putamen. Compared to the dorsal clusters, the ventral cluster had greater connections with primary somatosensory cortex, parietal operculum, and insula. *Conclusions:* The LMC is located in the dorsal portion of the ventral M1 and its connectivity profile follows that of a typical motocortical region. Conversely, the ventral location of neural activity, referred to as ventral LMC, is mapped to primary somatosensory cortex and

not M1. As such, the term ‘ventral LMC’ is misleading and should not be used when describing motocortical representation of laryngeal control. Future studies are warranted for clarification of the exact role of this ventral region in voice and speech control.

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Poster

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Topic: E.04. Voluntary Movements

Support: AI Center Postdoctoral Fellowship

Title: Towards a theoretical understanding of the control systems in speech perception and articulation

Authors: *R. MENDELSOHN[1], N. BERNAT[2], T. J. SEJNOWSKI[1], C. AMO ALONSO[3]; [1]Salk Inst. For Biol. Studies, San Diego, CA; [2]Comp. & Math. Sci., Caltech, Pasadena, CA; [3]AI Ctr., ETH, Zurich, Switzerland

Abstract: Speech production is one of the most sophisticated sensorimotor control (SMC) tasks performed by humans. Making intelligible speech requires a high degree of accuracy that can be achieved using sensory feedback-based error correction, yet speech is also performed at high speeds despite the time delays that should result from feedback control. Similarly to moving one's arm to reach a physical target, repetition of short sounds (either phonemes or syllables) can be modeled as reaching for internalized auditory or articulatory "targets". To address the longer sequences of syllables characteristic of natural language, most SMC speech models combine two control elements, a lower-level feedback controller keeps the system on target while a higher-level feedforward controller determines the sequence of targets. The architecture of how these elements interface with one another plays a significant role in determining how a syllable sequence is followed. However, comparing different control architectures represented by existing models poses a challenge because architectural assumptions can be difficult to separate from other features. To facilitate analysis of these key assumptions, we introduce a common framework which encompasses existing models and offers a general, simplified model. By distilling the models down to their architectures, we produce a modular structure in which simple functional components can be conceptualized, tested, and expanded upon. Simulations with the general model can be used to test novel and existing design features for their ability to handle different sequences, reproduce human speech phenomena, and operate realistically when connections are lesioned. Of specific interest is the relationship between the core computational elements and human neuroanatomy. To better describe the mechanisms behind speech and speech-related disorders, we simulate pitch perturbation experiments. By reproducing

experimental results with this highly abstract and simplified model, we offer a useful tool for understanding the fundamental computations involved. We explore how altered pitch responses in disorders like cerebellar degeneration, apraxia of speech, or conduction aphasia can be explained in a more detailed and differentiable manner by breaking feedforward and feedback controllers into key elements. Our framework will allow for the modeling of increasingly complex speech phenomena in an insightful manner. This is a key step in connecting SMC to the higher levels of cognition involved in speech, enabling understanding of how articulation and perception affect the formation of language.

Disclosures: R. Mendelsohn: None. **N. Bernat:** None. **T.J. Sejnowski:** None. **C. Amo Alonso:** None.

Poster

PSTR175: Speech and Oral Movements

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR175.13/J12

Topic: E.04. Voluntary Movements

Support: R01 NS 058487

Title: Speech unsteadiness and inconsistency in Parkinson's disease

Authors: *J. HUBBARD[1], J. J. KIM[1], S. DELMAS[1], R. J. MALIK[1], Y. CHOI[2], B. YACOUBI[1], E. A. CHRISTOU[1];
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Abstract: Two common clinical features predating a clinical diagnosis of PD are motor unsteadiness and speech impairment (e.g., hypokinetic dysarthria, articulatory decay, dysfluency, etc.). Although several previous studies have examined speech characteristics corresponding to Parkinsonism, none have decomposed speech signals to examine speech unsteadiness and inconsistency. Thus, the purpose of this study was to characterize differences in speech unsteadiness and inconsistency in PD and matched healthy controls. Here, we compared the structure of speech signals in PD (n=16), and healthy controls (n=15) when performing discrete phonations (i.e., "PA" (puh) and "PATAKA" (puh-tuh-kuh)). Participants performed one set of 30 trials for each phonation test. Similarly to how movement unsteadiness is quantified (first derivative of acceleration, Jerk), we quantified speech unsteadiness as the first derivative of the speech signal. We quantified speech inconsistency as the coefficient of variation of the speech unsteadiness signal across 30 trials. We used a wavelet analysis to sum the normalized power in four different frequency bins: 4-8, 8-12, 12-35, and 35-60 Hz. Our preliminary results indicate individuals with PD have greater speech unsteadiness ($p=0.039$) and greater speech inconsistency across 30 trials ($p=0.009$) when compared with healthy controls. The normalized power in each of the four frequency bins was similar for all individuals across groups. These

findings provide novel evidence that individuals with PD exhibit speech unsteadiness and inconsistency, which could potentially be used as markers for movement control impairments.

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Poster

PSTR175: Speech and Oral Movements

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR175.14/J13

Topic: E.04. Voluntary Movements

Support: NIH Grant R01DC014510

Title: Contributions of basal ganglia and cerebellar circuits in speech auditory-motor adaptation: DBS ON/OFF comparisons in patients with Parkinson's disease vs essential tremor

Authors: H. WANG[1], J. A. HERRON[2], G. CLER[1], A. KO[2], *L. MAX[1];
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Abstract: Different forms of motor learning are thought to involve distinct contributions of the cerebellothalamocortical (CBTC) and basal ganglia-thalamocortical (BGTC) circuits. Work on limb motor control suggests that the CBTC circuit specializes in implicit sensorimotor adaptation driven by sensory prediction error whereas the BGTC circuit is involved in explicit learning. However, direct evidence for the contribution of each circuit in speech motor learning has been sparse. Comparing patients with basal ganglia vs cerebellar disease in Deep Brain Stimulation (DBS) ON/OFF states provides an opportunity to investigate the roles of the CBTC and BGTC circuits in sensorimotor learning for this effector system. In essential tremor (ET), the implant target is usually the ventral intermediate nucleus of the thalamus (Vim), the recipient of cerebellar output in the CBTC circuit. DBS in Parkinson's disease (PD) usually targets the subthalamic nucleus (STN) or the internal globus pallidus (GPi), structures within the BG. Thus, both between-group comparisons (nature of the underlying neuropathology) and within-group comparisons (effects of the electrical stimulation) can provide insights into the respective circuits' role in behavioral task performance. Here, we tested the effects of BG and Vim DBS in speech auditory-motor adaptation paradigms with either a ramped or an abrupt introduction of an auditory feedback perturbation. Each patient performed these tasks twice: once ON DBS and once OFF. Both tasks involve perturbations that increase the first resonance frequency (F1) of the produced speech (e.g., produced "pet" is heard as "pat"). Learning is quantified by measures of opposing changes in the produced F1. The ramped and abrupt perturbation methods were chosen based on prior suggestions that the former mainly involves implicit learning driven by SPE whereas the latter may engage explicit learning processes. Our results to date suggest that ET patients, but not PD patients, show reduced speech auditory-motor adaptation as compared

with age-matched controls. In these initial data, neither the DBS ON vs OFF state nor the ramped vs abrupt introduction of the perturbation affected adaptation. Thus, the current results suggest that the cerebellar pathology underlying ET is detrimental for speech auditory-motor adaptation regardless of the perturbation schedule and that Vim electrical stimulation may not improve this impaired short-term motor learning. The basal ganglia pathology in PD, on the other hand, may spare affected individuals' ability for speech auditory-motor learning.

Disclosures: H. Wang: None. **J.A. Herron:** None. **G. Cler:** None. **A. Ko:** None. **L. Max:** None.

Poster

PSTR175: Speech and Oral Movements

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR175.15/Web Only

Topic: E.04. Voluntary Movements

Support: NSERC-Canada

Title: Enhancing speech motor learning through visual-acoustic biofeedback: efficacy and limitations

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Abstract: Previous clinical research has demonstrated that individuals with speech disorders may benefit from treatments involving the real-time visual presentation of speech motor or acoustic parameters (i.e., visual-articulatory or -acoustic biofeedback). The benefits are presumed to arise from increased perceptual salience of properties related to both the individual's speech output and the speech target. It remains unclear, however, whether beyond short-term improvements in speech accuracy, such tools would improve (or interfere with) retention and generalization of speech motor learning following removal of the visual feedback. In order to systematically explore the efficacy of visual-acoustic biofeedback in speech motor learning, the present study combines the visual feedback of vowel formants (F1 and F2) with a controlled speech adaptation task involving the real-time alteration of vowel acoustics in typical adult speakers. The protocol incorporates a number of features central to the clinical use of visual biofeedback, including a detailed pre-training procedure in which participants are familiarized with the visual acoustic display in combination with the auditory feedback manipulation. 30 participants (in 3 groups) produced monosyllabic words containing the mid-vowel /ɛ/. Following a series of baseline productions under normal auditory feedback, vowel formants were altered in real-time in a manner that was precisely adapted to each participant's vowel workspace (ensuring a comparable magnitude and direction of the required compensatory response in F1/F2 space). In one group, real-time visual feedback of F1 and F2, along with a visual target representing the participant's baseline [ɛ], was provided during the production of each word in the altered

feedback phase (100 tokens). Two control groups underwent the identical speech adaptation procedure without visual feedback of vowel formants (one with the detailed pre-training procedure and one without). The visual feedback group showed an enhanced compensatory response compared with controls — an effect that was successfully maintained following removal of the visual feedback and auditory perturbation. Generalization of speech adaptation to non-trained phonetic contexts, however, showed a more complex pattern between groups that suggests possible limitations of visually-guided speech motor learning. The results provide experimental evidence supporting the potential benefit of using visual-acoustic biofeedback to enhance speech motor learning, although more work is required to better understand its underlying mechanisms and possible limitations.

Disclosures: I. Hocine: None. **D.M. Shiller:** None.

Poster

PSTR175: Speech and Oral Movements

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR175.16/Web Only

Topic: E.05. Brain-Machine Interface

Support: JST FOREST Program (Grant Number, JPMJFR216W, Japan)

Title: Individual neural representations and functional connectivity during overt speech through electroencephalogram-based classification analyses.

Authors: *K. CHIN[1], Y. MARUYAMA[1], Y. OZAWA[1], K. NAKAMORI[1], S.-I. OSAWA[2], K. NIIZUMA[3], N. YOSHIMURA[1];
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Abstract: Neural representation of speech has classically been known to reside in Broca's area. However, recent researches that decoded speech information from implanted electrodes have demonstrated the effectiveness of utilizing signals from the motor cortex. In this study, we investigated how the neural representation of speech varies among individuals using electroencephalography (EEG), which allows for the measurement of brain activity across the entire brain.
Six native Japanese speakers were instructed to read aloud 68 Japanese phonetic characters displayed on a screen as quickly as possible. During the task, continuous recording of EEG with 64 channels took place.
To investigate the activity differences in anatomical brain regions, cortical current source signals were estimated from the acquired EEG. Eight statistical values of the time-domain signals (i.e., maximum, minimum, mean, variance, etc.) were computed for each source and used as feature values for the input of the support vector machine for the binary classification of the five vowels

corresponding to the characters.
The classification accuracies varied by class, participant, and current source. Three of the six participants had more signal sources in the right hemisphere than in the left hemisphere that could be significantly classified. The regions of high accuracy were mainly in the language area including the Broca's area, but different area, such as motor-related area and auditory area, could also significantly classified depending on the participant.
In order to investigate differences in functional connectivity between participants, the debiased weighted Phase Lag Index (dwPLI) was performed on the EEG. Some participants showed similar characteristics, but most of the differences between participants in networks were found in the temporal areas.

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Poster

PSTR175: Speech and Oral Movements

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR175.17/J14

Topic: E.04. Voluntary Movements

Support: Dysphonia International Foundation

Title: Initial evidence of usability of in-home vibro-tactile stimulation to treat voice symptoms of laryngeal dystonia

Authors: *S. AMINI[1], C. OZKUL[2], N. ELANGOVAN[3], J. KONCZAK[4];
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Abstract: Laryngeal dystonia (LD) - also called spasmodic dysphonia - is a chronic focal dystonia affecting the larynx, leading to involuntary muscle spasms that impair speech production and communication. LD is associated with somatosensory dysfunction. Underlying neurophysiological abnormalities include an excessive synchronization of neural activity over laryngeal sensorimotor cortex. Recent evidence from our group revealed that a one-time in-laboratory application of vibrotactile stimulation (VTS) improved voice/speech quality in 69% of patients. These behavioral improvements were associated with reduction in the abnormal synchronization of theta-band event-related spectral power. Building upon this evidence, the current study aimed to investigate the usability and feasibility of in-home laryngeal VTS as a symptomatic treatment for LD. We here report initial data of an ongoing clinical trial.
METHOD: A sample of 13 LD patients (age: 74.1 ± 7.3 years; 11 female) applied VTS at home over an 8-week period. Daily VTS dosage was 20 minutes. Participants progressively increased the number of VTS sessions from 3 days in week 1 to 6 days in week 4 (block1), followed by

four weeks of individually chosen VTS dosage (block 2), not exceeding six sessions/week, which allowed patients to self-determine an optimal weekly dose for effective symptom management in the second four weeks. Outcome measures were *perceived speech effort* (PSE) (scale 1-10, 10 = max. effort) and *duration (D)* of VTS induced effects on speech after each session. RESULTS: 1) Effects of VTS were immediate and 72% of participants responded positively to VTS. Effect magnitude was significant at each block (1^{st}: t= 14.02 p= 0.0001; 2^{nd}: t= 14.96 p= 0.0001). The absolute mean change in PSE was 0.88 (range: -2 to 5) for block 1 and 1.15 (range: -2 to 6) for block 2. The effect lasted for less than 1 hour in 33% of participants, and between 1 hour and 2 days for 66% of participants. DISCUSSION: The study results demonstrated that laryngeal VTS is feasible and can be used effectively as an in-home symptomatic treatment for the voice symptoms associated with LD. Initial analysis corroborated earlier findings showing that approximately two-third of LD patients may benefit from laryngeal VTS.

Disclosures: S. Amini: None. **C. Ozkul:** None. **N. Elangovan:** None. **J. Konczak:** None.

Poster

PSTR175: Speech and Oral Movements

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR175.18/J15

Topic: F.06. Autonomic Regulation

Support: JSPSKAKENHI 23K18356

Title: Involvement of P2X receptor in the initiation of swallows in anesthetized rats

Authors: *T. TSUJIMURA[1], M. YOSHIHARA[3,2], B. J. UNDEM[4], B. J. CANNING[5], M. INOUE[6];
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Abstract: Introduction: A previous study reported that purinergic P2X2/3 receptors in the small subset of vagal neurons that express P2RY1 receptors are involved in laryngeal water- and acid-evoked swallows. Here, we investigated whether P2X and P2RY1 receptors are involved in the initiation of swallows evoked by various natural stimuli. Methods: Experiments were carried out using urethane-anesthetized Sprague-Dawley male rats. A swallow was identified by digastric and thyrohyoid electromyographic bursts. Initially, we evaluated the effect of laryngeal topical application (3 µl) of P2X receptor agonist *αβ*-methylene ATP (1 µM-1 mM) on the initiation swallows. Next, we evaluated the effect of laryngeal application of P2X receptor antagonist PPADS (10 mM) on the initiation of swallows evoked by *αβ*-methylene ATP, distilled water

(DW), carbonated water (CW), citric acid (CA, 10^{-2}M), capsaicin (high dose: 10^{-5} M and low dose: 10^{-6} M), von Frey filament and airflow (strong: 40 ml/sec, weak: 25 ml/sec) stimulation to the larynx and electrical stimulation of the superior laryngeal nerve (SLN). Finally, we evaluated the effect of laryngeal application of P2RY1 antagonist MRS 2175 (10 mM) on the initiation of swallows evoked by DW, CW, CA and von Frey filament stimulation to the larynx Results: The number of swallows was increased in a dose dependent manner by *αβ*-methylene ATP application to the larynx. The bilateral transection of SLNs abolished *αβ*-methylene ATP-evoked swallows. The laryngeal challenge of PPADS significantly reduced the number of *αβ*-methylene ATP-, DW-, CW- and CA and low dose of capsaicin and weak airflow-evoked swallows. Additionally, PPADS slightly but significantly increased the swallowing threshold evoked by von Frey filament. On the other hand, PPADS failed to change the swallows evoked by high dose of capsaicin, strong airflow and electrical stimulation of the SLN. MRS 2175 did not change the number of DW-, CW- and CA-evoked swallows and the swallowing threshold evoked by von Frey filament. Conclusions: We speculate that P2X, but not P2RY1, receptor plays a role on the chemically and mechanically evoked swallows.

Disclosures: T. Tsujimura: None. **M. Yoshihara:** None. **B.J. Undem:** None. **B.J. Canning:** None. **M. Inoue:** None.

Poster

PSTR175: Speech and Oral Movements

Location: MCP Hall A

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Program #/Poster #: PSTR175.19/J16

Topic: E.04. Voluntary Movements

Support: NIH Grant 1R21NS135642
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Title: Grab a quick bite and chew it over: hand-jaw coordination as mice eat

Authors: *J. M. BARRETT[1], M. GAO[1], M. E. MARTIN[1], R. E. DRUZINSKY[3], J. A. MIRI[2], G. M. G. SHEPHERD[1];
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Abstract: Rodent jaws evolved structurally to support dual functionality, for either biting or chewing food. Rodent hands also function dually during food handling, for either actively manipulating or passively holding food. How are these oral and manual functions coordinated? We combined electromyography (EMG) and kilohertz kinematic tracking to analyze masseter and hand actions as mice handled food. Masseter activity was strikingly bimodal, and synchronized with bimodal hand movements. In holding/chewing mode, mastication occurred as rhythmic (~5 Hz) masseter activity while the hands held food below the mouth. In

oromanual/ingestion mode, bites occurred as lower-amplitude aperiodic masseter events that were precisely timed to follow regrips (by ~200 ms). Thus jaw and hand movements are flexibly coordinated during food handling: uncoupled in holding/chewing mode, and tightly coordinated in oromanual/ingestion mode as regrip-bite sequences. Computational analysis supported a model in which food handling actions are hierarchically orchestrated, with mode-switching under separate control from the intra-mode sequencing of actions (bites, regrips, chews). We serendipitously detected two additional masseter-related actions: tooth-sharpening, identified as bouts of higher-frequency (~13 Hz) rhythmic masseter activity, and eye displacement, including rhythmic proptosis, attributable to masseter contractions. Collectively, the findings exemplify how a natural, complex, and goal-oriented activity is organized as an assemblage of distinct modes and complex actions, adapted for the divisions of function imposed by anatomical structure. These results reveal intricate coordination of disparate effectors in the jaws and hands, and show how food handling can serve as model for understanding multi-body-part coordination in general.

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Poster

PSTR176: Rhythmic Pattern Generators: Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR176.01/J17

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant RFA NS18-023 (1UH3NS119844-01A1)

Title: Investigating the neurophysiological connectivity basis of tic generation

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Abstract: OBJECTIVES: Tourette syndrome is a neurodevelopmental disorder characterized by motor and phonic tics. We currently lack an ideal animal Tourette Syndrome (TS) model, and standard functional imaging modalities generally reveal normal brain architecture. The objective of this study was to enhance our understanding of the neurophysiological basis of tic generation in two common deep brain targets for stimulation: the centromedian thalamus (CM) region and the anterior globus pallidus internus (aGPi), and their interaction.
METHODS: Four consented patients with TS (4 females) underwent monthly clinical visits for collection of electrophysiology for a total of nine months. Participants were implanted with bilateral CM and aGPi electrodes that were connected to two neurostimulators, both with sensing capabilities. Preoperative MRI scans and post-operative CT scans were performed for localization of electrodes. After each therapy visit, participants completed daily surveys at home

to assess their tic and limbic symptoms in their activities of daily living.
RESULTS: Electrophysiological recordings from both brain regions revealed that the two brain regions engage in a low frequency activity (<10 Hz) neuronal interaction with different preferred directions. The flow of information from the aGPi to CM was seen to be greater than the flow of information from the CM to aGPi. The causal power of aGPi due to the influence of CM was also found to increase more significantly in frequencies below 10 Hz than the causal influence from aGPi to CM. This suggests that CM has a significant causal influence on aGPi.
CONCLUSION: We conclude that the CM and the aGPi engage in neuronal interactions in low frequencies known to be associated with tic electrophysiology. This could potentially address knowledge gaps in the field, revealing neurophysiological and connectivity evidence to identify pallido-thalamic network interactions of tic generation/suppression.

Disclosures: G. Lowor: None. **J. Gomez:** None. **K. Foote:** None. **M.S. Okun:** None. **A. Gunduz:** None.

Poster

PSTR176: Rhythmic Pattern Generators: Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR176.02/J18

Topic: E.07. Rhythmic Motor Pattern Generation

Title: The projectome of cardinal spinal cell types provides a new model of motor control

Authors: *S. A. KOMI, A. WINTHER, R. W. BERG;
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Abstract: Essential aspects of limbed movement are known to be generated in neural circuits located primarily in the spinal cord. However, despite decades of meticulous genetic classification of neuronal subtypes, the spontaneous emergence of concerted motor activity is still enigmatic, and the wiring of the responsible interneurons, the central pattern generator (CPG), remains largely unknown. Here, we propose a recurrent network model of the spinal cord built from first principles of neural dynamics and statistics. We first demonstrate that random balanced networks that produce oscillations consistently display a marked “Mexican-Hat” connectivity profile when embedded in a 1-dimensional space. When enforcing the Mexican hat through multiple inhibitory and excitatory subpopulations, this pattern offers a nearly independent control over amplitude, frequency and spatial organization of the emergent oscillations. Building on this observation, we then ask if such a structure is present in the cord and whether it is compatible with motor functions. We use spatial transcriptomics, single-cell RNA datasets and histological data from mice, to reconstruct the densities of cardinal cell types in the lumbosacral region of the cord. We sample hundreds of model networks from these distributions and impose either a random connectivity or literature-derived cell-type specific projections biases, which we call “the projectome”. Remarkably, the latter approach readily generates rhythms, rotational dynamics, and appropriate muscular coordination similar to

experiments. Furthermore, flexibility of motor output, such as modulation of period and amplitude as well as gait, was obtained by modulation of individual populations.
Finally, the model predicts the emergence of spatially propagating waves reminiscent of aquatic vertebrates generating undulatory locomotion. Hence, the model reconstitutes a functional framework for motor circuits, which share similar mechanisms across vertebrate species. We hence propose that genetic identities constrain spatial projection biases such that function emerges from spatially organized patterns.

Disclosures: S.A. Komi: None. **A. Winther:** None. **R.W. Berg:** None.

Poster

PSTR176: Rhythmic Pattern Generators: Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR176.03/J19

Topic: E.07. Rhythmic Motor Pattern Generation

Title: Rostro-caudal projections of interneurons in the rodent spinal cord

Authors: *G. HOUSER[1], R. W. BERG[2], S. A. KOMI[2], T. TOPILKO[3];
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Abstract: Within the spinal cord, distinct neuronal subtypes are essential for processing sensory information and coordinating motor responses. However, the detailed wiring and projection patterns of these neurons, forming the central pattern generator (CPG), remain incompletely understood. Our project employs a multifaceted approach that combines genetic targeting, viral tracing, tissue clearing, and advanced imaging techniques to map neuronal pathways originating in the spinal cord in detail across the whole central nervous system (CNS). We call this network mapping effort the "projectome."
Using transgenic mice expressing Cre recombinase in specific neuronal populations, we will identify and target pathways via a Cre-dependent anterograde adeno-associated virus (AAV) carrying a fluorescent reporter protein. In our initial investigations, wild-type mice received injections of an AAV virus expressing mScarlet under a neuron-specific promoter into the lumbar spinal cord. By optimizing tissue clearing and antibody labeling protocols, we have facilitated whole CNS imaging. We aligned brain regions within entire CNS samples to a common atlas using the open-source toolbox ClearMap, providing region-specific information on virus expression and spread in the brain. Additionally, we are actively developing methods to register spinal cords for comparative analysis.
Our ultimate goal is to construct comprehensive maps of the projectome, visualizing and comparing specific and broader neuronal projections within the CNS. By leveraging open-source tools for image registration and comparison, we aim to generate heat maps depicting the spread of infected cells. This will allow us to uncover how projection biases contribute to the

organization and interaction of different pathways within the spinal cord and help elucidate the functional framework for coordinated motor responses.

Disclosures: G. Houser: None. **R.W. Berg:** None. **S.A. Komi:** None. **T. Topilko:** None.

Poster

PSTR176: Rhythmic Pattern Generators: Connectivity

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Program #/Poster #: PSTR176.04/J21

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Human Frontier Science Program (RGP0060/2019)
ANPCYT (PICT 2016-2073)
UBACYT (20020150100179BA)

Title: Inhibitory premotor modulation in rhythmic motor control

Authors: *M. RADICE[1,2], L. SZCZUPAK[1,2];
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Abstract: The execution of rhythmic motor behaviors involves a complex interplay of control mechanisms that regulate the behavioral output, narrowing down the degrees of freedom of a system with multiple elements.
Leeches crawl on solid surfaces through a succession of elongation and contraction body waves, anchored on the posterior and anterior suckers. Their nervous system consists of a chain of 21 nearly identical segmental ganglia flanked by a head and a tail brain. Each segmental ganglion contains all the neurons required to produce this rhythmic motor pattern, including a pair of premotor NS (nonspiking) neurons connected to virtually every motoneuron through a central network that provides recurrent inhibitory signals onto the motoneurons. Our goal is to understand the role of NS in the context of crawling.
Experiments were performed in isolated ganglia where application of dopamine (75 µM) elicited fictive crawling. The rhythmic motor pattern was monitored by recording the alternated activation of DE-3 motoneuron that innervates longitudinal muscles and fires during the contraction phase. To obtain a comprehensive readout of the motor pattern we performed extracellular recordings of multiple segmental nerves and identified, through a spike sorting algorithm, different groups of motoneurons based on their activity profile relative to DE-3: In-Phase, Anti-Phase, or In-Phase-Early-Onset (n = 147 rhythmic units from 46 experiments).
Intracellular recordings show that NS neurons received inhibitory signals that are temporally correlated to the contraction phase of crawling, which we monitored through the DE-3 motoneuron. The amplitude of the inhibitory signal was correlated with the duration of the DE-3 burst but not with its frequency.
Transient removal of NS from the circuit enhanced the firing frequency of DE-3 and In-Phase

units. In contrast, the firing frequency of Anti-Phase units was not modified. Moreover, this maneuver stabilized the rhythmic activity of both In-Phase and Anti-Phase units.
Our data suggests that the premotor NS neuron exerts a phase-specific regulation of motoneuron output.

Disclosures: M. Radice: None. **L. Szczupak:** None.

Poster

PSTR176: Rhythmic Pattern Generators: Connectivity

Location: MCP Hall A

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Topic: E.07. Rhythmic Motor Pattern Generation

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Title: From anatomy to functions in locomotion - an optogenetic investigation on the organisation of V2a reticulospinal neurons

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Abstract: Locomotion is implemented with a modular structure in the spinal cord. Upstream, the reticulospinal neurons (RSNs) receive various sensory inputs, descending control signals and ascending feedback from spinal circuits to command specific kinds of movement. Due to the distributed anatomy of the reticular formation, the function and connectivity of RSNs among each other and onto spinal segment remains poorly understood. We investigate these questions in larval zebrafish focusing on the hindbrain excitatory neurons labelled by transcription factor *vsx2+* (V2a-derived) that have previously been associated with forward and turns. Most V2a RSNs have their soma located in the medulla and send ipsilateral descending axons in the spinal cord. Using one-photon optogenetics, we show that a subset of V2a RSNs in the caudal medulla reliably triggers forward swimming. To identify the connectivity patterns of these medullary V2a-derived RSNs, we combined two-photon holographic illumination with temporal focusing and population calcium imaging. We find that single cell stimulation of a forward V2a RSNs recruits a dozen of other V2a RSNs, mainly recruited on the ipsilateral side. Using spatially

confined photo conversion of V2a-derived RSN axons, we show that the axon of V2a RSNs in the forward cluster reach past the segments 15 along the rostro-caudal axis of the spinal cord. Our results are consistent with the existence of a recurrent connectivity among medullary V2a RSNs to trigger forward locomotion. Our findings provide a framework for comparative studies of the functional organisation of the RSNs across vertebrates and highlight a key command region for forward locomotion in the caudal medulla.

Disclosures: X. Jia: None. **M. Carbo Tano:** None. **C. Wyart:** None.

Poster

PSTR176: Rhythmic Pattern Generators: Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR176.06/J23

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH NINDS grant 1R01NS114007-01A1
NIH NINDS grant R01NS117749- 01
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R01NS112304

Title: Investigation of spinal commissural interneurons that receive afferent feedback and their role in locomotion

Authors: *A. J. SPENCE[1], E. V. ROOT[2], J. RAJAVONG[1], G. KOMA[1], T. J. CAMPION III[3], M. A. LEMAY[4], G. M. SMITH[5], S. M. DANNER[6];
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Abstract: Critical to successful legged locomotion is the selection and maintenance of gait. Spinal commissural interneurons (CINs) are crucial for the control of interlimb coordination and gait. CINs that receive afferent feedback and transmit it to the other side of the cord are likely critical for lateral stability and adaptation processes requiring a coordinated response of both limbs. V3 neurons were implicated in crossed reflexes and thus are a likely candidate population. Yet, V3 is a heterogenous population of neurons with local, commissural and long propriospinal projections. Here, we specifically target CINs that receive afferent feedback to investigate their role in locomotion. We hypothesized that this subpopulation of CINs serves to stabilize the phase relationship between legs by transmitting information about load or contact events contralaterally. As a result, we predicted that silencing of these CINs would result in a) more noisy gait, and b) a reduced ability for the animal to bring the hind-limbs in phase (closer to a

phase difference of zero) as speed increases. We used surgical viral approaches in the rat that allow more specific targeting of these CINs, and subsequently characterize them and test our hypotheses with histology and kinematics. By injecting AAV-retro-DIO-hM4Di-mCherry into the lumbar spinal cord and AAV1-AAV1-Nuc-Cre-GFP directly into the DRGs, we are able to selectively transduce only those interneurons in the lumbar enlargement that project contralaterally *and* receive direct afferent input. The hM4Di DREADDs construct allows us to temporarily and reversibly silence these interneurons in the behaving animal with subcutaneous injection of CNO. Preliminary treadmill kinematic data (n=3 rats) showed a shift in hind-limb phase differences towards walks with hind-limbs more in phase (2 of 3 rats; 16 cm/s), contradicting our hypothesis. At the highest speed tested (36 cm/s), the hindlimbs shifted slightly to be closer to perfect 180 degree anti-phase (towards a more perfect trot), which agrees with our hypothesis (we have inactivated CINs that hypothetically are working to push these limbs towards a phase difference of zero as speed increases). Ongoing work is adding kinematic data and performing histological characterization.

Disclosures: A.J. Spence: None. **E.V. Root:** None. **J. Rajavong:** None. **G. Koma:** None. **T.J. Campion:** None. **M.A. Lemay:** None. **G.M. Smith:** None. **S.M. Danner:** None.

Poster

PSTR176: Rhythmic Pattern Generators: Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR176.07/J24

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH grant 1 RF1 NS118606
DBI 2015317, part of NSF/CIHR/DFG/FRQ/UKRI-MRC Next Generation Networks for Neuroscience Program

Title: Nerve branch recordings reveal differential deployment of identified neurons in a muscle motor pool during Aplysia feeding behaviors

Authors: *J. M. MCMANUS, M. C. JENCKES, H. J. CHIEL;
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Abstract: To survive, animals must flexibly switch between different behaviors in response to changing environmental stimuli. Different behaviors are produced by activation of the same muscles in different ways, that is, by differential deployment of neurons in the muscles' motor pools. The marine mollusk *Aplysia californica* provides a tractable system for studies in this area. The muscles that generate *Aplysia*'s feeding movements are innervated by large motor neurons, most of which have been identified; therefore, differential deployment of single, identified neurons can be tied to different behavioral outputs. Recording directly from the cell body of a motor neuron provides the most information about its activity, but simultaneously recording from the cell bodies of many motor neurons is technically challenging, especially in a

preparation capable of performing feeding movements. Nerve recordings are easier to obtain, but most of the spikes recorded from a nerve that carries signals from a large pool of motor neurons cannot be unequivocally tied to their specific neurons. The I1/I3 muscle complex of the *Aplysia* feeding system (buccal mass) is innervated by buccal nerve 2 (BN2), a nerve that trifurcates into branches BN2-a, BN2-b, and BN2-c before entering the muscle. Major motor neurons for I1/I3 send signals to BN2-b, BN2-c, or both. Additionally, some motor neurons send signals to both ipsilateral and contralateral BN2s, whereas others send signals solely to the ipsilateral BN2. By recording simultaneously from BN2 and its branches bilaterally in a semi-intact preparation capable of producing feeding movements, we can unequivocally tie many more spikes to specific neurons than what has previously been done using nerve recordings. This has provided new insights into the differential deployment of the I1/I3 motor pool in different behaviors. For example, during the protraction phase of feeding behaviors, there appears to be a switch between deployment of motor neuron B38 in swallowing behaviors and deployment of motor neurons B47 and B82 in other behaviors. Furthermore, during the retraction phase, when many I1/I3 motor neurons are active together, it was previously thought that different behaviors were characterized mainly by differences in strength of activation of the motor pool, but our results reveal that different elements of the motor pool are preferentially active in egestive vs. ingestive behaviors. By using a novel technique to identify activity of individual neurons from nerve recordings, our results shed light on how an animal differentially deploys a motor pool to produce multiple distinct behaviors using the same musculature.

Disclosures: J.M. McManus: None. **M.C. Jenckes:** None. **H.J. Chiel:** None.

Poster

PSTR176: Rhythmic Pattern Generators: Connectivity

Location: MCP Hall A

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Program #/Poster #: PSTR176.08/J25

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIMH MH046742-26
NINDS R35 NS097343

Title: Sensitivity analysis of intrinsic and synaptic conductances in a small rhythmic circuit

Authors: *Z. FOURNIER[1], L. M. ALONSO[2], E. E. MARDER[2];
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Abstract: Neural activity is driven by the contribution of both intrinsic and synaptic conductances. In models of neural circuits, different combinations of maximal conductances can give rise to similar activity, and this in turn endows circuits with robustness to perturbations. We compared the robustness of a neural circuit to perturbations in their intrinsic versus synaptic conductances. To address this, we performed a sensitivity analysis on a population of

conductance-based models of the pyloric network from the crustacean stomatogastric ganglion (STG). The model network consists of three neurons with nine currents: a sodium current (Na), three potassium currents (Kd, KCa, A-type), two calcium currents (CaS and CaT), a hyperpolarization-activated current (H), a non-voltage-gated leak current (leak), and a neuromodulatory current (MI). The model cells are connected by seven synapses of two types, glutamatergic and cholinergic. We produced one hundred models of the pyloric network that displayed similar activities with values of maximal conductances distributed over a wide range. We then evaluated the robustness of each model to perturbations of increasing strength. We found that different models have different sensitivities to perturbations, both in their intrinsic and synaptic conductances. As expected the models become less robust as the strength of the perturbations increase, but they do so in different ways, and we characterized this variability quantitatively. We found that in all cases, the model networks are more sensitive to the perturbation of their intrinsic conductances than their synaptic conductances.

Disclosures: Z. Fournier: None. **L.M. Alonso:** None. **E.E. Marder:** None.

Poster

PSTR176: Rhythmic Pattern Generators: Connectivity

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR176.09/J26

Topic: E.07. Rhythmic Motor Pattern Generation

Support: CIHR grant 14392
CIRH grant 180522

Title: Assessment of the contribution of the trigeminal main sensory nucleus to the masticatory function

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[1]Dept. of Neurosciences, Univ. de Montréal, Montreal, QC, Canada; [2]Ctr. Interdisciplinaire de Recherche sur le Cerveau et l'Apprentissage (CIRCA), Montreal, QC, Canada; [3]Dept. of Stomatology, Fac. of Dentistry, Univ. de Montréal, Montreal, QC, Canada

Abstract: Mastication arises from a circuitry referred to as a central pattern generator (CPG) that produces a rhythmic output and that is composed of still ill-defined elements of the trigeminal system. Our previous work led to the hypothesis that the rhythmogenic core of the CPG lies in the dorsal part of the trigeminal main sensory nucleus (NVsnpr) where neurons were found to change their firing patterns, shifting from tonic to rhythmic, in response to a decrease of extracellular Ca^{2+}. This shift in firing pattern results from the enhancement of a sodium persistent current (I_{NaP}; involving Nav1.6 channels) caused by the decrease of extracellular Ca^{2+}, which in turn is produced by the release of S100β, an astrocytic Ca^{2+}-binding protein. However, these findings were obtained *in vitro*, and it is still unknown whether NVsnpr is indeed essential to produce mastication *in vivo* and if yes whether it acts alone or in concert with other trigeminal

neurons. Here we use optogenetics in transgenic mice expressing channelrhodopsin (ChR2) or Archrhodopsin (ArchT) under the control of the VGluT2 promoter to elicit or block rhythmic jaw movements (RJM). Photostimulation (by activation of ChR2) of two cortical locations referred to as the anterior and posterior cortical masticatory areas (CMA) elicited RJM. Bilateral injection of 4,9-anhydroTTX, a Nav1.6 channel blocker (n=3) in NVsnpr greatly reduced the frequency and amplitude of cortically-induced RJM, while that of an antibody against S100β induced an increase of movement latency. Inversely, bilateral photostimulation of NVsnpr neurons produced robust RJM (n=4), with hand-to-mouth paw movements in two of them. Photostimulation of two other areas located medial (JuxtV, n=1) and ventral (PCRt, n=1) to the trigeminal motor nucleus also produced jaw movements but not as coordinated as those produced by stimulation of NVsnpr. We currently examine the effects of photoinhibition of NVsnpr and other peritrigeminal areas on cortically-induced RJM and are developing probes to measure changes of concentration of S100β in NVsnpr during mastication using Surface-enhanced Raman scattering (SERS). These results will help resolve the composition of the masticatory CPG and establish the role of astrocytes in rhythmogenesis.

Disclosures: D. Falardeau: None. **E. Maljean:** None. **A. Kolta:** None.

Poster

PSTR176: Rhythmic Pattern Generators: Connectivity

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Program #/Poster #: PSTR176.10/J27

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Craig H. Neilsen Foundation (award number: 993926)
Canada Research Chairs (CRC)
Canada Institution of Health Research (CIHR)
University of Manitoba

Title: Characterizing the activity of thoracic sympathetic preganglionic neurons during tonic and rhythmic motor activity in the neonatal mouse spinal cord

Authors: *L. E. DOMINGUEZ-RODRIGUEZ, C. V. NWACHUKWU, N. SHAHSAVANI, J. GARCIA, J. W. CHOPEK, K. C. COWLEY;
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Abstract: During sustained rhythmic locomotion, sympathetic and motor systems must be activated in concert to ensure appropriate metabolic support and to maintain body homeostasis. Lumbar V3 interneurons (INs) are vital for stable and robust locomotion and are responsible for left-right coordination during high-speed locomotion. We recently showed lumbar V3 INs provide direct excitatory synaptic input onto thoracic sympathetic preganglionic neurons (SPNs), demonstrating the presence of an ascending excitatory coupling between lumbar locomotor and thoracic sympathetic autonomic systems. This ascending excitatory drive may help increase

activation of body tissues and organs providing metabolic and homeostatic support during movement and exercise. After spinal cord injury the disruption of these spinal networks and loss of descending input results in motor-sensory deficits and autonomic dysfunctions. Thus, understanding ascending locomotor drive on thoracic SPN activity during locomotion is critical. We propose that thoracic SPNs exhibit a range of rhythmic oscillatory responses during locomotor activity, dependent upon their rostrocaudal location in the isolated mouse spinal cord. Spinal cords were isolated from neonatal mice (P0-5) in two series: thoracic SPNs from C57Bl/6 mice labelled by calcium dye crystals (Ca-green conjugated dextran amine, CGDA; 3000MW) applied to the cut ends of thoracic ventral roots (VRs); and ChAT-Cre/GCaMP6 mice bred to endogenously express the genetically encoded calcium indicator GCaMP6 in thoracic SPNs. In both series, oblique cuts were performed to visualize SPNs at different levels (T3-T13). Fluorescent Ca-images of thoracic SPNs were recorded at baseline, and during tonic and rhythmic ventral root (VR) activity. In some experiments, fictive locomotion was induced by whole cord bath application using combinations of NMDA, serotonin and dopamine. In other experiments, we determined the effect of the ascending lumbar locomotor drive on SPN activity, using a split bath chamber to apply drugs only to lumbar regions. We demonstrate that thoracic SPNs can exhibit rhythmic oscillations at baseline while other SPN show oscillations during tonic and rhythmic lumbar locomotor activity. Our results also demonstrate a somatotopic organization of thoracic SPN activity arising from lumbar locomotor activity, with differential changes in the relative intensity of calcium and the appearance of rhythmic oscillations of thoracic SPNs depending on their thoracic level during both tonic and rhythmic VR activity.

Disclosures: L.E. Dominguez-Rodriguez: None. **C.V. Nwachukwu:** None. **N. Shahsavani:** None. **J. Garcia:** None. **J.W. Chopek:** None. **K.C. Cowley:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.01/J28

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSERC Grant: RGPIN-2018-04699

Title: Investigating the rapid effects of locally synthesized estrogens in social recognition within the bed nucleus of the stria terminalis (BNST) of male mice

Authors: *A. VARATHARAJAH[1], D. ASPESI[2], L. STIMSON[1], Z. BROWN[1], E. CHOLERIS[1];
[1]Dept. of Psychology and Neurosci. Program, Univ. of Guelph, Guelph, ON, Canada; [2]Neurosci. Inst. Ctr. for Behavioral Neurosci., Georgia State Univ., Atlanta, GA

Abstract: Social recognition (SR) is a trait that is critically implicated in the recognition and differentiation between familiar and unfamiliar conspecifics, enabling the display of social

behavioural responses. Sex steroids, such as testosterone (T) and 17β-estradiol (E2) are produced by the gonads and locally within the brain to regulate SR. In gonadectomized (GDX) male mice, it has been found that exogenous administration of E2 facilitates SR within a rapid timeframe in the bed nucleus of the stria terminalis (BNST). T can also be rapidly aromatized to E2 locally within the brain by the enzyme aromatase. Blocking local synthesis of E2 through aromatase inhibition within the dorsal hippocampus impairs long-term object memory and consolidation in GDX male mice, and short-term social memory in GDX female mice. Conversely, in gonadally-intact male mice, this effect was not seen, suggesting a protective role of circulating androgens against the loss of local E2 production within the hippocampus. Currently, there is limited research available regarding whether the local production of estrogens affects short term memory in a similar manner within the BNST of male mice. The current project is investigating the role of locally synthesized E2 within the BNST of both GDX and intact male mice. The BNST is crucially involved in male social behaviour and has high expression of the G protein-coupled estrogen receptor (GPER) as well as ERα and ERβ receptors, the latter two being co-expressed with aromatase mRNA. Currently, a range of doses of letrozole, an aromatase inhibitor, is being infused into the BNST of GDX and intact CD-1 male mice. 15 minutes before being exposed to a SR paradigm that is completed within a rapid timeframe (40 min from treatment). It is hypothesized that aromatase inhibition will dose dependently impair SR in GDX male mice and that intact male mice may require higher doses of letrozole in comparison to GDX mice due to the protection exerted by circulating androgens. Investigation of the differing role of locally synthesized E2 within both GDX and gonadally-intact male mice can elucidate the interplay of the local synthesis of estrogens with circulating hormones. This can provide insights into the biological function of brain synthetized estrogens in male social behavior and possibly help understand social disorders with sexually differentiated incidence and manifestation.

Disclosures: A. Varatharajah: None. **D. Aspesi:** None. **L. Stimson:** None. **Z. Brown:** None. **E. Choleris:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.02/J29

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: R01DA046403

Title: The role of social housing on nucleus accumbens dopamine in male and female rats using fiber photometry

Authors: *N. BASS[1], J. B. BECKER[2];
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Abstract: Environmental conditions such as social housing and isolation influence rodent behavior and dopamine (DA) activity in the nucleus accumbens (NAc). NAc DA regulates many motivated behaviors. There are sex differences in the effect of social housing conditions on motivated behaviors and responses to stressful situations such that in comparison to males, socially housed females showed attenuated drug self-administration and responded better to stressful situations. Previous researchers from our laboratory integrated fast-scan cyclic voltammetry (FSCV) to investigate DA activity in the NAc between individually and socially housed male and female rats, and reported higher levels of electrically stimulated DA activity in females than males. With microdialysis there is greater methamphetamine-induced DA release in pair-housed vs individually housed females. Microdialysis has low temporal resolution but greater accuracy. FSCV produces high-resolution DA recordings and measures rapid dynamic DA fluctuations. In this study we use fiber photometry that has greater temporal resolution and scan rates and is more efficient in differentiating DA neurons from other neurotransmitters compared with FSCV. To further investigate the effect of social housing on NAc DA activity in female and male rats and determine whether fiber photometry replicates other techniques, we utilized the adeno-associated dopamine sensor $GRAB_{DA}$ with fiber photometry to monitor DA release in the NAc of freely behaving rats. Specifically, $GRAB_{DA}$ was injected into the NAc directly below an implanted optic fiber that was connected to the fiber photometry system during experimentation. Endogenous DA activity in individually and socially housed rats within and between sexes is compared as rats interact with conspecifics of each sex. We find greater NAc DA activity when male rats interact with female versus male conspecifics. Findings from this study will contribute to the understanding the role of social housing on NAc DA circuitry and set the foundation for later studies that will investigate the role of social housing on drug-self administration and NAc DA activity between and within sexes.

Disclosures: N. Bass: None. **J.B. Becker:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.03/J30

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSERC (Grant: RGPIN-2018-04699)

Title: Medial Amygdala Estrogen Receptors Interplay with Oxytocin Receptors in the Rapid Facilitation of Social Recognition in Female Mice

Authors: *D. CANTINI[1], C. SCHMIDT[1], E. CHOLERIS[2];
[1]Univ. of Guelph, Guelph, ON, Canada; [2]Psychology, Univ. of Guelph, Guelph, ON, Canada

Abstract: The estrogen, 17β-estradiol (E2), has been shown to rapidly facilitate social recognition in various regions within the social-brain-network. The medial amygdala is heavily

involved in the processing of social odours in mice and expresses the three known estrogen receptors (ER): ERα, ERβ, and G Protein-Coupled ER (GPER). Selective agonists for each of the three ERs rapidly facilitate social recognition in the medial amygdala of female CD1 mice. The neuropeptide oxytocin is also crucial for the processing of social information. Fully functional oxytocin receptors (OTR) within the medial amygdala are necessary for the rapid effects of E2 on social recognition, suggesting an E2/OTR interplay. Here, we elucidate how the three ERs interplay with OTRs in the medial amygdala to elicit rapid facilitation of social recognition. Female mice were ovariectomized and had bilateral cannulae implanted into the medial amygdala. A sub-effective dose of OTR antagonist (i.e. the highest dose of the antagonist that does block social recognition per se) was infused into the medial amygdala 2 minutes before the infusion of one of the respective ER agonists (ERα agonist PPT; ERβ agonist DPN; GPER agonist G1). A difficult social recognition paradigm designed to measure the rapid facilitating effects of treatment was employed. The results demonstrated that a sub-effective dose of OTR antagonist administered before each respective ER agonist prevented the facilitation of social recognition by each ER agonist. This shows that ERα, ERβ, and GPER require fully functional OTRs to rapidly facilitate social recognition in the medial amygdala of female mice and suggests an interplay between ERs and OTRs in the regulation of social cognition.

Disclosures: D. Cantini: None. **C. Schmidt:** None. **E. Choleris:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.04/J31

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Canada Foundation for Innovation
Ontario Research Fund
Canada Research Chair
McMaster startup fund
NSERC PGS-D
CALM Seed Grant

Title: Sex differences in calcium activity of oxytocin neurons and neighboring astrocytes in the hypothalamic paraventricular nucleus during social and stress stimuli.

Authors: *K. SANDOVAL[1], J. RYCHLIK[3], K. Y. CHOE[2];
[1]Psychiatry & Behavioural Neurosci., [2]Psychology, Neurosci. & Behaviour, McMaster Univ., Hamilton, ON, Canada; [3]Neurosci., McMaster Univ. Neurosci. Grad. Program - Minds, Hamilton, ON, Canada

Abstract: Oxytocin (OXT) neurons within the hypothalamic paraventricular nucleus (PVN) are known to be strongly activated by social stimuli and serve as anxiolytic facilitators during stress.

Whether PVN OXT neuronal responses to social and stress stimuli vary by sex remains largely unexplored. Furthermore, it remains unknown how these stimuli affect the activity of neighboring astrocytes, a major regulator of OXT neuronal activity. To record *in vivo* population activity of PVN OXT neurons and neighboring astrocytes, we expressed genetically encoded calcium (Ca) reporters in OXT neurons (RCaMP2) and astrocytes (GCaMP6f) and performed dual-color fiber photometry. During these recordings, freely behaving male (n=5) and female (n=6) adult mice underwent reciprocal social interaction test (familiar or unfamiliar conspecifics) and looming shadow stress test. We observed a sex-dependent increase in Ca levels in OXT neurons during social sniffing of unfamiliar conspecifics, particularly in female mice. In astrocytes, we observed no robust Ca changes in all groups except for a slow and steady decrease during unfamiliar interactions in male mice. Looming shadow stress stimuli reliably evoked run or freeze responses in both male and female mice. Interestingly, the run response was associated with sustained Ca elevations in OXT neurons in both sexes. In astrocytes, a robust and short-lived increase in Ca activity was observed in both sexes during the run response, while only males demonstrated a significant increase in Ca activity during the freeze response. Stimuli-associated change in Ca levels were significantly correlated between OXT neurons and neighboring astrocytes during social interactions, except for male mice during unfamiliar interactions. In contrast, during the looming shadow stress test, a significant correlation in Ca responses was only observed in female mice during the run response. Our results reveal sex-dependent variations in OT neuron and astrocyte responses within the PVN, offering crucial insights into the neural dynamics of social interactions and stress.

Disclosures: K. Sandoval: None. **J. Rychlik:** None. **K.Y. Choe:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.05/J32

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant P20 RR16462

Title: Prolactin increases cell proliferation but not neurogenesis in the dentate gyrus of adult male rats.

Authors: *M. D. SPRITZER, U. U. IROH, R. J. GROCELA, E. B. CUNNINGHAM, I. Z. CADDEAU, K. A. BLEK;
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Abstract: Neurogenesis occurs throughout adulthood in the dentate gyrus region of the hippocampus, and a variety of hormones have been shown to influence hippocampal cell proliferation and survival. Past studies with rodents suggest that prolactin enhances cell proliferation in the dentate gyrus and that resulting changes in neurogenesis may play a

functional role in social recognition. Prolactin is a large peptide hormone produced within the anterior pituitary, and it binds to receptors located throughout the body to have a variety of effects. We tested the effects of acute and chronic injections of a wide dose range of prolactin upon cell proliferation and neurogenesis within the dentate gyrus of adult male rats. Rats received either an injection of saline (control) or recombinant rat prolactin (5, 10, 50, 100, 150, or 800 μg/kg). In the first experiment, a single injection of prolactin was immediately followed by an injection of bromodeoxyuridine (BrdU), and brain tissue was collected 24 h after injections to assess prolactin's effects upon cell proliferation. Two subsequent experiments involved either acute (4 injections at 12 h intervals prior to BrdU injection) or chronic (14 daily injections starting 24 h after BrdU injection) treatment of with prolactin, and brain tissue was collected 15 days after BrdU injections. Peroxidase immunohistochemistry was used to visualize BrdU-labeled cells and assess cell proliferation and survival. All labeled cells in every 10th section throughout the dentate gyrus were counted using light microscopy. A low dose of prolactin (10 μg/kg) caused a significant increase in the density of BrdU-labeled cells that were 24 h old relative to the control group. In contrast, neither acute injections of prolactin during the cell proliferation period nor daily injections during the cell survival period had significant effects on the number of BrdU-labeled cells that survived to 14 days old. Subsequently, immunofluorescent double-labeling (NeuN and BrdU) and confocal microscopy were used to determine the percentage of new cells that developed into neurons. In both of the 15-day experiments, about 90% of new cells were determined to be neurons, and prolactin treatments had no significant effect on this percentage. We conclude that an acute low dose of prolactin enhances cell proliferation in the dentate gyrus of adult male rats, but neither acute nor chronic dosing with prolactin influences adult neurogenesis. These somewhat contradictory results suggest that there may be a selective process, unrelated to prolactin levels, which determines which newly proliferated cells stimulated by prolactin will survive to become neurons.

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Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.06/J33

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant 5R01AG069970-03

Title: Investigating the Effect of Systemic Estrogen Loss on Cognition: Preliminary Results from Female Rabbits

Authors: *E. IREWOLE, D. WANG, L. BAYS, N. LAL, M. SMITH, C. SMITH-BELL, B. G. SCHREURS;
Neurosci., West Virginia Univ., Morgantown, WV

Abstract: Menopause, a phase marked by systemic estrogen loss, is a physiological process that occurs in most women, either naturally or surgically. Observational studies have reported a correlation between systemic estrogen levels and cognition in postmenopausal women, highlighting the need to investigate whether menopause directly affects cognitive function. To examine the effect of menopause on cognition, systemic estrogen loss was surgically induced via gonadectomy (GDX) in sexually matured female rabbits. These rabbits were divided into three experimental groups. The first group received GDX with subcutaneous estradiol (ES) implants (GDX+ES). The second group received GDX with vehicle (V) implants (GDX+V), and the third group received sham GDX with vehicle implants (sGDX+V). These rabbits received their respective implants for eight weeks before being subjected to trace eyeblink conditioning (tEBC), which was used to assess their cognitive performance. Cognitive performance was assessed by the percentage of conditioned responses (%CR, the primary index of learning), the CR onset latency (an index of processing speed), and delayed recall (an index of episodic memory). Based on the preliminary results, the GDX+ES group had a terminal average CR of about 40%, closely comparable to the sGDX+V group, with an average CR of 41% at the end of the acquisition session of tEBC. However, the GDX+V group had a terminal average CR of about 15%, indicating a possible cognitive impairment in the group. The GDX+V group also showed longer CR onset latency (slower cognitive processing speed) and lower delayed recall (impaired memory recollection). The data collected so far also revealed that administering ES to the GDX rabbits rescued cognitive deficits to a level comparable to that of the sham animals. These preliminary results suggest that systemic estrogen loss (induced via GDX) may impair cognitive performance.

Disclosures: E. Irewole: None. **D. Wang:** None. **L. Bays:** None. **N. Lal:** None. **M. Smith:** None. **C. Smith-Bell:** None. **B.G. Schreurs:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.07/J34

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R01NS082179

Title: Influence of Neuroestrogens on Auditory Circuit Dynamics in Zebra Finches

Authors: *H. KANG, L. REMAGE-HEALEY;
Neurosci. and Behavior, Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Neuroestrogens, estrogens synthesized within the brain, are abundant in the forebrain auditory circuits of vocal learning species such as humans and songbirds. In humans, estrogens influence auditory perception and memory, though the specific cellular and synaptic mechanisms are not fully understood. In zebra finches, neuroestrogens are produced in the NCM

(caudomedial nidopallium) in response to conspecific songs, and they rapidly enhance neuronal firing and modulate inhibitory synaptic transmission. Our ex vivo whole-cell voltage clamp recordings in the NCM, targeting distinct cell types, reveal that estrogens mainly reduce inhibitory transmission onto excitatory neurons. Additionally, blocking the G-protein coupled estrogen receptor 1 (GPER1), predominantly expressed in excitatory neuronal membranes, abolishes neuroestrogen-induced changes in synaptic transmission, indicating GPER1's critical role in these effects. To further explore these mechanisms at the level of intact in vivo circuits, we used 'Retrodrive' recordings, combining in vivo electrophysiology with retrodialysis. We observe a reduction in neuronal responses to conspecific songs during neuroestrogen synthesis blockade, while exogenous estrogens do not fully reverse these effects. Ongoing analyses are determining whether estrogen synthesis inhibition and exogenous estrogen application differ by cell type. We hypothesize that neuroestrogens selectively influence auditory coding in excitatory compared to inhibitory neurons in the NCM. Understanding how neuroestrogens impact auditory processing could illuminate fundamental sensory perception and neural circuit mechanisms.

Disclosures: H. Kang: None. **L. Remage-Healey:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.08/J35

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF Grant IOS 2050260
NSF Grant IOS 2050230

Title: The effects of aromatase inhibition and estradiol replacement on the hippocampal transcriptome of the zebra finch

Authors: *R. ANDRADE[1], Y. NAU[2], E. BASHAW[1], D. HANAUER[4], D. J. BAILEY[4], J. BRACHT[3], C. J. SALDANHA[1];
[1]Neurosci., [3]Biol., [2]American Univ., Washington, DC; [4]Biol., St. Norbert Col., De Pere, WI

Abstract: Estrogens like 17β-estradiol (E2) are synthesized from testosterone (T) by the enzyme aromatase. In vertebrates, aromatase is expressed in ovary, adipose, bone, brain, and other tissues. In the songbird brain, the hippocampus (HP), a telencephalic structure critical for learning and memory, is a major site of E2 synthesis where aromatase is readily detectable in both sexes. In the HP, aromatase is particularly abundant at synaptic loci and surprisingly sparse at other neuronal compartments. Decreases in local E2 synthesis via the aromatase inhibitor 1,4,6-Androstatriene-3,17-dione (ATD) in the zebra finch (*Taeniopygia guttata*) HP impairs spatial memory performance to the same extent as in HP-lesioned birds. Aromatase inhibition with concomitant E2 replacement, however, restores memory function back to control levels, as does treatment with the GPER agonist, G1. These data suggest that local, perhaps synaptic

aromatization, may be a crucial modulator of memory function in songbirds, an effect that is dependent upon the membrane E2 receptor GPER. The genomic mechanisms and gene clusters underlying these observations, however, are completely unknown. This project seeks to understand the transcriptional changes that may contribute to the poor spatial memory performance in zebra finches with low local E2 synthesis in the HP. mRNA from three treatment groups (control, ATD, and ATD with E2 replacement) was sequenced (Illumina) and a traditional RNAseq analysis pipeline (HISAT, Stringtie, and DESeq2) was performed. Weighted gene correlation network analysis (WGCNA) sorted 33,739 genes into 80 modules identifying correlations of sex and treatment, most notably, creating two distinct modules of genes for either sex. Network connections were created with Cytoscape to identify hub genes in each module. Current work involves performing gene ontology analysis to understand functional pathways involved in changes of the local HP endocrine environment following inhibition of HP aromatase and replacement with E2.

Disclosures: R. Andrade: None. **Y. Nau:** None. **E. Bashaw:** None. **D. Hanauer:** None. **D.J. Bailey:** None. **J. Bracht:** None. **C.J. Saldanha:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.09/J36

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R01NS082179

Title: Neuroestrogen inhibition destabilizes neuronal ensembles in songbird auditory forebrain.

Authors: *F. CINI[1], L. REMAGE-HEALEY[2];
[1]Univ. of Massachusetts, Amherst Neurosci. and Behavior Program, Hadley, MA; [2]Neurosci. and Behavior, Univ. of Massachusetts, Amherst, MA

Abstract: Neuroestrogens rapidly modulate brain activity and support performance in learning tasks. Inhibiting estrogen synthesis leads to changes in electrophysiological activity of single neurons. Furthermore, neuronal ensembles, groups of co-activated cells (within a brief 6 ms time window), enhance information processing compared to single neuron activity. However, the effects of hormonal modulation on ensembles is virtually unknown. Therefore, we hypothesize that inhibiting neuroestrogen synthesis will: 1) change neuronal auditory responses, and 2) modify ensemble stability in the songbird zebra finch (Taeniopygia guttata). The caudomedial nidopallium (NCM), an auditory forebrain region of songbirds, is rich in aromatase (estrogen synthase). Using silicon probes for in-vivo electrophysiological recordings, combined with microdialysis, we recorded neuronal activity in NCM while administering aCSF (control) or fadrozole (FAD - an aromatase inhibitor). Single units, based on spike waveform, were classified as putative inhibitory or excitatory. Additionally, we used a combination of principal and

independent component analyses to identify coordinated neuronal ensembles (range 3-10 neurons per ensemble). Our results showed that exposure to FAD induced a reduction in song-evoked single unit firing rate. This difference was driven by a stronger reduction in inhibitory unit response in the FAD condition, with excitatory units experiencing a more modest reduction in firing rate. Moreover, we found that half of the ensembles in the FAD condition underwent dynamic changes with repeated auditory stimuli, while the majority of the ensembles in the aCSF condition (> 90 percent) remained stable. These results suggest that inhibiting neuroestrogen synthesis destabilizes the coordination of membership in neuronal ensembles, driven by changes in inhibitory cell activity. Understanding more about how neuroestrogens modulate neuronal circuitry for auditory processing and learning, could unveil new therapeutic targets for conditions involving hormonal dysregulation or cognitive impairments.

Disclosures: F. Cini: None. **L. Remage-Healey:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.10/Web Only

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Hippocampus-synthesized estrogen and androgen modulate dendritic spines and LTP in non-genomic manner

Authors: *S. KAWATO;
Univ. of Tokyo, Tokyo, Japan

Abstract: We have demonstrated (1) hippocampal synthesis of estrogen and androgen, and (2) non-genomic synaptic modulation by these sex-steroids. [Synthesis] We showed expression as well as neuronal/synaptic localization of essential enzymes in the adult male rat hippocampus. Mass-spectrometric analysis demonstrated that hippocampal levels of estradiol (E2), testosterone (T) and dihydrotestosterone (DHT) are about 8 nM, 17 nM and 7 nM, higher than those in plasma. Rapid E2 synthesis with in the hippocampus can be measured via blocking of LTP (long-term potentiation) by 20 min perfusion of Aromatase inhibitor in hippocampal slices. Castration did not decrease male hippocampal E2, because E2 is synthesized from hippocampal T. Castration, however, significantly decreased T and DHT in the hippocampus, indicating a transport of T via the blood circulation. Female hippocampal levels of E2 (0.5-4 nM), and T (1 nM) are less than male, but much higher than those in plasma. [Synaptic Modulation] E2-induced rapid non-genomic modulation (1 h) was demonstrated by analysis of dendritic spines and LTP of adult rat hippocampal ‘acute’ slices (steroid-depleted slices). Dendritic spine analysis was performed for CA1 pyramidal neurons in hippocampal slices. Spine density and spine head diameters were obtained by mathematical software Spiso-3D which identifies spines by calculating geometrical parameters. E2 at 1 nM rapidly increased the density of small-head spines. T and DHT at 10 nM increased the density of small-head and large-head spines.

Signaling pathways are: synaptic ERalpha or AR → LIMK, MAPK, PKA, PKC, Src → cofilin or cortactin → actin polymerization→ new spine formation. LTP analysis showed that 1 nM E2 induced full-LTP (E2-LTP) upon sub-threshold stimulation, although without E2 the sub-threshold stimulation did not induce full-LTP. Kinase inhibitors against MAPK, PKA, PKC, LIMK, Src blocked E2-LTP. References: Kimoto et al., 2001 Endocrinol, Hojo et al., 2004 PNAS, Mukai et al., 2007 J. Neurochem, Hojo et al., 2009 Endocrinol, Mukai et al. 2011 Cereb Cortex, Ooishi et al. 2011 Cereb Cortex, Okamoto et al., 2012, PNAS, Kato et al., 2013, Frontier Neurosci. Hasegawa et al., 2015 Brain Res., Hatanaka et al., 2015 Brain Res., Murakami et al., 2015 Brain Res., Soma et al., 2018 Frontier Neurosci., Hojo and Kawato 2018 Frontier Neurosci.

Disclosures: S. Kawato: None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.11/J37

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF Grant IOS 2050260
NSF Grant IOS 2050230

Title: Aromatase-dependent transcriptome of the zebra finch hippocampus suggests a molecular sex dimorphism in gene clusters

Authors: J. SPURGEON[1], A. ST JEAN[1], C. HARVEY[1], E. PRYOR[1], R. ANDRADE[1], Y. NAU[1], D. HANAUER[2], D. J. BAILEY[2], J. BRACHT[1], *C. J. SALDANHA[3];
[1]American Univ., Washington, DC; [2]Biol., St. Norbert Col., De Pere, WI; [3]Neurosci., American Univ., Washington, DC

Abstract: In the songbird brain, aromatase, the enzyme that synthesizes estradiol (E2), can be found in either soma or synaptic boutons in various brain areas. In the hippocampus, aromatase is almost exclusively localized in synaptic boutons, and aromatase-expressing presynaptic boutons are more abundant in males than females. Differences in synaptic aromatization can modulate neuronal function and sex-specific behavior, though the specific downstream effects of these mechanisms remain unknown. Our aim was to examine differential gene expression and pathways dependent on sex and estradiol manipulation in the songbird hippocampus. Samples from three treatment groups were used: silastic implant (n=8), aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD) (n=8), and ATD with supplemented estradiol (n=8). All three treatment groups were split evenly by sex (n=12 per sex). From the RNA-seq (Illumina) count data normalized for depth of sequencing, we used the DESeq2 package to determine differential gene expression between treatment groups and sex. A likelihood ratio test was done to determine effects of ATD and ATD+E2 treatments for single-sex cohorts or effects of sex regardless of treatment. Finally, gene ontology analysis for known gene names examined biological process

(BP), molecular function (MF), and cellular compartment (CC) pathways, and these enrichment results were compared to known steroid-mediated processes. Several uncharacterized genes were identified in all treatment groups, and sex comparisons, indicating a gap in the literature regarding potential steroid-mediated signaling. In addition, for every treatment group analyzed, unique sets of upregulated genes were separately identified for male and female birds. Enrichment for cytoskeletal reorganization genes were found in female ATD-treated birds, but not male. Further, autophagy-linked genes were enriched in ATD+E2-treated males but not females. Overall, these data suggest a molecular, E2-dependent sex dimorphism of gene clusters in the zebra finch hippocampus.

Disclosures: J. Spurgeon: None. **A. St Jean:** None. **C. Harvey:** None. **E. Pryor:** None. **R. Andrade:** None. **Y. Nau:** None. **D. Hanauer:** None. **D.J. Bailey:** None. **J. Bracht:** None. **C.J. Saldanha:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.12/K1

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF IOS 2207023
NIH T32 AG 044402

Title: Effect of Estrogen and Progesterone Loss on Neurogenesis-related Spatial Learning and Search Strategies in Aging Female Rats

Authors: *G. M. WINTER[1], M. J. CORENBLUM[2], S. PILLUTLA[3], J. R. MEREDITH[4], P. WENE[5], N. MENAKURU[6], S. L. COWEN[1], L. MADHAVAN[7,8];
[1]Dept. of Psychology, Univ. of Arizona, Tucson, AZ; [2]Dept. of Neurol., Univ. of Arizona, Tucson, AZ; [3]Univ. of Arizona, Tucson, AZ; [4]Mol. and Cell. Biol. Undergraduate Program, Univ. of Arizona, Tucson, AZ; [5]Col. of Agr. and Life Sci., Univ. of Arizona, Tucson, AZ; [6]Neurosci. and Cognitive Sci. Undergraduate Program, Univ. of Arizona, Tucson, AZ; [7]Neurol., Univ. of Arizona, Tucson, AZ; [8]Bio5 Institute and Evelyn F. McKnight Brain Institute, Tucson, AZ

Abstract: The adult mammalian brain contains active neural stem progenitor cells (NSPCs) that generate nerve cells throughout life, and neurogenesis is compromised with advancing age. Our previous work identified a specific critical period of decline in the neurogenic ability of NSPCs, (13-15 months) in aging F344 rats. These studies focused on male rats and included the characterization of deficits in different neurogenesis-relevant behaviors. In the current study, we utilized different groups of aging F344 female rats (2, 6, 9, and 14 months old) to examine changes in cognitive flexibility, a correlate of the neurogenic function of hippocampal NSPCs present in the subgranular zone (SGZ) of the dentate gyrus, using Reversal Learning on the

Morris Water Maze task (RMWM). To probe the role of the female sex hormones, 17b-estradiol (E2) and progesterone (P4), on aging NSPC function, a group of female rats also underwent ovariectomy (OVX) 2.5 weeks prior to experiments. Standard Morris Water Maze (MWM) learning for a fixed escape location was assessed over the initial 4 days of training followed by 2 days of training on the reversal version of the task where the platform was moved 180 degrees from the original location (RMWM). To support a fine-grained analysis of the search strategy employed by each animal, we utilized an automated system for strategy identification (Rtrack by Rupert Overall). This approach allows the identification of 9 unique search strategies during water maze performance. These strategies are classified into non-goal oriented, procedural, or allocentric search strategies. Preliminary analysis of our 2-month-old cohort (OVX n = 13, SHAM n = 11) looked at Corrected Integrated Path Length (CIPL), and Search Strategy. Two-way ANOVA using CIPL scores revealed no significant difference between groups in either the MWM ($p = 0.15$) or RMWM ($p = 0.78$). Further, we found no meaningful group-wise effect in Search Strategy during MWM or RMWM performance in 2-month-old rats. These preliminary results indicate that estrogen and progesterone loss at young ages has no demonstrable effect on learning. Analysis of our 6-, 9-, and 14-month-old cohort data is ongoing and will also be presented.

Disclosures: G.M. Winter: None. **M.J. Corenblum:** None. **S. Pillutla:** None. **J.R. Meredith:** None. **P. Wene:** None. **N. Menakuru:** None. **S.L. Cowen:** None. **L. Madhavan:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.13/K2

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSERC

Title: Effects of estrogen and progesterone replacement on the learning of anticipatory nausea in ovariectomized female rats

Authors: *M. HUYNH[1], V. MATIC[2], I. BISHNOI[3], K.-P. OSSENKOPP[4], M. KAVALIERS[4]; [1]Western Univ., Vaughan, ON, Canada; [2]Univ. of Western Ontario, Niagara Falls, ON, Canada; [3]Western Univ., London, ON, Canada; [4]Univ. Western Ontario, London, ON, Canada

Abstract: Anticipatory nausea (AN) is a classically conditioned phenomenon often experienced in chemotherapy patients, where aversive feelings of nausea are paired with neutral stimuli associated with the treatment such as the hospital setting. Successive rounds of chemotherapy may result in a hospital environment becoming a conditioned stimulus that induces feelings of nausea prior to treatment. Compared to males, female patients are at a greater risk of developing AN, a sex difference that is similarly observed in rodent models of AN. Recently estrous cycle-dependent levels of estrogen and progesterone have been implicated in these sex differences.

Therefore, the goal of this study is to investigate the potential role of gonadal hormones estrogen and progesterone in the learning of AN in ovariectomized female rats. The ovariectomy was needed to eliminate the sex difference, allowing the use of a hormone replacement procedure. 72 rats were assigned either a hormone treatment or control (estrogen, progesterone, or sesame oil), and conditioned with either the nausea-inducing agent lithium chloride (LiCl) or the control saline (NaCl). Following 10 consecutive days of hormone treatment and four 48-hour interval conditioning trials in a novel environmental context, rats were re-exposed to the environmental context and gaping behaviour was measured as an index of AN. Ovariectomized female rats treated with estrogen and conditioned with LiCl displayed significantly less gaping behaviour compared to other LiCl-conditioned rats treated with either progesterone or sesame oil. These results suggest that estrogen could play an impairing role in the conditioning of AN, whereas progesterone doesn't appear to play a significant role. Further research on the role of hormones in the learning of AN can help discover the underlying mechanisms behind the sex differences in AN that have been frequently observed.

Disclosures: M. Huynh: None. **V. Matic:** None. **I. Bishnoi:** None. **K. Ossenkopp:** None. **M. Kavaliers:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.14/K3

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIA Grant R01-AG076030

Title: Hippocampal regulation of dopamine system function as a novel therapeutic target in the VCD rat model of perimenopause

Authors: *K. LILLY[1], A. M. BOLEY[1], D. J. LODGE[2], S. M. PEREZ[3];
[1]Pharmacol., UT Hlth. San Antonio, SAN ANTONIO, TX; [2]UTHSCSA, San Antonio, TX; [3]Pharmacol., UTHSCSA, San Antonio, TX

Abstract: Perimenopause, the transitional period leading to menopause, is a period of increased risk for developing a psychiatric disorder or experiencing exacerbated symptoms of a pre-existing disorder. Psychiatric symptoms are debilitating and significantly diminish the quality of life for women and their families. Thus, understanding the neurocircuitry contributing to these conditions is critical for the discovery of novel therapeutics. Of particular interest is the dopamine system, which has been highly implicated in the regulation of mood, addiction, psychosis, and cognitive function. Here we demonstrate aberrant dopamine system function in the 4-vinylcyclohexene diepoxide (VCD) model of perimenopause. This model has translational relevance, as daily administration of VCD induces a progressive loss of ovarian follicles and accompanying hormonal changes that closely resemble the hallmarks of perimenopause

occurring in women. Further, the aberrant dopamine system function in this model appears secondary to hippocampal hyperactivity. We posit that MP-III-022, a positive allosteric modulator selective for α5-$GABA_A$ receptors, will decrease hippocampal hyperactivity and normalize downstream dopamine signaling, leading to a reversal of deficits in related behaviors. Indeed, aberrant dopamine system function in VCD rats was normalized by the systemic administration of MP-III-022. VCD rats also displayed deficits in cognition, as measured by the novel object recognition task, which was also reversed by MP-III-022. Taken together, these data suggest that targeting hippocampal regulation of dopamine signaling may be a potential novel therapeutic for the treatment of psychiatric alterations during perimenopause.

Disclosures: K. Lilly: None. **A.M. Boley:** None. **D.J. Lodge:** None. **S.M. Perez:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

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Program #/Poster #: PSTR177.15/K4

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Wisconsin Partnership Program at the University of Wisconsin School of Medicine and Public Health

Title: The puberty blocker, leuprolide, reduces sex differences in structural plasticity markers, GFAP and ARC mRNA levels, within the adolescent amygdala.

Authors: *A. NGUYEN, A. P. AUGER;
Psychology, Univ. of Wisconsin, Madison, WI

Abstract: Gonadotropin-releasing hormone agonists, also known as puberty blockers, are used in treating gender dysphoria, in part, by suppressing puberty in transgender and nonbinary adolescents. It is also known that adolescent development is a time of increased mental health risk. Therefore, it is important to understand the impact of pausing puberty on brain and behavior during postpubertal development. Previously, we examined the impact of leuprolide on play and anxiety-related behaviors in juvenile rats. Upon finding changes, we are now focusing on central gene expression to understand the role of amygdala gene expression and behavior. Juvenile Sprague Dawley rats (24 male and 24 female) were injected with either 25 ug/kg of leuprolide or saline from postnatal day (P) 27-39. Anxiety-like behavior was assessed (P27-37, P38-39). Brain and trunk blood were collected on P40. Leuprolide treatment reduced anxiety-like behavior in adolescent rats. We then measured levels of glial fibrillary acidic protein (GFAP) mRNA, an astrocyte structure marker, and activity-regulated cytoskeleton-associated protein (ARC) mRNA, an immediate early gene involved in cellular plasticity, within the amygdala. We found that control females had higher ARC and GFAP mRNA levels than control males ($p = 0.006$), and leuprolide eliminated this sex difference. Interestingly, ARC mRNA levels negatively correlated with testosterone, and positively correlated with corticosterone and androstenedione ($p = 0.027$,

p = 0.014, p = 0.006, respectively). ARC is also negatively correlated with anxiety-like behavior (p = 0.005). Our findings suggest that the puberty blocker, leuprolide, reduces sex differences in markers of structural plasticity within the adolescent amygdala. These data suggest that sex differences continue to be refined during early adolescence and that pausing puberty also reduces sex differences within the development amygdala.

Disclosures: A. Nguyen: None. **A.P. Auger:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

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Program #/Poster #: PSTR177.16/K5

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: MBRS-SCORE-1SC2DA047809
NIGMS-1R16GM149491
NIGMS-RISE-5R25GM061151-22
R25NS080687

Title: Increased BDNF expression in the nucleus accumbens and hippocampus for the extinction of morphine place preference across the estrous cycle in females

Authors: *F. Z. ROSADO;
UPR Recinto Río Piedras, San Juan, PR

Abstract: Increased BDNF expression in the nucleus accumbens and hippocampus for the extinction of morphine place preference across the estrous cycle in females
[1]Rosado-Rodríguez, Fabiana Z., [2]Richiez-Mateo, Wilma V., [3]Arraiza-Truust, Helena, [4]Bonilla-Rullán, Pedro, [3]Schrils-Soto, Ricardo, [5]Barreto-Estrada, Jennifer L. [1]Department of Psychology, University of Puerto Rico-Río Piedras Campus, Río Piedras, 00925-253; [2]Department of Biology, University of Puerto Rico-Bayamón Campus, 00959-1919; [3]Department of Biology, University of Puerto Rico-Río Piedras Campus, Río Piedras, 00925-253; [4]Department of Social Sciences, School of Public Health, University of Puerto Rico, Medical Sciences Campus, 00936, [5]Department of Anatomy and Neurobiology, University of Puerto Rico-Medical Sciences Campus, 00936.
Women are emerging as the demographic with the fastest-growing rate of substance abuse in the United States. Like men, women are prone to developing addiction disorder, characterized by chronic drug-seeking behaviors. This behavior can impact neuronal neuroplasticity, affecting the functioning of the corticomesolimbic reward system. To understand the behavioral and neural basis of opioid addiction in females, we have established an animal model of addiction for the extinction of maladaptive behaviors. Previously, we demonstrated that females that extinguished drug-seeking behaviors after undergoing both morphine-induced conditioned place preference (CPP), and extinction training, showed increased expression of BDNF in the hippocampus

(HPC), whereas the amygdala (AMY) remained unaltered. In this study we look at other brain regions, such as the ventral striatum/nucleus accumbens (VS/NAc) and the medial prefrontal cortex (mPFC) for the expression of both BDNF, and its receptor TrkB. In addition, the estrous cyclicity was determined during the extinction test. Results showed increased BDNF expression in the VS/NAc in animals that extinguished morphine place preference, similar to the previous finding in the HPC. The mPFC showed no differences. TrkB expression in the HPC showed a slight decrease in the extinction group, while the mPFC was unaffected. Also, preliminary results showed that female rats in the extinction group were in the estrous stage during the extinction test day. Together our data suggest that extinction of drug-related behaviors might improve with increased expression of BDNF in the HPC and VS/NAc, while in low levels of estrogen.
Support: MBRS-SCORE-1SC2DA047809, NIGMS-1R16GM149491, NIGMS-RISE-5R25GM061151-22 and R25NS080687 NeuroID.

Disclosures: F.Z. Rosado: 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.; MBRS-SCORE-1SC2DA047809, R25NS080687, NIGMS-1R16GM149491, NIGMS-RISE-5R25GM061151-22.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.17/K6

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: GSU Second Century Initiative Neurogenomics Fellowship

Title: The effects of ovarian hormone loss on amyloid pathology in a transgenic model of Alzheimer's disease

Authors: *C. LEVINE[1], E. P. HARRIS[2], V. NGO-VU[2], U. ZAFAR[3], D. A. BANGASSER[2], M. B. PARENT[3];
[1]Georgia State Univ., Atlanta, GA; [2]Neurosci. Instititute, Georgia State Univ., Atlanta, GA; [3]Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Alzheimer’s disease (AD) comprises 60-70% of all dementia cases and currently affects an estimated 6.9 million Americans over the age of 65. Without intervention to delay or prevent AD, this number is projected to increase to 12.7 million by 2050. Decades before the onset of dementia, AD neuropathology such as demyelination, neuroinflammation and amyloid-β, first emerges in the absence of overt symptoms (i.e., the preclinical stage) followed by mild cognitive impairment (i.e., the prodromal stage). The preclinical and prodromal stages are a vulnerable period when exposure to AD-risk factors profoundly influences the trajectory of the disease. Biological sex is major risk factor for AD, with women composing 67% of patients and

having nearly twice the lifetime risk of AD as men. Genetic risk factors such as APOE & SERPINB1 affect women more strongly than men, and following the onset of clinical symptoms, women experience more severe AD burden than men, but live longer with the disease. Menopause transition and subsequent menopause coincide with prodromal AD and facilitate the development of AD neuropathology, leading peri- and postmenopausal women to accumulate more tangles and exhibit more severe hippocampal atrophy than age-matched males. Women who have undergone surgical menopause following bilateral oophorectomy are at even greater risk than those who have gone through spontaneous menopause, highlighting a role for ovarian hormone loss distinct from the impact of age. Collectively, this evidence has led us to hypothesize that ovarian hormone loss potentiates AD pathology. To test this, we are investigating the impact of ovariectomy (OVX) on dorsal hippocampal (dHC) AD pathology and dHC-dependent memory in female TgF344-AD rats, which are currently the most comprehensive model of AD available. Female TgF344-AD rats and their wildtype littermates are given OVX or sham surgery during the prodromal period (6-6.5-months of age). dHC-dependent memory (spontaneous alternation and novel object location) will be tested after a short and longer period of ovarian hormone loss and then rats will be euthanized and AD-like neuropathology, such as proinflammatory cytokines, myelin status, and Aβ will be assessed. Our preliminary data indicate that 1 month of ovarian hormone loss does not affect spontaneous alternation nor dHC $A\beta_{42}$. We are currently assessing the impact of OVX on other markers of early AD pathology and determining whether a more prolonged period of ovarian hormone loss will exacerbate AD trajectory.

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Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.18/K7

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: K99MH127244
PI startup funds

Title: Leveraging CRISPR/dCas9 tools to examine cell-type specific mechanisms underlying the memory-enhancing effects of estradiol in the rat dorsal hippocampus

Authors: *R. BORAWKE[1], J. NATARAJAN[1], J. J. DAY[2], J. J. TUSCHER[1];
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Abstract: The sex steroid hormone estradiol (E2) is a key modulator of synaptic plasticity and memory formation throughout the lifespan. E2 enhances hippocampal-dependent memory in

male and female rodents, yet the specific molecular mechanisms underlying its beneficial effects remain poorly understood. This gap in knowledge has hindered the development of therapies targeting memory dysfunction in neuropsychiatric and neurodegenerative disorders. Traditional behavioral pharmacology approaches have provided invaluable insights regarding the receptors and cell-signaling pathways through which E2 modulates hippocampal memory. However, these methods impact a diverse array of cell populations and are therefore limited in their ability to disentangle the cell-type specific molecular mechanisms through which E2 exerts its effects. The dorsal hippocampus is composed of a heterogeneous mixture of cell types, many of which express estrogen receptors (ERs). Understanding how E2 acts within each of these discrete cellular populations is critical for refining our understanding of the cellular and molecular mechanisms through which E2 mediates hippocampal function. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/dCas9 technologies enable precise interrogation of cell-type specific gene targets *in vitro* and *in vivo*. Here, we developed guide RNAs (gRNAs) that allow for targeted repression of classical receptors ERα and ERβ selectively in neurons using a CRISPR interference (CRISPRi) approach. *In vitro* validation confirmed successful repression of these receptors in primary rat neuron cultures. Ongoing experiments are examining the role of neuron-specific ERs in mediating hippocampal memory in male and female rats *in vivo*. Ultimately, our goal is to systematically dissect how E2 engages neuronal and non-neuronal populations to promote neuroplasticity and support cognitive function. In doing so, we hope to identify potential therapeutic targets for reducing memory dysfunction in patients with neuropsychiatric disorders such as Alzheimer's disease.

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Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.19/K8

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: CONAHCYT 1148428

Title: Decreased memory in postnatal age offspring 21 of mothers with hypothyroidism

Authors: *G. SOTO PORTILLA[1], M. ALVARADO[2], A. ANAYA-HERNÁNDEZ[3], A. GUTIERREZ GARCIA[1], L. HERNANDEZ SALAZAR[1];
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Abstract: Introduction: Thyroid hormones such as triiodothyronine and thyroxine play a crucial role in energy metabolism and participate in the development and maturation of the nervous system. During pregnancy and lactation, thyroid hormone depletion has been linked to altered hipocampal neurodevelopment in the rat pups during early ages. One of the main functions of the

hippocampus is the processing of short- and long-term memory. One way to assess memory at the end of lactation is by the T-maze test. Purpose: To evaluate the effect of maternal hypothyroidism on the memory of rat pups at the end of lactation at postnatal age 21 (P21). Method: We used a total of 24 offspring (n = 24), distributed in 2 groups: control (n = 12) and experimental (n = 12), with 6 females and 6 males in each group. Statistical analysis: Student's T test. The experimental group we obtained from mothers with hypothyroidism with oral pharmacological induction (methimazole) during pregnancy, from embryonic day 10 (E10) until the end of lactation at P21. We evaluated the locomotor activity of the pups at postnatal age P19 with the open field test for 5 minutes. Subsequently, we performed the assessment of short-term memory at postnatal age 20 (P20) and long-term memory at P21. The variables assessed in the T-maze test were: the number of entries to the choice arm and the latency to the stimulus. During the T-maze test, two sessions were carried out: in the first session the pups had access to the arms with the milk and water stimulus, in the second session (10 minutes later) they had free choice. Results: No significant differences were found between the of control and maternal hypothyroid groups in the open field test. At age P20, the results of the first session of the T-maze indicated that the offspring of mothers with hypothyroidism had a longer latency to choice of the correct stimulus (milk). At age P21, the group of offspring with maternal hypothyroidism had a longer latency and a lower number of hits for the choice of the correct stimulus (milk) compared to the control group. Conclusion: The offspring of mothers with hypothyroidism, at the end of lactation, presented a decrease in short- and long-term memory.

Disclosures: G. Soto Portilla: None. **M. Alvarado:** None. **A. Anaya-Hernández:** None. **A. Gutierrez garcia:** None. **L. Hernandez Salazar:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.20/K9

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: R01NS128123 (KMF, TJJ)
R01MH122414 (TJJ, KMF)
UWM Distinguished Dissertator Fellowship (SBB)
Support for Undergraduate Research Fellow award from the UWM Office of Undergraduate Research (KAF)

Title: Examining a role for estradiol-induced regulation of ubiquitin proteasome system activity at hippocampal synapses following object training in ovariectomized female mice.

Authors: *S. BEAMISH[1], K. A. FRANK[1], T. J. JAROME[2], K. M. FRICK[1];
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Abstract: 17β-estradiol (E_2) enhances hippocampal function and long-term memory formation, yet the molecular mechanisms through which E_2 exerts its effects remain unclear. E_2 regulates hippocampus-dependent memory by activating cell-signaling cascades in the dorsal hippocampus (DH) to promote the synthesis of proteins that support structural changes at DH synapses. However, this work has overlooked the equally important contributions of protein degradation mediated by the ubiquitin proteasome system (UPS) in synaptic remodeling. In this system, proteins are tagged with ubiquitin and become substrates for degradation by the 26S proteasome complex. Our preliminary data indicate that proteasome activity is necessary for E_2 to enhance hippocampus-dependent spatial and object recognition memory consolidation in ovariectomized (OVX) female mice. Although these findings suggest that E_2 requires proteasomal protein degradation to enhance hippocampal memory, it is unclear when UPS-mediated protein degradation activity occurs and whether post-training administration of E_2 potentiates training-induced effects on UPS activity. Thus, this work examined the extent to which E_2 regulates training-induced UPS activity at DH synapses in OVX mice. Adult female C57BL6/J mice were bilaterally OVX, implanted with bilateral guide cannulae targeting the DH, and then assigned to homecage+vehicle, train+vehicle, or train+E_2 groups. Trained mice accumulated 30 s exploring two identical objects in an open field. Immediately after training, mice received a bilateral DH infusion of vehicle or E_2 and DH tissue was collected 5 min, 1 h, and 4 h later. These timepoints were chosen because they represent distinct periods where protein synthesis and protein degradation activity peak (5 min, 4 h) and trough (1 h) during consolidation phase of long-term memory formation. DH tissue was fractionated to obtain synaptic fraction and measures of UPS-mediated protein degradation activity will be measured via western blot and proteasome activity assays including: 1) K48 polyubiquitination, representing the degree to which proteins are tagged for proteasomal degradation, 2) RPT6 phosphorylation, representing activity-dependent state of the proteasome, and 3) 20S proteasomal activity, representing catalytic activity of the proteasome. To determine the extent to which E_2-induced UPS activity overlaps with E_2-induced local protein synthesis following object training, we will also measure phosphorylation of mTOR and downstream effectors 70S6K and 4E-BP1. This work will reveal novel insights into the mechanisms that regulate E_2-induced enhancements in object memory consolidation.

Disclosures: S. Beamish: None. **K.A. Frank:** None. **T.J. Jarome:** None. **K.M. Frick:** None.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01MH107886
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UWM Discovery Innovation Grant 101x418
UWM Distinguished Dissertation Fellowship

UWM Office of Undergraduate Research
UWM College of Letters and Sciences

Title: Mechanisms regulating mPFC estradiol synthesis in object recognition memory consolidation in female mice

Authors: *M. SCHWABE, H. A. BEATY, J. XU, K. M. FRICK;
Univ. of Wisconsin - Milwaukee, Milwaukee, WI

Abstract: The hormone 17β-estradiol (E2) regulates hippocampal synaptic plasticity and enhances the consolidation of memories dependent on the dorsal hippocampus (DH). De novo E2 synthesis via the enzyme aromatase in DH is essential for object recognition (OR) and object placement (OP) memory consolidation in ovariectomized (OVX) female mice (Tuscher et al., 2016). Other regions, such as medial prefrontal cortex (mPFC), play critical roles in these types of memory, but less is known about the involvement of mPFC E2 synthesis in mPFC function and memory. We previously found that intra-mPFC infusion of the aromatase inhibitor letrozole impaired OR and OP consolidation in young ovariectomized female mice (unpublished data), supporting a key role for de novo E2 synthesis in mPFC during memory consolidation. However, the extent of aromatase expression in mPFC and mechanisms regulating its activity are not well known. Here, we studied the effects of object training on mPFC aromatase expression during the early consolidation period. Female C57BL/6 mice (10-12 weeks of age) underwent ovariectomy (OVX) or sham surgery to compare effects of training on mPFC aromatase expression levels in mice in diestrus to those deprived of ovarian hormones. After recovery, vaginal lavage was used to establish estrous cycling in sham mice and lack of estrous cycle in OVX. On the day of diestrus, sham mice underwent object training (or remained in homecage as a control) and were euthanized 1 hour later with OVX mice day-matched with shams. Brains were flash frozen, sectioned and mounted to slides, and then dual RNAscope ISH/immunohistochemistry was performed to identify the aromatase gene cyp191a and c-fos protein, respectively. Ongoing analyses will examine whether there is a spatial relationship between cells activated after object training and cyp19a1-positive and aromatase protein-expressing cells.

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Poster

PSTR177: Hormones, Cognition, and Social Behavior

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Alzheimer's Association (ABA-22-973796)
NIA grant R43AG079715
AD Strategic Fund
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Title: Effects of systemic oral treatment with novel estrogen receptor beta agonist (EGX358) on memory and hot flash-like symptoms in an EFAD mouse model of Alzhiemer's disease

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Abstract: Estrogen levels plummet at menopause, contributing to a symptoms including hot flashes, memory loss, and increased risk of Alzheimer's disease (AD), the most common type of dementia affecting 78 million individuals worldwide. Genetic factors, such as the apolipoprotein E allele ε4 (*APOE4*), contribute to at least 80% of late-onset AD cases, and older women bearing 2 copies of *APOE4* are at the highest risk of AD relative to *APOE4*+ men or those bearing 1 or 2 copies of the neural risk allele *APOE3*. Menopausal estrogen loss increases brain amyloid levels and risks of cognitive decline and dementia, however, traditional estrogen therapies carry risks of cancer due to the cell proliferative effects of estrogen receptor alpha (ERα). Estrogen receptor beta (ERβ) does not cause cancer cell proliferation yet enhances memory formation in rodents, suggesting that highly selective ERβ agonists may be a viable option for improving memory and alleviating hot flashes in women with AD. We previously showed that daily oral gavage of the highly potent and selective ERβ agonist EGX358 (>750-fold more selective for ERβ) for 3 months promotes memory and reduces the magnitude of a drug-induced hot flash in ovariectomized (OVX) female mice. We also found that acute infusion of the highly potent estrogen 17β-estradiol (E_2) into the dorsal hippocampus promotes memory and synaptic morphology in AD mice (human $APOE^{+/+}$/5xFAD$^{+/-}$) mice bearing 2 copies of *APOE3* (E3FAD) or 1 copy of *APOE3* and *APOE4* (E3/4FAD), but not 2 copies of *APOE4* (E4FAD). Although unclear why E4FAD mice are less responsive to E_2, ERα levels, but not ERβ levels, are aberrantly high in E4FADs, and ERα in the AD brain is largely non-functional, suggesting potential efficacy of selectively targeting ERβ in E4FAD females. Our pilot data suggest that oral EGX358 enhances object recognition, but not spatial memory, in OVX E3FAD and E3/4FAD females, but only reduces the magnitude of a drug-induced hot flash in E3FADs. Here, we tested effects of long-term oral EGX358 treatment on OVX 4-5 month-old female E3FAD, E3/4FAD, and E4FAD females (n=14/group) to assess efficacy of EGX358 in all 3 genotypes. Mice were treated daily with vehicle (10% DMSO), E_2 (0.2 mg/kg), or EGX358 (0.5 mg/kg) via oral gavage for 2 months prior to behavioral testing and then during testing. Memory was assessed using object placement and object recognition memory tasks. Thermal imaging of tail skin temperature assessed hot flashes after acute injection of vehicle or senktide, an NK-3 tachykinin receptor agonist that induces heat dissipation from the tail. Body and uterine weights were also recorded. Data collection is ongoing and results will be discussed.

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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.; Alzheimer's Association (ABA-22-973796). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Estrigenix.

Poster

PSTR177: Hormones, Cognition, and Social Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.23/K12

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIA Grant P01AG071746

Title: Metabolic dysfunction impedes estradiol treatment-mediated potentiation of neurovascular coupling in middle-aged ovariectomized female mice

Authors: *Z. PLUMLEY[1], J. CALVO IGLESIAS[2], I. FERNANDEZ UGIDOS[2], A. WALKER[2], I. PIRES DOS SANTOS[3], H. TAYLOR[3], R. MOSTANY[2];
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Abstract: The menopause transition places females at elevated risk of age-related cognitive decline relative to males. In animal models of menopause, estradiol treatment initiated within a short period following sex hormone depletion has protective effects for cognition. However, clinical trials of post-menopausal hormone therapy have shown mixed results. Neurovascular coupling (NVC) is dysregulated in rodent models of menopause and metabolic disease, which has downstream consequences for neuronal metabolism, synaptic plasticity, and cognition. The presence of metabolic syndrome prior to menopause has been hypothesized to occlude the neuroprotective effects of estrogen by altering function of the neurovascular unit. To test this hypothesis, *in vivo* two-photon imaging was used to assess NVC in 11-13 month old female C57BL/6J mice following ovariectomy (OVX) and treatment with 17β-estradiol (E2). Experimenters were blinded to hormone treatment to reduce bias of results. Cranial windows were surgically implanted over the somatosensory cortex barrel field, a model cortical circuit that can precisely measure the effects of sensory input on penetrating arteriole vasodilation. To induce metabolic syndrome, mice were fed a high-fat diet (HFD) or low-fat control diet (CD) for at least 11 weeks prior to OVX. Dual x-ray absorptiometry scans and glucose tolerance tests were completed to characterize metabolic status. HFD animals given vehicle (VEH) after OVX exhibited glucose intolerance and increased fat mass deposition relative to HFD animals given E2, which indicated that HFD successfully induced a metabolic syndrome and that E2 treatment rescued the negative effects of HFD in the periphery. E2 treatment after OVX enhanced NVC amplitude and slope in CD-fed mice relative to VEH-treated controls, consistent with previous findings. Interestingly, NVC amplitude and slope of HFD-fed mice treated with E2 after OVX

were not different from controls treated with VEH, indicating a blunted response to E2 treatment. These findings suggest that metabolic dysfunction prior to OVX is sufficient to occlude the beneficial effects of E2 treatment on NVC. Taken together, our data indicate that early post-menopausal E2 treatment can improve the middle-aged female metabolic profile and enhance the function of the neurovascular unit, with baseline health status as a rate-limiting factor for the magnitude of benefit E2 can confer. Future experiments will explore the molecular machinery that underlies the observed HFD-induced deficits in neurovascular coupling.

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Poster

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant P01AG071746

Title: The role of estradiol in hippocampus function in control and high fat diet-fed ovariectomized mice

Authors: *V. FELINTRO[1], I. PIRES DOS SANTOS[1], H. TAYLOR[1], Y. ZHA[1], S. OFFOR[1], L. D. DESMOULINS[2], C. DUGAS[2], A. ZSOMBOK[2,3], L. A. SCHRADER[1,3];
[1]Cell and Mol. Biol., Tulane Univ., New Orleans, LA; [2]Physiol., Tulane Univ., New Orleans, LA; [3]Brain Institute, Tulane University, LA

Abstract: Menopause-associated estradiol (E2) decline has been correlated to cognitive impairment and memory loss. Studies involving postmenopausal healthy women indicate that hormone therapy enhances cognitive function. However, contrasting clinical data across various health conditions, including cardiovascular disease and metabolic disorders, present divergent effects of hormone treatment. To elucidate the mechanisms behind these divergent effects, we investigated the impact of E2 and diet manipulation in middle-aged female mice. Our hypothesis is that E2 treatment improves spatial memory and cognitive functions in control diet-fed mice but is less effective in high fat diet-fed animals. Female C57BL/6J mice seven month of age were fed either a high-fat diet (HFD, BioServ #F3282) or a control diet (CTD, BioServ #F4031) for 10 to 14 weeks, followed by ovariectomy (OVX). Mice were randomly allocated to receive a silastic tube implant that administered either 17β-estradiol (E2) or a vehicle (VEH, cholesterol), all the while maintaining their respective diets. Animals were divided into four groups (HFD+VEH, HFD+E2, CTD+VEH, CTD+E2). The novel object location test (NOL) was conducted to assess memory performance. To investigate potential synaptic mechanisms underlying the effects of E2 on memory, we analyzed the impact of E2 treatment on long-term potentiation (LTP) in the hippocampus of OVX mice fed with HFD and CTD. Considering the

crucial role of the phosphoinositide 3-kinase (PI3K) pathway in various physiological brain functions, we evaluated the protein levels and phosphorylation status of PI3K in the hippocampus using western blotting. Surprisingly, the results from the NOL test revealed that E2 significantly enhanced NOL memory in both CTD- and HFD-fed mice (two-way ANOVA, Fisher LSD post hoc). Preliminary results from extracellular field recordings conducted in the Schaffer collateral-CA1 pathway in hippocampus slices indicated that E2 treatment had no effect on the magnitude of LTP induced after theta burst stimulation in either group. Moreover, while the diet and E2 showed no significant effect on either total or phosphorylated PI3K individually, analysis of the pPI3K/PI3K ratio suggests a potential augmentation in phosphorylation attributed to E2. Nonetheless, these findings are preliminary, and further experiments are required to increase the sample size. In summary, our findings show that E2 treatment enhances cognitive processes in CTD- and HFD-fed mice possibly through an increase in activation of the PI3K pathway.

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Poster

PSTR177: Hormones, Cognition, and Social Behavior

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR177.25/K14

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH P01AG071746

Title: Divergent effects of estrogen on peripheral versus brain vascular health

Authors: *C. RICHARD[1], B. VISNIAUSKAS[2], I. FERNANDEZ UGIDOS[2], Z. DIAZ[2], A. MCNALLY[2], R. MOSTANY[2], S. H. LINDSEY[2];
[1]Tulane Brain Inst., Tulane Univ., New Orleans, LA; [2]Pharmacol., Tulane Univ., New Orleans, LA

Abstract: Loss of ovarian function and the presence of cardiovascular disease both disrupts neurovascular coupling (NVC), and defects in this mechanism are associated with cognitive decline. However, the interaction between these two conditions and the ability of estrogen to restore NVC in the presence of cardiovascular disease is unknown. This current study hypothesized that estrogen improves NVC in normotensive but not hypertensive conditions. Female C57BL/6J mice were ovariectomized at 10.5 months of age to mimic menopause in humans and randomized to vehicle or 17β-estradiol (E2) treatment. In addition, mice were randomized to control or hypertension induced by infusion of angiotensin II (AngII; 700 ng/kg/min). Tail cuff plethysmography and pulse wave velocity were used to monitor blood pressure and arterial stiffness, while cranial window two-photon microscopy was used to

measure cerebral blood flow through penetrating arterioles of the somatosensory cortex. In the absence of AngII, E2 did not impact blood pressure or arterial stiffness but improved NVC (124% versus 116% peak vasodilation, P=0.02). AngII induced hypertension in the vehicle group (143 vs 111 mmHg, P< 0.001), but not in the E2 group (109 vs 107 mmHg, P=0.78). Arterial stiffness, measured as pulse wave velocity, followed the same pattern as blood pressure, with AngII increasing stiffness in the vehicle group (3.0 versus 1.7 m/s, P=0.02), but not the E2 group (1.3 versus 1.7 m/s, P=0.65). Lastly, AngII impaired NVC to the same level in both vehicle and E2 groups (both 108% peak vasodilation, P=0.91). We found that E2 improved NVC in control conditions but not in the presence of Ang II, despite the fact that E2 was protective against AngII-induced hypertension and arterial stiffness. These data indicate that AngII has detrimental effects on the brain that are independent of peripheral vascular health. Funding provided by NIH P01AG071746.

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Poster

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Program #/Poster #: PSTR177.26/K15

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant P01AG071746

Title: The ability of different regimens of estradiol treatments to enhance the cognitive aging trajectory in aging ovariectomized rats is independent from their ability to affect cardiometabolic aging

Authors: C. MONTANARI[1,2], ***M. MAROTEAUX**[1,2], E. L. DONG[2,3], S. SRINIVASAN[2,3], A. OLIVEIRA LEITE[4,2], R. MENON[4,2], A. B. WALKER[5,2], A. F. DELARGE[1,2], L. D. DESMOULINS[5,2], S. H. LINDSEY[4,2], A. ZSOMBOK[5,2], J. M. DANIEL[1,2,3];
[1]Psychology Dept., [2]Tulane Brain Inst., [3]Tulane Neurosci. Program, [4]Pharmacol. Dept., [5]Physiol. Dept., Tulane Univ., New Orleans, LA

Abstract: In rodent models of menopause, estradiol administration positively impacts cognition and blocks weight gain induced by loss of ovarian function. Weight gain can be associated with an unhealthy phenotype. The goal of the current study was to determine if the ability of different regimens of estradiol treatment to impact health status in aging female rats was related to its ability to impact cognitive aging. At 8 months of age, Long-Evans rats were trained on a radial-maze spatial memory task and baseline cognitive function was determined for each subject by averaging performance of the last 4 of 24 days of training. Following behavior training, baseline cardiometabolic assessment was completed. Measures included body composition using dual energy X-ray absorptiometry, glucose tolerance test, and blood pressure using tail-cuff

plethysmography. Following baseline assessment, animals were ovariectomized and implanted with Silastic capsules that delivered vehicle or estradiol. Capsules were replaced after 40 days and again after 5 months resulting in the following treatment groups: 1) Continuous Vehicle (received only vehicle capsules; modeling women who never use menopausal estrogen therapy, 2) Continuous Estradiol (received only estradiol capsules; modeling women who take and remain on estrogen therapy), 3) Previous Estradiol (received estradiol for 40 days followed by vehicle; modeling women who take estrogen therapy for a few years and then stop), and 4) Delayed Estradiol (received vehicle for 5 months followed by estradiol; modeling women who begin taking estrogens years after menopause). Cognitive aging trajectories (change in radial-maze performance from baseline) and cardiometabolic health assessment were determined at middle (18 months) and old age (22 months). Results revealed no relationship between the effects of the different estradiol treatments on cognition and their effects on health outcomes. For example, Continuous Estradiol enhanced the cognitive aging trajectory from middle to old age and had positive impacts on health status (body mass/fat, systolic blood pressure, and basal glucose levels). Previous Estradiol treatment also positively impacted the cognitive aging trajectory from middle to old age but had no impact on health measures. Delayed Estradiol treatment had no impact on the cognitive aging trajectory, but positively impacted basal glucose levels. These results indicate that impacts of estradiol on memory and the aging brain are not secondary to its effect on cardiometabolic health.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.01/K16

Topic: F.03. Stress and the Brain

Support: Wellesley College Staley Fellowship (MJT)

Title: Stress, menstruation, and contraceptive use in young adults: Insights from a microbiome study

Authors: J. KAPOOR[1], J. S. LUNIA[2], C. E. PLASENCIA[3], C. M. DEVENEY[4], A. G. COPE[6], N. CHIA[8], M. R. S. WALTHER-ANTONIO[7], C. W. PATTANAYAK[5], ***M. J. TETEL**[2]; [1]Data Sci. Program, [2]Neurosci. Dept., [3]Neurosci., Wellesley Col., Wellesley, MA; [4]Psychology, Wellesley Col., Wellelsey, MA; [5]Quantitative Analysis Inst., Wellesley Col., Wellesley, MA; [6]Obstetrics and Gynecology, [7]Dept. of Obstetrics & Gynecology; Dept. of Surgery; Microbiomics Prog., Mayo Clin., Rochester, MN; [8]Argonne Natl. Lab., Lemont, IL

Abstract: Stress is associated with a variety of factors, including lifestyle factors, hormonal states and the microbiome. The gut microbiome, composed of the microorganisms that reside in the gut and their metabolites, impacts stress and many other aspects of human health and disease. The vaginal microbiome has been implicated in vaginal health and the pathology of several diseases, including bacterial vaginosis, and endometrial and ovarian cancers. However, little is known about the possible interactions between the gut and vaginal microbiomes and the relationship between these interactions and stress, hormones, and lifestyle factors. To explore these understudied interactions, we are investigating associations between stress, hormones (menstruation, hormonal contraception), lifestyle factors (e.g. diet, activity, sleep), and the gut and vaginal microbiomes. In the present study, volunteer college students who were assigned female at birth (n=63) provided daily fecal and vaginal swab samples for up to 10 weeks. Daily diet and nutritional information, menstruation, contraceptive use, and weekly stress ratings were recorded with a mobile application. Birth control is categorized as none, local progestin, systemic progestin-only, or systemic estrogen and progestin combined. Sleep and activity data were collected by FitBit. Across time, participants who menstruated were significantly more stressed than participants who did not menstruate (p=0.05). Furthermore, participants using systemic progestin-only birth control exhibited lower stress scores over the semester compared to those not using any birth control (p=0.01). Microbiota and fungal data from daily fecal and vaginal swab samples will be analyzed to determine if stress, hormones and/or lifestyle factors impact crosstalk between the gut and vaginal microbiomes. These findings will provide a more in depth understanding of the regulation of these two important microbiomes and their impact on women's health and disease.

Disclosures: J. Kapoor: None. **J.S. Lunia:** None. **C.E. Plasencia:** None. **C.M. Deveney:** None. **A.G. Cope:** None. **N. Chia:** None. **M.R.S. Walther-Antonio:** None. **C.W. Pattanayak:** None. **M.J. Tetel:** None.

Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.02/K17

Topic: F.03. Stress and the Brain

Support: ARI Grant # W911NF-22-1-0226

Title: Understanding physiological response and reactivity to stressful environment

Authors: *R. PATEL[1], K. LAFOLLETTE[2], B. MACNAMARA[2];
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Abstract: Understanding how our body adapts under stress is pertinent to both our physical and psychological health. The sympathetic and parasympathetic nervous systems activated during

stress and recovery from stress respectively, are largely regulated by the baroreflex. Serving as a mechanical sensor for blood pressure, baroreceptors fire nerve impulses through the carotid sinus nerves reaching cardiovascular regulatory centers in the medulla oblongata. In response to high blood pressure, vagal neurons are activated to induce vasodilation, decreasing heart rate, and eccrine sweat gland activity. The baroreflex measure is often overlooked in psychophysiology studies, in favor of simpler stress reactivity measures, such as heart rate variability, respiratory sinus arrhythmia, electrodermal activity, and blood pressure. Measured independently, these indices only capture certain components of stress reactivity. The baroreflex is a more holistic measure of stress response, and psychophysiology studies can indirectly measure the baroreflex by integrating multiple measures of stress response. In this study, we demonstrate this by analyzing electrodermal activity, heart rate, and blood pressure changes in response to an acute psychophysiological stressor. Participants ($N = 62$; μ age = 19.0, $\sigma = 1.131$; F: 34, M: 27, NR: 1) were assigned at random to either a stress or stress-free control condition. The stress condition was administered the Maastricht Acute Stress Test (MAST; Smeets et al., 2012), a validated stress manipulation in which participants submerged a foot in ice water and completed a serial subtraction task. The control condition was administered a variant procedure with room temperature water and a simple counting task. Electrocardiogram, electrodermal activity, and blood pressure measures were collected at rest and throughout the MAST administration. We find a pattern of results supporting the stress manipulation having an effect on the function of the baroreflex. Specifically, we find that induced stress is associated with increased blood pressure, decreased heart rate, and a greater number of skin conductance responses. Our results illustrate a change in baroreflex sensitivity in response to stress and support a composite of psychophysiology measures as a promising index of psychophysical stress reactivity.

Disclosures: R. Patel: None. **K. LaFollette:** None. **B. Macnamara:** None.

Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.03/Web Only

Topic: F.03. Stress and the Brain

Support: The Scientific and Technological Research Council of Turkey (TÜBİTAK) 1001 - Grant Number: 219K089

Title: The psychophysiological response to psychosocial stress is correlated with the structural integrity of the amygdala and putamen

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Ankara, Turkey; [5]Department of Interdisciplinary Neuroscience, Institute of Health Sciences, Ankara University, Ankara, Turkey; [6]Brain Research Center, Ankara University, Ankara, Turkey

Abstract: The aim of this study was to examine the relationship between the cortisol response triggered by psychosocial stress and the structural connectivity of the brain.
The study involved 47 healthy participants aged between 18 - 35. The diffusion tensor imaging (DTI) was performed using a 3T MRI scanner. The images were analyzed using the FSL. Preprocessed DTI images were used to generate fractional anisotropy (FA) maps for each participant. Subsequently, TBSS (Tract - Based Spatial Statistics) analysis was performed to create a mean FA skeleton mask. Sixteen ROIs (hippocampus, caudate, putamen, amygdala, insula, supplementary motor area, intraparietal sulcus, dorsolateral prefrontal cortex) were selected bilaterally based on the literature. Participants were instructed to improve task performance to enhance physiological stress response. Saliva samples were collected at five-time points during the MR scan. Cortisol response was measured colorimetrically by ELISA method using the Cortisol Saliva ELISA kit (DiaMetra, DKO020). The rate of cortisol increase was calculated by comparing t_0 with subsequent samples. FA metrics from the ROIs were correlated with the cortisol increase rate using Pearson correlation analysis.
The findings of this study revealed a negative correlation between the cortisol increase rate and the fractional anisotropy values of the putamen ($r = -0.317$, $p = 0.034$) and amygdala ($r = -0.383$, $p = 0.009$) regions of the left hemisphere.
These findings show that cortisol response to psychosocial stress is associated with the structural integrity of the amygdala and putamen regions in the left hemisphere, suggesting a complex interplay between physiological stress response and the brain's neural connectivity.
The authors declare no conflicts of interest.

Disclosures: S. Altinok: None. **G. Vatansever:** None. **S. Üstün:** None. **F. Cetinkaya:** None. **G. Gumus-Akay:** None. **M. Çiçek:** None.

Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.04/K18

Topic: F.03. Stress and the Brain

Support: MSCA ITN Grant 955684

Title: Identifying the monosynaptic inputs to the locus coeruleus - basolateral amygdala pathway and their involvement in the response to acute stress

Authors: *D. FUSCO[1,2,3,4], M. LLORCA-TORRALBA[5,2,3], D. P. FINN[6,7,8], M. M. ROCHE[9,7,8], E. BERROCOSO[1,2,3];
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Abstract: The Locus Coeruleus (LC) is a small brainstem nucleus that represents the most relevant source of noradrenaline within the entire central nervous system (CNS). This nucleus sends a wide range of projections throughout the CNS and plays an active role in the modulation of several physiological processes, such as arousal, cognition, memory, pain, and stress responses. Recently, it has been shown that the hyperactivation induced by stress and chronic pain of projections from the LC to the basolateral amygdala (BLA) leads to an anxiety-like phenotype. Also, this effect is mediated by the release of noradrenaline and the consequent interaction with the β-adrenergic receptors in the BLA. Despite these findings, the anatomical basis of the neural circuit that triggers the activation of the LC-BLA pathway remains unknown. With this study, we hypothesised that there are upstream brain regions that send monosynaptic inputs to the LC-BLA pathway and activate the pathway under stress conditions. To test our hypothesis, we used male and female TH-Cre mice (N=20). The cTRIO technique (cell-type-specific tracing of the relationship between input and output), using a combination of viral vectors, was employed to identify the inputs to the LC within the LC-BLA pathway. Additionally, the animals were exposed to acute restraint stress for 30 minutes and 90 minutes after were euthanized. A histological study was performed to verify and quantify the expression of mCherry+ neurons in the LC (LC neurons projecting to the BLA) and GFP+ neurons (green fluorescent protein - inputs to the LC-BLA pathway). Immunohistochemistry was used to detect c-Fos protein expression induced by restraint stress in the whole brain. cTRIO showed that both sexes had the highest input density in the caudal regions of the rostral axis (bregma -6.12mm to -3.80mm), while the density of GFP+ cells progressively decreased towards the more rostral areas. Specifically, in both sexes, a high number of GFP+ inputs were observed from the gigantocellularis nuclei at the pons level and from the raphe and periaqueductal grey nuclei at the midbrain level. In both sexes, we found increased c-Fos expression in the ventrolateral periaqueductal grey (**p=0.0079) and dorsal raphe (**p=0.0079) of stressed mice, compared to the non-stressed animals. Surprisingly, despite the high number of c-Fos+ cells in areas with high GFP+ cell density, no co-localisation between the two markers was found. This result suggests that in the acute restraint stress model, the stress response is mediated by different neuronal populations than those sending projections to the LC-BLA pathway.

Disclosures: D. Fusco: None. **M. Llorca-Torralba:** None. **D.P. Finn:** None. **M.M. Roche:** None. **E. Berrocoso:** None.

Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.05/K19

Topic: F.03. Stress and the Brain

Support: Indiana University
National Science Foundation Graduate Research Fellowship

Title: Parallel activation in stress-sensitive brain regions during acute and chronic stress in male and female rats

Authors: *H. STORSVED[1], I. BLAIR[2], D. HASEMAN[4], M. LIU[5], C. L. WELLMAN[3];
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Abstract: Chronic stress produces sex-specific changes in the structure and function of rat medial prefrontal cortex and leads to differential responsiveness in stress-sensitive regions of the brain to subsequent stressors. A possible explanation for these sex-dependent effects is that the HPA axis response to restraint is different in males and females. To test this hypothesis, we assessed serum corticosterone concentrations and expression of c-Fos in stress-sensitive brain regions in adult male and female rats during either acute or chronic stress. Acutely stressed rats were placed in restrainers for 0, 60, or 180 minutes, followed immediately by blood sampling and euthanasia. Chronically stressed rats underwent 180 minutes of restraint stress for 9 days; on day 10, rats were placed in restrainers for 0, 60, or 180 minutes, followed by blood sampling and euthanasia. Rats were perfused, and brains were removed, fixed, sectioned, and immunohistochemically stained for c-Fos. To quantify activation neural activation, c-Fos-positive cells were counted stereologically in the prelimbic cortex (PL) and the paraventricular nucleus of the hypothalamus (PVN). Serum was separated and corticosterone concentrations determined via ELISA. In acutely stressed rats, c-Fos expression was significantly elevated at 60 and 180 minutes relative to 0 minutes in both PL and PVN. In chronically stressed rats, c-Fos expression in PL and PVN peaked at 60 minutes of restraint stress then decreased to baseline by 180 minutes. Interestingly, the pattern of c-Fos expression did not differ between males and females. Serum corticosterone paralleled c-Fos expression, increasing over the duration of the stressor in the acutely stressed rats and peaking at 60 minutes before decreasing at 180 minutes in the chronically stressed rats, both independent of sex. These findings suggest that, despite previous evidence of sex dependent dendritic remodeling after chronic stress, male and female rats exhibit similar, partial habituation to 10 days of chronic restraint stress. Thus, the sex-dependent downstream morphological and behavioral effects chronic restraint stress are not likely due to differential HPA axis activation during the stressor.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.06/K20

Topic: F.03. Stress and the Brain

Support: R01NS125589

Title: Social isolation lowers synaptic mitochondrial respiration in female, but not male, California mice

Authors: *A. WEGENER[1,2], H. FULENWIDER[3], H. STADTLER[3], E. R. GLASPER[4], G. N. NEIGH[3];
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Abstract: The neural mechanisms that facilitate the maladaptive consequences of loneliness are not fully understood. Loneliness and social isolation are not synonymous in humans, but exploring the mechanisms affected by social isolation in a social and genetically monogamous rodent may provide insight into loneliness as a stressor. As mitochondria are implicated in the stress response, we investigated the influence of social isolation on mitochondria respiration in the monogamous*Peromyscus californicus* (California mouse). Adult male and female California mice were placed in same sex pairs of 2-3 at weaning. Mice remained with their cage mates until reaching adulthood (approximately 90 days). Three housing paradigms were investigated for both sexes: same sex paired housing (n=19), 10-day social isolation (n=18) or a 30-day social isolation (n=20). All mice were tested for anxiety-like behavior in a 20-minute open field assay at the end of the housing paradigm. Housing status did not influence open field outcomes for males or females. The day following open field, functional mitochondria were isolated from the hippocampus and terminal organ weights were assessed. Peripheral metrics were altered by housing status in males, such that isolated males displayed a higher normalized adrenal and spleen weight. These changes were not observed in females. Hippocampal mitochondrial respiration was assessed using Agilent's Cell Mito Stress test. Housing status did not alter synaptic mitochondria respiration in male California mice. Females did exhibit a change in hippocampal synaptic mitochondria respiration, with 10- and 30-day separated females displaying an overall decrease across mitochondrial dynamics ($p<0.05$): basal, maximal, proton leak, and spare capacity. These data indicate that California mice respond to changes in their housing status through peripheral and central mechanisms, but males and females are not mutually affected. Males are more sensitive to the influence of housing status on peripheral changes, while females showed a robust decrease in hippocampal respiration, but neither is associated with detectable changes in anxiety-like behavior. These studies support the use of California mice for investigating social stressors on neural outcomes and the importance of using both sexes. As we did not observe evidence of anxiety-like behavior, future work should determine if the social isolation periods leveraged in this study were perceived as a maladaptive chronic social stressor or a prompt to adapt to new circumstances.

Disclosures: A. Wegener: None. **H. Fulenwider:** None. **H. Stadtler:** None. **E.R. Glasper:** None. **G.N. Neigh:** None.

Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

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Program #/Poster #: PSTR178.07/K21

Topic: F.03. Stress and the Brain

Support: DGAPA-PAPIIT IN208722

Title: Behavioral and molecular characterization of the stress response in Octopus maya

Authors: *H. SANCHEZ-CASTILLO[1], N. TRUJILLO GUTIÉRREZ[1], F. SÁNCHEZ[1], J. F. VERGARA-OVALLE[2], D. A. GONZÁLEZ-NAVARRETE[3], D. B. PAZ-TREJO[1]; [1]Univ. Nacional Autonoma de Mexico, Mexico City, Mexico; [2]Biochem., UNAM, Cd de Mexico, Mexico; [3]Univ. Nacional Autónoma De México, Ciudad de México, Mexico

Abstract: Octopuses exhibit a stress response similar to that of other animals, involving both physiological and behavioral changes. Increased levels of cortisol, a stress-related hormone in vertebrates, have been observed in response to stressors such as human handling or environmental changes. Additionally, octopuses may display defensive or avoidance behaviors in stressful situations. It has been suggested that stress can adversely affect the health and well-being of octopuses. This highlights the importance to understand the stress response not only for ensuring proper care in captivity but also for comprehending the principles governing this response in an animal with a complex behavioral range and a nervous system lacking structures traditionally associated with stress, such as the amygdala or the pituitary. However, there is still much to be understood regarding the behavioral and physiological components of the stress response in cephalopods. Therefore, in the present study, we aimed to evaluate the stress response in Octopus Maya when exposed to various stressors. To accomplish this, we exposed the octopuses to a range of stressors such as high intensity light, non substrate tanks, and high levels of nitrogenous components. Then, we evaluated corticosterone levels in the skin mucus and brain of the octopuses. Additionally, we analyzed the behavioral responses of the subjects, particularly focusing on the frequency and duration of locomotion and protective behaviors. Our results revealed increased levels of corticosterone in stressed subjects, along with modifications in the frequencies and durations of locomotion behaviors. Importantly, these results showed variations depending on the type of stressor.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

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Topic: F.03. Stress and the Brain

Support: The State of São Paulo Research Foundation (FAPESP # 2018/04899-1; 2021/00148-4; 2021/04572-5; 2021/06709-8; 2023/00306-4, 2023/15852-4).

Title: Investigating the impact of Daun02 inactivation in the ventral hippocampus on cardiovascular, autonomic and neuroendocrine responses during acute restraint stress in male and female c-fos Lacz transgenic rats

Authors: *L. GIATTI[1], B. SCARAMBONE[2], L. GOMES-DE-SOUZA[2], L. B. DE SOUZA[2], F. M. CORREA[3], J. P. HERMAN[4], F. C. CRUZ[6], C. CRESTANI[5], C. BUSNARDO[7];
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Abstract: Stressful stimuli cause an increase in mean arterial pressure (MAP), heart rate (HR) and corticosterone and adrenocorticotropin (ACTH) plasma levels. Moreover, the ventral hippocampus (vHip) is implicated in regulating stress responses. Present work used Daun02 inactivation method in order to evaluate the effect of vHip on cardiovascular, autonomic and neuroendocrine responses related to acute restraint stress (RS) in c-Fos LacZ transgenic female (f) and male (m) rats. The present study was approved by The Ethics Committee on Animal Use (CEUA) of FCFAr-UNESP (protocol #02/2021). Guide cannulas were implanted into the vHip. We submitted female and male rats to RS (2h) and studied the effect of vehicle or Daun02 microinjection into the vHip on RS-induced blood pressure and heart rate (Vehicle: f: n=4; m: n=5; Daun02: f: n=4; m: n=6), tail skin temperature responses (Vehicle: f: n=4, m: n=4; Daun02: f: n=4, m: n=4) and ACTH and corticosterone plasma levels (Vehicle: f: n=5, m: n=6; Daun02: f: n=5, m: n=6). Data were analyzed by Two-way ANOVA followed by Sidak's post-test. RS caused increases in MAP and HR, and drop in skin temperature (ST) in male rats. Bilateral microinjection of Daun02 into the vHip reduced RS-induced tachycardiac response [$F(1, 680) = 66.01$, $P < 0.0001$] and the drop in ST [$F(1, 54) = 4.62$, $P = 0.036$], but did not change pressor response [$F(1, 680) = 1.46$, $P = 0.23$] in male rats. Besides, RS did not change MAP response but increased HR and decreased the ST in female rats. However, bilateral microinjection of Daun02 into the vHip reduced MAP [$F(1, 602) = 8.59$, $P = 0.0035$] and the drop in ST [$F(1, 54) = 18.05$, $P < 0,0001$] during RS but did not change RS-induced tachycardiac response [$F(1, 513) = 0.018$), $P = 0.89$] in female rats. RS caused a slight increase in ACTH and corticosterone plasma levels, being greater in females than males. However, bilateral microinjection of Daun02 into the vHip did not change hormones plasma levels in male [ACTH: $F(1, 28) = 0.36$, $P = 0.55$; Corticosterone: $F(1, 29) = 0.56$, $P = 0.46$] and female [ACTH: $F(1, 24) = 0.89$, $P=0,36$; Corticosterone: $F(1, 23) = 0.74$, $P = 0.40$] rats. Results show that vHip participates differently sex-dependent in the neural pathway which is involved with RS-induced cardiovascular responses. vHip modulates the sympathetic nervous system-mediated redistribution of blood flow in the same direction in both sexes. Nevertheless, the vHip does not seem to play a role in the neural circuitry responsible for neuroendocrine responses elicited by acute stress in both sexes.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.09/K23

Topic: F.03. Stress and the Brain

Support: MH127835
MH119814

Title: Neurocircuitry of Stress Responsive Grooming in F344 rats

Authors: *A. GLORIUS[1], J. P. HERMAN[2];
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Abstract: Obsessive Compulsive Disorder (OCD) is a highly disruptive chronic condition that affects approximately 1.2% of the adult U.S. population. Rodent models of OCD typically examine stereotyped behaviors such as sequenced (cephalocaudal) self-grooming. Self-grooming increases in the presence of stress in numerous rodent models. However, mechanisms underlying this process remain to be delineated. We propose that a stressor triggers activation of the BLA, synapses in the nucleus accumbens core, which in turn drives a nigral projecting pathway known to induce grooming. We used EPM (Elevated Plus Maze) and modified SEFL (Stress Enhanced Fear Learning) models to test self-grooming behavior. We used DREADD approaches in Fischer 344 rats to assess our proposed circuit, examining the potential to initiate grooming behaviors via CNO dosing. Rats received bilateral injections of rgAAV (Cre expressing) in the nucleus accumbens and Cre-dependent DREADD in the BLA. Subsequent experiments examined grooming of Fischer 344 rats (in multiple contexts) as a parameter for stress-responsive behavior, as a means of identifying a reproducible model of stress grooming. CNO treated animals were split into CNO and Saline groups to test impact of DREADD actuation. Our studies indicate that drive of the BLA to NAc circuit by Gq-DREADD actuation results in enhanced home-cage grooming, suggesting a role of this projection is control of baseline grooming behavior. In the context of home cage behaviors prior to stress exposure, CNO dosing of DREADD infused F344 rats significantly increased grooming behavior. However, CNO dosing of DREADD infused rats during EPM and SEFL testing eliminated grooming increases observed in naïve rats. These observations suggest that activation of the BLA◊NAc may have opposing effects on baseline vs, stress-induced grooming, and suggest a role for this circuit in controlling putative stress-coping behaviors.

Disclosures: A. Glorius: None. **J.P. Herman:** None.

Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

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Topic: F.03. Stress and the Brain

Support: HMRF 09203236
HKRGC-GRF 14115821
HKRGC-CRF C4012-22GF

Title: The midline thalamic nucleus reuniens promotes compulsive-like grooming in rodents

Authors: *R. GOH[1], M. MU[1], Y. KE[1], W. YUNG[2];
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Abstract: Obsessive-compulsive disorder (OCD), a disabling and notoriously treatment-resistant neuropsychiatric disorder, affects 2-3% of the general population and is characterized by recurring, intrusive thoughts (obsessions) and repetitive, ritualistic behaviors (compulsions). Although dysfunction within the cortico-striato-thalamic-cortical (CSTC) circuits has long been associated with OCD, the thalamic role in OCD pathogenesis remains highly understudied in the literature. Here, we identified a rat thalamic nucleus—the reuniens (NRe)—that mediates persistent, compulsive-like self-grooming behavior. Optogenetic activation of this nucleus triggers immediate, excessive grooming characterized by strong irresistibility and negative affective valence. A thalamo-hypothalamic pathway linking the NRe to the dorsal premammillary nucleus (PMd) was discovered to mediate excessive self-grooming behavior and render it a defensive coping response to stress, mirroring the obsessions faced by OCD patients. Given the close resemblance between this self-grooming behavior and the clinical manifestations of OCD, the results from this study highlight the role of NRe in mediating OCD-like behaviors. This can be attributed to the NRe's position at the nexus of an extensive frontal-striato-thalamic network regulating cognition, emotion, and stress-related behaviors, suggesting the NRe as a potential novel target for intervention.

Disclosures: R. Goh: None. **M. Mu:** None. **Y. Ke:** None. **W. Yung:** None.

Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

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Topic: F.03. Stress and the Brain

Support: Huck Institutes of the Life Sciences
Department of Biobehavioral Health, Penn State University
Biotechnology and Biological Sciences Research Council BB/R021317/1

Title: Hypothalamic and hippocampal ADCYAP1 gene expression associated with stable behavioral traits

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Abstract: Stress exposure activates many physiological pathways that can influence behavior. One of the central stress response systems is the hypothalamic-pituitary-adrenal (HPA) axis which is responsible for maintaining homeostasis and is associated with glucocorticoid synthesis, a steroid hormone secreted by the adrenal glands and regulated by corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH). When chronically activated, this response leads to over-synthesis of glucocorticoids that can have negative impacts on health including maladaptive behaviors related to fear, memory, and anxiety. The adenylate cyclase activating polypeptide 1 (ADCYAP1) gene encodes for the pituitary adenylate cyclase-activating polypeptide (PACAP) which is found in stress-associated brain regions. This gene is reported to upregulate CRH expression in the paraventricular nucleus of the hypothalamus, and PACAP modulates stress-induced behaviors, such as anxiety-related exploratory behavior and reward-seeking behaviors. Consequently, chronic exposure to stress can lead to higher activation of ADCYAP1 in the hypothalamus, leading to a higher synthesis/secretion of CRH and glucocorticoids, and failure to return to homeostasis, which can have negative health consequences. In this observational study, we investigated whether consistent behavioral traits (i.e. temperaments) previously associated with glucocorticoid secretion are associated with altered hypothalamic or hippocampal basal expression of ADCYAP1 and other HPA-regulation genes. In 54 outbred adult Sprague Dawley male rats we quantified multiple temperaments and brain gene expression. Temperament was characterized based on rat responses to 5 different behavioral tests, each conducted three times. Lastly, RT-PCR was used to measure expression of ADCYAP1, Glucocorticoid Receptor (GR), and FKBP5 (a negative regulator of GR) relative to two reference genes. Our results indicate a negative correlation between ADCYAP1 expression and one particularly stable temperament trait; socially bold individuals, who display decreased glucocorticoid reactivity to stressors, had decreased central ADCYAP1 expression. These results suggest that naturally occurring temperaments are associated with altered basal expression of a genes that can have a significant impact on physiological stress reactivity.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.12/K26

Topic: F.03. Stress and the Brain

Support: MH132680
MH096889
The Bren Foundation.

Title: Targeting corticotropin-releasing hormone receptor type 1 neurons with FlpO: Validating the specificity of a novel transgenic mouse

Authors: *M. HARDY[1], Y. CHEN[2], T. BARAM[3], N. J. JUSTICE[4];
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Abstract: Background: Corticotropin-releasing hormone (CRH) is peptide that functions as an essential molecular regulator for neuroendocrine, autonomic, and behavioral stress responses via its cognate receptors, CRHR1 and CRHR2. However, how activation of these receptors produces transient or long-term changes to circuit function remains unknown. **Methods:** Using a bacterial artificial chromosome (BAC) and recombineering techniques, we have developed a novel transgenic mouse, the CRHR1-FlpO mouse, that expresses codon-optimized flippase recombinase (FlpO) driven by the *Crhr1* promoter. To assess specificity within this transgenic mouse, we examined the expression and anatomical distribution of viral and transgenic Flp-dependent constructs. First, we injected CRHR1-FlpO mice with one of two separate adeno-associated virus (AAV) vectors that require Flp-mediated recombination to express fluorescent reporter molecules. We then applied immunocytochemistry (ICC) to label CRHR1 and viral reporters in three brain regions (i.e. hippocampus, nucleus accumbens, and cortex) where CRHR1 is known to exert important biological functions and displays characteristic immunolabeling patterns. Using confocal microscopy, we visualized the co-expression of CRHR1 and these viral reporters within single cells. The number of cells that expressed both CRHR1 and the viral reporter (i.e. colocalizing cells) vs the number of cells that expressed the viral reporter only (i.e. non-colocalizing cells) was quantified. As a second method of validating the mouse, we crossed CRHR1-FlpO with a transgenic reporter line, Ai65F(RCF-tdT), where tdTomato expression is suppressed in cells by an *frt*-flanked STOP codon. After Flp-mediated removal of the STOP codon, tdTomato is constitutively expressed. This allowed us to indirectly visualize FlpO distribution throughout the whole mouse brain. **Results:** Focusing on three principal CRHR1-expressing regions, cortex, hippocampus and the amygdala, Flp expression was highly specific to cells that also express CRHR1. Within these regions the anatomical distribution of tdTomato, corresponding to the presence of Flp recombinase, recapitulated known anatomical patterns of CRHR1 in the mouse brain. **Conclusions:** These results confirm that the CRHR1-FlpO mouse is a highly specific tool that enables genetic access to CRHR1-expressing neurons in the mouse brain via viral delivery of genetic material or through breeding crosses with compatible transgenic lines. **Funding:** Supported by MH132680; MH096889 and the Bren Foundation.

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Poster

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Topic: F.03. Stress and the Brain

Title: Investigating Neurobiological Profiles of Vigilance and Wariness: Comparisons of Wild-Trapped and Laboratory Rattus norvegicus

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Abstract: Despite the extensive use of laboratory-bred rodents in neuroscience research, neurobiological characteristics of wild *Rattus norvegicus* remain poorly understood. Recently, our lab reported heavier brains and higher cerebellar neuronal densities in wild rats compared to their laboratory counterparts, as well as higher levels of fecal corticosterone (CORT) metabolites, heavier adrenal glands, and spleens (Jacob et al., 2022). In a second cohort of wild-trapped and laboratory-control female rats, we were interested in neural variables contributing to heightened vigilance and wariness associated with survival in wild habitats (Koizumi et al., 2018). Various neural targets associated with survival behaviors were assessed including the auditory, motor, somatosensory, and pyriform cortical areas (involved in sensory and movement responses); further, brain areas associated with threat cue detection and coping/appraisal such as the lateral habenula, hippocampus, and amygdala were investigated. For brain analyses, wild female rats were trapped in Richmond VA and compared to weight-matched laboratory counterparts (n=7 for each group). Compared to laboratory control females, neural quantification revealed distinct neurobiological profiles in the wild female rats including increased Glucocorticoid-immunoreactivity in CA2 hippocampus, increased neuronal density in the lateral habenula, increased glial density in the pyriform cortex, and increased neuronal and glial density in the auditory cortex ($p<.05$ for all variables). Additional analyses incorporating male rats (n=4 for wild and lab rat groups) confirmed higher fecal CORT metabolites and larger adrenal glands, both indicative of heightened stress responses. An additional cohort of wild male and female rats housed individually in large outdoor cages for appx. five days indicated a rapid habituation of the CORT response to a mere 5% of the original values, a finding suggestive of adaptive emotional regulation mechanisms. Together, these results indicate that wild rats have different neurobiological profiles than selectively bred laboratory rats. Increased neuronal density in the lateral habenula may be involved in the rat's ability to assess environmental threats-- an ability that is necessary for survival in a wild or wild-type environment. Further, altered profiles in cortical processing areas and critical limbic areas may facilitate the rapid processing of threat

cues in the varied and dynamic environments of wild animals. Overall, our study highlights the importance of considering wild rats as a valuable model for investigating stress physiology and adaptive behaviors.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

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Topic: F.03. Stress and the Brain

Support: R56 MH130006

Title: Steroid 5α-reductase 2 in the prefrontal cortex mediates acute stress response via allopregnanolone synthesis

Authors: *C. BRANCA[1], R. CADEDDU[1], G. BRACCAGNI[1], G. FLORIS[2], G. PINNA[3], S. SCHEGGI[4], M. BORTOLATO[1];
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Abstract: Acute stress elicits several rapid brain responses aimed at facilitating adaptive coping mechanisms. One such response involves the increased biosynthesis of the neurosteroid allopregnanolone (AP), which exerts anxiolytic, neuroprotective, and antidepressant properties; however, the neurochemical mechanisms underlying this process remain unclear. Given that the enzyme 5α-reductase (5αR) catalyzes the rate-limiting step in AP synthesis, we investigated the role of its two major isoenzymes in acute stress response. Different types of acute stress led to a rapid, selective increase in the mRNA and protein levels of 5αR type 2 - but not 1 - in male rats. These effects were not observed in females, irrespective of the estrous cycle phase. Targeted downregulation of 5αR2, but not 5αR1, in the medial prefrontal cortex (mPFC) markedly reduced the responsiveness of males, but not females, to both stressful and arousing stimuli. Similarly, a newly generated line of 5αR2 knockout rats exhibited abnormal reactions to stressful and incentive environmental stimuli. Notably, while 5αR1 was found to control AP synthesis under baseline conditions, 5αR2 enabled AP synthesis only in response to acute stress. In line with this finding, the behavioral alterations in rats with mPFC 5αR2 down-regulation were reversed by acute AP administration. Single-nuclei transcriptomic analyses of the mPFC showed that, while acute stress increased protein synthesis in pyramidal neurons and glia, these processes were dramatically downregulated in rats with 5αR2 mPFC knockdown. Collectively, these findings indicate that 5αR2 serves as a sexually dimorphic, inducible enzyme orchestrating acute

stress adaptation by enabling rapid AP synthesis in the mPFC. These data point to 5αR2 as a crucial determinant in sex differences with respect to stress reactivity.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

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Program #/Poster #: PSTR178.15/K29

Topic: G.04. Emotion

Support: NIH R01MH132795

Title: Prefrontal stress ensemble mediates maladaptive avoidance in a mouse model of Tuberous Sclerosis Complex

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Abstract: Emotions are crucial features that allow us to react adaptively to internal and external stimuli in the environment and serve to promote self-preservation. The inability to control the attributes and intensity of one's emotion is one of the debilitating symptoms in neurodevelopmental disorders (NDDs). Tuberous sclerosis complex (TSC) is an autosomal dominant NDD caused by the loss-of-function mutations in two TSC genes (*Tsc1* or *Tsc2*) and is associated with pathological anxiety, which is characterized by a pervading sense of unrealistic worry about normal life situations. Psychological stress is a major risk factor for the initiation and progression of several neurodevelopmental and neuropsychiatric disorders. Moreover, due to the fundamental need for social connection in social animals, prolonged involuntary isolation can act as a stressor, inducing a sustained negative affective state that can precipitate maladaptive avoidance, a hallmark of pathological anxiety, in genetically susceptible individuals. The medial prefrontal cortex (mPFC) is an important cortical region that integrates information from cortical and subcortical areas and converges updated information to output structures, thereby regulating emotional processing, and may be a potential therapeutic target for anxiety disorder in TSC. TSC proteins constitute the TSC complex which acts as a molecular brake on mammalian target of Rapamycin complex I (mTORC1) and integrated stress response, which are two opposing pathways regulating nascent protein synthesis. We hypothesized that selectively inactivating *Tsc2* in mPFC neuronal ensembles activated during social isolation will precipitate maladaptive anxiety-like phenotypes. To test this, we used viral-mediated cell type-specific deletion of Tsc2 in the mPFC and examined the effect of prolonged social isolation on behavior in male and female mice. We further examined the effects of drugs targeting downstream TSC effectors in rescuing the observed phenotypes. Our findings indicate that the lack of social buffering

precipitates maladaptive anxiety-like phenotypes, specifically in signaled active avoidance paradigm where animals learn to shuttle in response to the threat-predictive auditory cue for avoiding the footshock. We also tested the effect of social isolation on mice with global heterozygous deletion of Tsc2. Finally, we were able to tag neuronal ensembles in the mPFC that are activated during social isolation, which might be a potential therapeutic target for stress-mediated emotional dysregulation in TSC and other NDDs.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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Topic: F.03. Stress and the Brain

Support: Psychology startup fund for NCL

Title: The effects of social isolation on anxiety-like behaviors and perineuronal nets in the insular cortex

Authors: *J. J. VENEGAS[1,2], A. S. BRINKS[1,3], O. A. NABELSI[1,2], R. E. HERRINGSHAW[1,2], N. C. GORDON[1,2], N.-C. LIANG[1,2,3];
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Abstract: Although the negative impacts of social isolation on emotional and metabolic health are well-documented, the underlying mechanisms remain largely unclear. Perineuronal nets (PNNs) may be involved in social isolation-associated increases of anxiety-like behavior. Given that studies examining this question in rodents during late adulthood are scarce, we explored the effects of social isolation in middle-aged rats on anxiety-like behavior, insulin response, and PNN density in the anterior insular cortex (AIC), a region implicated in modulating anxiety. We hypothesized that social isolation would decrease PNNs in the AIC and lead to increased anxiety-like behavior compared to group housed rats. Additionally, we expected that socially isolated rats would exhibit a decreased insulin sensitivity, suggesting an increased susceptibility to developing type 2 diabetes. To test these hypotheses, both sexes of rats that were never socially isolated prior to 11 months of age were individually or group housed for two months followed by assessments of anxiety-like behavior and insulin response while remaining in their assigned housing conditions. Anxiety-like behavior was assessed through an open field test, novelty suppressed feeding test, elevated plus maze (EPM), and a marble burying task. Finally, all rats underwent an insulin tolerance test (ITT) to assess their insulin sensitivity. Brains were collected and processed for PNN quantification in the AIC. We observed a sex-dependent effect in the EPM, where female rats made more entries into the center of the maze compared to males.

Female rats also exhibited lower blood glucose levels than males 90 minutes after an insulin injection. Contrary to our hypotheses, anxiety-like behaviors and blood glucose levels following the ITT in isolated and group housed rats were not statistically different. However, preliminary results (n = 4/group) reveal that compared to isolated rats, group housed rats had a higher PNN density in the AIC. These results suggest that social isolation for two months in middle-aged rats may not be sufficient to change anxiety-like behaviors despite altering PNN density in the AIC. Future studies are warranted to elucidate the extent to which anxiety-like behaviors and PNNs are affected by a longer duration of isolation in aging rats that have not previously been housed alone.

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Poster

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Topic: G.04. Emotion

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BEPE FAPESP Award - Process number 2022/10168-5
UCSD Triton Research & Experiential Learning Scholarship

Title: Trapping ensembles for predator stress

Authors: *Y. LOU[1], C. FAVORETTO[2], A. NGUYEN[3], S. RANJAN[4], L. BERTOTTO[5], K. LIN[6], F. C. CRUZ[7], E. P. ZORRILLA[8];
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Abstract: Chronic stress globally impacts human behavior and health. While the striatum and prefrontal cortex are implicated in neurobiological responses to stress, regional ensemble differences therein between acute vs. repeated psychosocial stress are unknown. A behaviorally-validated model of predator stress where TRAP2-Ai9 mice (n=9-21/group) received 4-hydroxytamoxifen (4OHT; 40mg/kg) or vehicle after acute (1st) or repeated (10th) exposure to predator stress (odorous rat bedding) was used. Controls received clean bedding. Activated, CreER-expressing neurons during 4-hydroxytamoxifen injections undergo recombination, permanently expressing tdTomato fluorescent reporters. Eighteen days after the 10th, mice

received a final (11th) exposure and were euthanized. Brain slices were imaged under an LSM710 confocal microscope. Activated cells at sacrifice (green Fos immunofluorescence) vs. after acute (1st) or repeated (10th) stress (TRAPped red tdTomato) were counted, and Fos-tdTomato co-localization was assessed using ImageJ.The dorsomedial striatum (DMS) and anterior cingulate (aCg) had more tdTomato-positive TRAPped cells after the 1st than 10th exposure to both conditions, indicating a Time effect. Greater neuronal activation occurred after the 10th stress exposure compared to control, displaying a Stress effect. In the aCg, Fos-positive cells from the 11th, separate re-exposure co-localized more with tdTomato-positive ensembles TRAPped after one acute exposure than ten repeated exposures. Cellular activation in the DMS and aCg habituated after repeated exposure to both stimuli, predominantly in controls, with stress evoking a greater response than control conditions. Unexpected stress induced the greatest response, indicated by ensembles after the 1st exposure having the greatest activation and most co-localization to a separate re-exposure.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

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Topic: G.05. Mood Disorders

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Title: Proteomic alterations in the medial prefrontal cortex of mice following learned helplessness

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Abstract: Acetylcholine (ACh) signaling is implicated in the etiology of depression, but is also important for learning, memory, and attention, suggesting that optimal levels are beneficial, while excessive increases are detrimental to affective health. Prolonged ACh signaling during highly stressful events could therefore lead to a negative encoding bias, in which stressful experiences are both attended to, and encoded, more potently, leading to increased depressive symptoms. In agreement with this hypothesis, mice experiencing inescapable stressors as part of the learned helplessness (LH) paradigm display elevated medial prefrontal cortex (mPFC) ACh transients during LH training. Although both sexes displayed escape deficits in an active avoidance test following LH training, the intensity of ACh signaling during training correlated positively with escape deficits during testing only in males. In addition, chemogenetic excitation

of mPFC cholinergic terminals increased escape deficits in both male and female mice, while inhibition also increased escape deficits in females but decreased the proportion of helpless male mice, demonstrating the importance of mPFC ACh signaling in active avoidance learning following LH training. To identify molecular mechanisms by which mPFC ACh activity could mediate LH outcomes, we conducted proteomics on mPFC synaptosomes from mice following completion of LH. We identified significant changes in pathways involved in synaptogenesis and ACh receptor signaling. Regarding ACh signaling, we identified alterations in levels of the muscarinic ACh receptors Chrm1, Chrm2, and Chrm4. Chrm4 was only downregulated in mice that had undergone two days of LH training followed by active avoidance testing, indicating potential involvement of this receptor in LH outcomes. In contrast, LH training or active avoidance testing alone resulted in downregulation of Chrm1. Interestingly, Chrm2 was differentially regulated in male and female mice, with large decreases observed in the inescapable group in female mice indicating a potential target for the sex-specific responses observed in our behavioral study (Abdulla et al. 2023), and polymorphisms in Chrm2 have been associated with depression in women. Together, these data implicate mPFC cholinergic signaling as an important regulator of later active avoidance learning in the LH model and point to several targets for future explorations into the mechanism by which ACh mediates LH outcomes.

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Poster

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Topic: G.05. Mood Disorders

Support: CIHR

Title: Stress-induced functional adaptations in NAc and VTA projecting cells elicited by somatostatin and parvalbumin interneurons

Authors: *M. D'ANGELO[1], C. PROULX[2], B. LABONTÉ[3];
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Abstract: The medial prefrontal cortex (mPFC) is a major hub in the control and elaboration of stress responses. It does so by integrating and processing information from different brain regions before redirecting it to limbic structures controlling different aspects of emotional behaviors. Somatostatin (SST) interneurons and parvalbumin (PV) maintain a homeostatic balanced activity in the mPFC by controlling the inputs and the output of pyramidal cells, respectively. Work from our group suggests that chronic stress impairs the excitatory and inhibitory (E/I) balance in the mPFC. Here, we tested whether chronic stress changes with the morphological and functional properties of SST and PV GABAergic interneurons to disrupt the

E/I balance in the mPFC of male and female mice. We used 21 days of chronic variable stress (CVS) to induce an emotional stress response in PV-cre and SST-flpo transgenic mice. A trans-sectional viral approach was used to label SST and PV cells and assess neuronal complexity and functional alterations in stressed male and female mice. Connectivity and functional properties of both SST and PV GABAergic interneurons in male and female mice were addressed using the mGRASP viral approach. Our results show a reduction in the complexity of SST interneurons in both male and female stressed mice compared to controls. On the other hand, we observed an increase in the number and complexity of PV interneurons in stressed female mice compared to controls, while stressed males exhibited reduced complexity compared to controls. Interestingly, this was accompanied by a concomitant increase in the dendritic arborization of PV interneurons in stressed female mice, while stressed male mice showed reduced dendritic arborization. SST interneurons showed a reduction of the dendritic arborization in stressed males and females compared to controls. Our results provide a better understanding on how chronic stress might affect morphological and functional properties of SST and PV interneurons and their relationship with excitatory pyramidal neurons in modulating behavioral stress responses in a sex-specific way.

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Poster

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Title: Long and very-long chain ceramides promotes an anxiety-like behavior in female mice through microglia activation in cortex

Authors: *S. BERNAL[1,2], A. CAMACHO[3,2];
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Abstract: Anxiety disorders are characterized by excessive and enduring fear, commonly associated with an exacerbated excitability state in the nervous system, affecting 4% of the global population. Risk factors encompass stress, genetics, substance use and fetal exposure to adverse factors. Neuroinflammation and sphingolipid metabolism alterations contribute to anxiety pathogenesis. Clinical and animal studies link plasma ceramides to anxiety symptoms, yet their exact molecular mechanisms remain unclear (Bernal-Vega, 2023). Maternal exposure to

high-energy-dense nutrients induces anxiety-like behavior and increases plasma levels of long-chain ceramides in offspring. Furthermore, ceramides can modulate proinflammatory pathways in microglia, suggesting a role in anxiety. This project aims to identify the effect of ceramides on the induction of anxiety-like behavior in mice through microglia activation. C57/BL6J 3-month-old male and female mice received intravenous ceramides (C16:0, C18:0, C22:0, C24:0, C24:1), and behavioral tests were conducted. Microglia cells were obtained from hippocampus, striatum, and cerebral cortex and phenotyped by flow cytometry. Phagocytic activity was determined in microglia immortalized cultures following ceramide stimulation by using fluorescent latex beads, cells were analyzed using a flow cytometer. For the elevated plus maze we found a significant increase in the time spent on the closed arms in female subjects administered with ceramides. In male mice, we did not observe a significant difference in any of the analyzed parameters. In the open field test, there were no significant differences in any of the assessed parameters for both female and male mice. For the dark/light box test we found a significant increased latency to first enter the light compartment in female mice administered with ceramide. In male subjects no statistically significant differences were detected. In the novelty suppressed feeding test, we found that female mice administered with ceramides experienced longer latency to reach the food pellet in the center of the arena. We did not observe statistically significant differences in the analyzed parameters for males. We analyzed if ceramides promote changes in microglia phenotype. Ceramides increased proinflammatory CD86+ cells in the cortex and decreased anti-inflammatory CD206+ cells across striatum, cortex and hippocampus. In vitro, ceramides enhanced microglial phagocytic activity. These findings suggest long and very long-chain ceramides induce anxiety-like behavior in female mice and promotes cortical microglial activation, potentially mediating anxiety.

Disclosures: S. Bernal: None. **A. Camacho:** None.

Poster

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Klingenstein-Simons Fellowship in Neuroscience

Title: Investigating the impacts of early life adversity on developmental behaviors and neuron-microglia interactions in the NAc

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Abstract: Early life adversity (ELA) is a major risk factor for developing mood and anxiety disorders, with an effect size outweighing any known genetic factor. Studies in humans and animal models suggest that exposure to ELA disrupts the development of key emotion centers in the brain, including the nucleus accumbens (NAc), leading to threat-sensitization, dysfunctional reward processing, and maladaptive behaviors later in life. However, most preclinical studies of ELA examine the behavioral and biological consequences of early life stress in adult animals. As such, the cellular mechanisms through which ELA impacts *developing neural circuits* in the NAc, altering its function and conferring a long-lasting susceptibility to psychiatric disease, remain poorly understood. Moreover, it's unknown whether ELA leads to maladaptive changes in *developmental behavior patterns*, which might precede behavioral changes observed in adults. In this study, we use a limited bedding and nesting (LBN) mouse model of early life adversity, in combination with immunohistochemistry and viral labeling techniques to examine how ELA impacts cells within the NAc at time points spanning from infancy to early adulthood (P12-P70). We focus on microglia, the brain-resident immune cells. Microglia are responsive to external stress and play crucial roles in synaptogenesis and synapse pruning. We hypothesized that ELA in the form of resource scarcity and caregiver stress (LBN) alters developmental microglial phenotypes and circuit-refinement activity in the NAc, resulting in maladaptive circuit maturation that underlies ELA-induced behavioral dysfunction. We discovered that there is a selective, significant increase in microglial density in the NAcs of juvenile LBN mice compared to age matched, standard-reared mice. Ongoing analyses will determine whether this abnormal increase in microglial densities is associated with changes to microglial lysosomes, phagocytic cups, and morphology, as well as altered spine densities in the NAcs of juvenile mice. Additionally, we observed sex-specific differences in anxiety-like behaviors between LBN mice and age-matched controls at adolescent and adult time points. This suggests that ELA-induced behavioral changes first appear during developmental periods in a sexually dimorphic manner. Overall, this project will reveal the neurobiological mechanisms through which ELA impacts developing reward circuits in the brain and alters related behaviors. In doing so, we can gain insight into how ELA increases susceptibility to mood disorders later in life and potentially open new strategies for therapeutic interventions.

Disclosures: S.V. Blagburn-Blanco: None. **C. Christensen:** None. **L.A. DeNardo:** None.

Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.22/L1

Topic: F.03. Stress and the Brain

Support: Sponsored research award from Ono Pharmaceutical Co., Ltd
Postdoctoral fellowship from Fundacion Ramon Areces

Title: Astrocyte molecular changes during basolateral amygdala-related emotional behaviors

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Abstract: Astrocytes tile the central nervous system (CNS) and have fundamental roles in physiology and disease, yet there remain open questions about their contributions to physiological and pathological processes in the CNS. The basolateral amygdala (BLA) integrates rewarding and aversive stimuli and is integral to mood regulation and emotional behaviors. As many past studies have focused on neurons in the BLA, the contribution of BLA astrocytes to emotional behaviors is largely unknown. A major bottleneck has been the paucity of reliable tools to explore astrocyte functions and molecular mechanisms in vivo. This shortfall has been addressed by recent developments in the field reporting approaches to systematically explore the role of astrocytes with improved tools. Here, we used several such tools to elucidate BLA astrocytic changes during emotional behavioral responses. Applying several mouse models of stress (negative emotional triggers) and astrocyte-specific adeno-associated viruses (AAVs), [Ca^{2+}] imaging, RNA sequencing, and proteomics, we explored the role of astrocytes at the molecular and cellular level in the BLA. We analyzed the data to evaluate how astrocytes might be exploited in vivo to improve behavioral outcomes in emotion-related disorders such as anxiety and depression.

Disclosures: S. G. Pelaz: None. **K. Mitsui:** None. **R. Kawaguchi:** None. **V. Pandey:** None. **J. Wohlschlegel:** None. **B.S. Khakh:** None.

Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.23/L2

Topic: G.05. Mood Disorders

Support: CIHR Grant PJT-186290

Title: Examining sex-specific activation of immature neurons during chronic stress-induced negative cognitive bias

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Abstract: Major depressive disorder (MDD) is a debilitating illness that affects 20% of the global population. About one third of MDD patients are treatment-resistant, leaving an enormous

unmet need. Sex differences in the prevalence and manifestation of MDD exist, with females twice as likely to be diagnosed with MDD as males and often presenting with different symptom profiles. MDD is associated with decreased hippocampal neurogenesis and aberrant neural activity in the dorsal and ventral hippocampus (dHPC and vHPC). Negative cognitive bias (NCB), or the interpretation of ambiguous stimuli as negative, is a treatment-resistant cognitive disruption that predicts first onset and relapse of MDD. To model cognitive bias, we train rats to discriminate between shocked context A and non-shocked context B. After 16 days, they freeze in anticipation of a shock in context A, with minimal freezing in context B. Rats are then tested in ambiguous context C with a mix of cues from contexts A and B; high freezing indicates NCB. Chronic unpredictable stress (CUS) reliably produces depressive-like endophenotypes in both male and female rats such as decreased sucrose preference and increased passive coping behavior, reduced neurogenesis, and altered functional connectivity. Pilot data from our lab indicate that CUS also increases NCB in both sexes. Furthermore, we have found that despite similar levels of NCB, CUS-induced changes to neural activity in the HPC are sex-specific. In this study, we sought to examine whether there are sex differences in the CUS-induced reduction of neurogenesis and the activity of new neurons during NCB. We exposed young adult rats to 21 days of CUS or no-CUS, then administered the NCB task. In line with previous data, we found that CUS increased NCB in both female and male rats, with no sex differences in freezing behavior. Brain sections including vHPC and dHPC were stained for zif268, a neural activity-dependent immediate early gene implicated in synaptic plasticity and memory, and doublecortin (DCX), which labels immature neurons. We hypothesize that activation of immature neurons (zif268/DCX co-expression) in the HPC will differ by sex and stress despite similar levels of NCB. Based on previous findings, we expect that female CUS animals will be less likely to use immature neurons during NCB testing vs. no-CUS females whereas male CUS animals will be more likely to use immature neurons. Better understanding the neurobiology underlying negative cognitive bias could suggest sex-specific and much needed drug targets for the development of novel antidepressants.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.24/L3

Topic: F.03. Stress and the Brain

Support: UNAM. PAPIIT IN305622

Title: Role of striatal glucocorticoid receptors in memory acquisition under restraint stress and corticosterone inyections

Authors: *E. A. RENDON-OCHOA[1], L. N. CEDILLO ZAVALETA[2], A. O. FLORES SÁNCHEZ[3], M. GONZALEZ LOPEZ[4], S. E. CRUZ-MORALES[5];
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Abstract: Stress exposure releases several molecules destined to maintain homeostasis. Corticosterone (CORT) is the main hormone secreted by HPA axis, modulating neurochemical and behavioral responses, like learning and memory. The behavioral outcome is determined by the type and duration of the stressor, and the type of stage of memory when stress is applied. Exits evidence that stress applied before training could impair memory acquisition, and stress applied after training could enhance memory consolidation. Nevertheless, several evidences indicate no changes in memory acquisition and consolidation in procedural memory; the exact mechanism for this discrepancy is controversial. In this work we explore the participation of dorsal striatal glucocorticoid receptors in the modulation of memory acquisition in the elevated T-maze (ETM). The first objective was to determine whether 15 min of restrain (RES) or CORT ip (CORT) could impair memory acquisition and whether a glucocorticoid synthesis inhibitor, administrated 30 min before stress paradigms, could prevent memory modulation induced by stress. Under these conditions, RES and CORT ip, produced a reduction in the latencies in the ETM, indicating an impairment in procedural memory acquisition. Previous injection of metyrapone effectively prevented the impairment, indicating that glucocorticoid affected memory acquisition and their posterior retention.
As a next objective and to keep searching the role of glucocorticoid receptors in memory, we wanted to determine whether antagonism of glucocorticoid receptors located in dorsal striatum is sufficient to prevent the impairment in memory acquisition produced by RES or CORT. We performed stereotaxic surgeries to implant cannulas directly in dorsal striatum, structure highly related to procedural memory, to deliver and antagonist of glucocorticoid receptor, mifepristone 5 min before RES and CORT injections. Under these conditions, we found that RES or CORT ip failed to decreased latencies in ETM, indicating that dorsal striatal glucocorticoid receptor activation could yield procedural memory impairment. To further confirm the previous findings, we delivered CORT directly into dorsal striatum in naïve rats. We observed that CORT in situ impaired acquisition, reducing latencies in ETM like those observed in RES and CORT. Altogether, these results showed the participation and their critical role of striatal glucocorticoid receptors in modulating memory acquisition.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.25/L4

Topic: F.03. Stress and the Brain

Support: R01MH051399
R01MH129306
NIH T32-MH087004
The Hope for Depression Research Foundation
F31 MH133297-02

Title: Resilient Specific Sex-Conserved Transcriptomic Networks in the Nucleus Accumbens Following Chronic Social Defeat Stress in Mice

Authors: *T. GYLES[1], E. M. PARISE[1], L. HOLT[1], A. M. MINIER-TORIBIO[1], T. MARKOVIC[1], L. PARISE[1], R. DURAND-DE CUTTOLI[1], A. RAMAKRISHNAN[1], Y. YIM[1], C. J. BROWNE[1], A. GODINO[1], A. M. CARDONA-ACOSTA[2], M. ESTILL[1], C. A. BOLANOS-GUZMAN[3], S. J. RUSSO[1], E. J. NESTLER[1];
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Abstract: Major depressive disorder (MDD) is a leading cause of disability and a leading contributor to suicide according to the World Health Organization. Chronic stress is a primary risk factor for MDD and is modeled in rodents using the chronic social defeat stress (CSDS) paradigm. This paradigm allows for identifying animals across a continuum of responses from those that develop depression-like behavioral abnormalities, termed susceptible, to those that maintain mostly normal behavioral function, termed resilient. This approach has proven to be highly useful but has been mostly examined in male mice, given that depression is more prevalent in women, it is crucial to investigate potential sex-specific molecular mechanisms underlying susceptibility vs. resilience. To address this gap, we conducted RNA-seq on female mice subjected to an adapted model of CSDS and identified transcriptional changes associated with stress susceptibility-resiliency across multiple brain regions. Initial comparison of this new dataset with published findings on male mice replicated earlier findings of striking sexual dimorphism in adaptations associated with resilience in female vs. male mice in the brain regions studied. Despite this sexual dimorphism, we identified a cluster of genes uniquely upregulated in the NAc of resilient mice that overlapped ~40% across sex. To further investigate this convergence, we performed Weighted Gene Co-Expression Network Analysis (WGCNA) on both resilient male and female mice. After the creation of a co-expression network, we compared gene modules (comprised of genes with similar co-expression) across sex to identify convergent modules across sexes in resilient mice. We found a pair of modules that display 25% percent overlap, the highest of all possible module combinations across sex. Both modules have the highest enrichment for differentially expressed genes that are upregulated in the NAc of resilience mice. Furthermore, within these gene modules, two key driver genes, *Gprin1* and *Stx1a*, were predicted to regulate other module genes and positively correlate with behavioral resilience in both sexes. Bilateral overexpression of GPRIN1 or STX1a in all NAc neurons of male mice induces a pro-resilient effect and ongoing research is characterizing effects on transcriptional, neuronal, and circuit function that underlie the promotion of behavioral resilience. This study provides novel insight into the molecular mechanisms underlying stress resilience and the importance of considering sex-specific factors in understanding depression and developing targeted interventions.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.26/L5

Topic: F.03. Stress and the Brain

Support: NIMH
Hope for Depression Research Foundation
Boehringer Ingelheim Fonds

Title: Transcriptional mechanisms of female stress susceptibility

Authors: *C. A. MCLAIN[1], O. ISSLER[2], A. CUNNINGHAM[1], M. ESTILL[1], L. HOLT[1], G. ROJAS[1], E. J. NESTLER[1];
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Abstract: Chronic stress produces sex-specific neurobiological and behavioral outcomes which are associated with greater stress susceptibility in female animals and a higher prevalence of depression in women. Our understanding of the molecular mechanisms that drive sex-differentiated stress responses is limited. In this project, we map the distinct contributions of female sex hormones versus sex chromosomes to stress-induced transcriptional regulation in the nucleus accumbens (NAc) and medial amygdala (MeA), brain regions implicated in stress and sex differences, and in stress-induced behavioral outcomes. To investigate the effects of these two biological sex variables independently, we use the four core genotypes mouse model, in which sex chromosomes are dissociated from gonadal sex by expressing the *Sry* gene autosomally. Here, we show that a hormonal difference produces more discrepancy in gene expression and behavior after chronic stress than a sex chromosomal difference. In a separate sequencing cohort, we show that ovariectomy surgeries in wild-type adult females dramatically alter transcriptional profiles in the NAc following stress. Finally, we are identifying DNA-binding sites of estrogen receptor alpha by use of CUT&RUN-sequencing in our brain regions of interest in control and stressed conditions, and we highlight genes that are consistently regulated by female sex hormones and stress across datasets. These experiments will define the molecular basis of the dramatic sex differences seen in chronically stressed rodents and in depressed humans, which will help define sex-specific approaches for novel therapeutics for human stress disorders.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.27/L6

Topic: G.05. Mood Disorders

Support: University of Wisconsin-Milwaukee

Title: The Role of Estrogen Receptor Beta in Stress-Induced Sleep Disturbances and Maladaptive Behavioral Responses in mice

Authors: *P. BENDIS, S. ZIMMERMAN, P. GEORGIOU;
Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Depression is a prevalent mental health disorder that significantly impacts daily functioning and quality of life. Among the various physiological factors implicated in the etiology and progression of depression, sleep disturbances are consistently recognized as both a symptom and a contributing factor. Poor sleep quality, including difficulties in falling asleep, staying asleep, or experiencing non-restorative sleep, has been linked to increased severity of depressive symptoms, reduced responsiveness to treatment, and higher relapse rates. Conversely, improvements in sleep quality are often associated with better outcomes for depression. Understanding the mechanisms through which sleep impacts depression could unveil potential therapeutic targets and improve intervention strategies. One possible mechanism underlying sleep impairments in depression is the dysregulation of estrogen receptor beta (ERβ). Accumulating evidence suggests that ERβ activation may play a protective role against the neurobiological impacts of stress on sleep patterns, potentially offering a targeted avenue for mitigating sleep disturbances associated with stress-related disorders, including depression. In support of this, our preliminary data using RNAseq and complex bioinformatics and gene ontology analysis demonstrated a downregulation in core promoter sequence-specific DNA binding, which includes the ERβ gene and sleep-associated genes such as RORA and NR1D1 in the ventral hippocampus following chronic stress in mice. Moreover, to investigate this in vivo, we performed sleep recordings in ERβ knockout mice following acute stress. We observed an increase in rapid-eye movement sleep during the daytime in ERβ knockout mice following acute stress, accompanied by a simultaneous decrease in sucrose preference. Additionally, the sleep impairments were accompanied by an increase in delta power during the day in ERβ knockout mice. These findings suggest that ERβ may underlie the development of sleep impairments following stress and the subsequent development of maladaptive behaviors.

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Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.28/L7

Topic: H.08. Learning and Memory

Support: R01-DA057767
R01-HL163965

Title: Fiber photometry reflects enhances adrenergic expression in the brain of in vivo mice after intermittent hypoxia

Authors: *B. M. BROWE[1], A. J. GARCIA, III[2];
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Abstract: The locus coeruleus (LC) is a key node of the sympathetic nervous system serving as the principal source of norepinephrine (NE) in the brain, which serves to facilitate autonomic neurophysiology and cognitive performance. Intermittent hypoxia (IH), a hallmark sleep apnea, causes neurocognitive deficit and leads to persistent elevation of sympathetic activity. However, the impact of IH on LC activity and central noradrenergic neuromodulation remains poorly resolved. We hypothesize that IH leads to increased LC activity that coincides with an enhanced central adrenergic tone. In this ongoing study, we use fiber photometry in freely behaving mice to measure gCAMP and $GRAB_{NE}$ fluorescence to assess LC activity and intrahippocampal (HPC) adrenergic tone, respectively. Measurements were made in freely moving mice while the animals were breathing room air (Fi_{O2} =21%) and in response to hypoxia (Fi_{O2} =10%) before and following IH. IH led to an increased number of LC events when breathing room air, yet during hypoxia, LC activity is unchanged. Many LC events in room air correlated with the occurrence of high frequency respiratory transients (HFRT) which increased in proportion after IH. Prior to IH, hypoxia led to a decoupling of HFRTs and LC events. However, after IH exposure there is an increased correlation between LC and HFRTs in hypoxia as well. Like LC, HPC adrenergic tone is correlated to HFRT events in room air. IH exposure enhances tonic NE tone in both room air and hypoxia. However, prior to IH $GRAB_{NE}$ expression has a sustained increase in the HPC in response to hypoxia. After IH, there is an increased adrenergic response to hypoxia. Rise time and peak response to hypoxia are elevated. In post hypoxic room air recovery, NE tone declines to baseline levels. The decay rate and NE reduction after IH are enhanced compared to the recovery rates prior to IH. The elevated response to hypoxia of LC and HPC adrenergic expression after IH, indicates a sustained remodeling of adrenergic drive in the CNS. IH exposure increases coupling of respiration and adrenergic drive during hypoxic events. These results implicate LC-NE modulation alters brain state to align with respiratory circuits affecting downstream signaling higher brain regions, such as the HPC. Brain state modulation alters responses to blood gas oscillations during disease states not just in cardiorespiratory centers and cellularly but throughout the CNS.

Disclosures: B.M. Browe: None.

Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.29/L8

Topic: F.03. Stress and the Brain

Title: Protein kinase C alpha regulates molecular, cellular, and behavioral aspects associated with posttraumatic stress disorder

Authors: *Z. LI, Q. DING, Y. GAO, L. AN, H. WANG;
Michigan State Univ., East Lansing, MI

Abstract: Genetic variations in protein kinase Cα (PKCα) are potentially associated with the risk of post-traumatic stress disorder (PTSD). However, the precise mechanism by which PKCα contributes to the emergence of PTSD remains elusive. We used western blot and immunohistochemistry to demonstrate that PKCα is expressed in multiple brain regions, including the striatum, cerebellum, prefrontal cortex, and hippocampus, with the hippocampus showing the highest expression of PKCα. Since the hippocampus is also a key region related to fear generalization, which is a fundamental clinical characteristic of PTSD, we further focused on the hippocampus and detected that PKCα is mainly expressed in Cornu Ammonis 1 (CA1) and Cornu Ammonis 3 (CA3) of the hippocampus, but is rarely expressed in the dentate gyrus (DG). Although PKCα deficiency was not suggested to affect neuronal development in the hippocampus, our RNA sequencing revealed an upregulation of genes associated with synaptic plasticity and adaptive behavior in the hippocampus of PKCα-deficient mice. Based on this, we tested the behavioral function of PKCα in PKCα-deficient mice and found that PKCα deficiency led to hypoactivity and anxiety. A contextual fear conditioning paradigm was used to show that PKCα deficiency caused higher freezing compared with wild-type mice in response to foot shock on the training day, and higher fear retrieval and less fear extinction on the extinction days. These results collectively indicate that PKCα may play a pivotal role in various responses associated with PTSD, underscoring its potential as a significant contributor to the disorder's pathogenesis.

Disclosures: Z. Li: None. **Q. Ding:** None. **Y. Gao:** None. **L. An:** None. **H. Wang:** None.

Poster

PSTR178: Anatomy, Physiology, Neurochemistry of the Stress Response

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR178.30/L9

Topic: F.03. Stress and the Brain

Support: MH126534
BBRF31820

Title: Cellular mechanisms underlying parity programming and postpartum stress effects on the maternal brain

Authors: *J. CHAN, G. DI SALVO, S. DUTTA, E. BRINDLEY, I. S. MAZE;
Mount Sinai Sch. of Med., New York, NY

Abstract: Pregnancy represents an incredible physiological stressor, yet while the effects of psychosocial stresses are well-documented, how reproductive experiences produce lasting impact on the maternal brain remain unknown. Moreover, prior pregnancies (parity) are risk factors for several disorders including peripartum mood and affective disorders, yet not all individuals who experience pregnancy and childbirth develop brain disorders later in life. Therefore, there is great need to understand the biological processes that both orchestrate and disrupt parity effects in the brain to promote later disease susceptibility. Using female mice, we performed brain-wide transcriptional profiling to determine region sensitivity to prior pregnancy and postpartum exposures. We identified the dorsal hippocampus (dHpc) as the region exhibiting greatest plasticity 1 month post-offspring weaning, followed by other regions of the dopaminergic circuitry. These transcriptional changes suggest altered neuronal and glial composition compared to nulliparous females, and were associated with improved performance on context-dependent fear conditioning, a behavioral task reliant on dHpc function. To determine the specific contributions of pregnancy and pup interactions on parity programming of the dHpc, we examined pregnancy only vs. pup sensitized females. We show that while pregnancy is the major contributor of neuroplasticity changes, complete parity programming of the dHpc involves additional postpartum exposures. Thus, we next tested whether disruptions over the postpartum period could impact dHpc changes. We utilized a model of maternal separation stress that incorporated limited nesting and daily pup separations over ten days. Our results show that this postpartum stress model disrupted both the transcriptional and behavioral changes observed in control dams. To comprehensively define the impact of parity vs. postpartum stress effects, we performed single nuclei RNA-sequencing. Analysis of dHpc neuronal subclusters revealed pregnancy-specific effects in D1 and D2 receptor expressing interneuron populations that are dysregulated by postpartum stress. Ongoing work will test the role of dopaminergic dysregulation in postpartum stress effects on parity programming of the maternal brain. Overall, we suggest pregnancy and postpartum exposures elicit long-term behavioral adaptations, which are vulnerable to disruptions by postpartum stress. These studies provide insight into the molecular mechanisms contributing to long-term effects of parity on the brain, and the environmental triggers that may interact to influence maternal brain health.

Disclosures: J. Chan: None. **G. Di Salvo:** None. **S. Dutta:** None. **E. Brindley:** None. **I.S. Maze:** None.

Poster

PSTR179: Hypothalamus and Whole Brain Networks

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR179.01/L10

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH Grant GM127251 to AMK
NIH Grant MH114961 (AMK co-I)
COURI MERITUS Award to ID

Title: Navigating lateral hypothalamic networks: Mapping inputs and outputs of the ventral lateral and ventral medial subdivisions in the adult male rat

Authors: *V. I. NAVARRO, I. DELGADO, M. GUILLEN, A. M. KHAN;
Biol. Sci., The Univ. of Texas at El Paso, El Paso, TX

Abstract: The hypothalamus, an essential brain structure, regulates body temperature, hormonal balance, sleep/wake cycles, and motivated behaviors to help maintain physiological homeostasis and orchestrate adaptive responses to internal and external stimuli. Within this intricate network, the lateral hypothalamic area (LHA,) the largest component of the hypothalamus, is now believed to not only integrate physiological signals, but aid in cognitive functions as well. Once perceived as homogenous, the LHA harbors distinct cytoarchitectural subregions as delineated by Swanson (*Brain Maps 3.0*; 2004) and subsequently substantiated by studies highlighting their unique neuronal connections and functional roles. Here, we build upon previous investigations by exploring the neuroanatomical inputs and outputs of the ventral lateral and ventral medial subdivisions (LHAvl/vm) of the LHA in male rats, spanning *Brain Maps 4.0* atlas levels 5-40 (*BM4.0*; Swanson, 2018; *J Comp Neurol*). Utilizing the anterograde and retrograde tracers, *Phaselous vulgaris*-leucoagglutinin and cholera toxin B subunit, respectively, we mapped these connections at high-spatial resolution, elucidating differential connectivity patterns which may inform potential functional distinctions among these subregions. Thus far, our preliminary observations reveal some variations and overlap in the connectional profiles of the LHAvl and LHAvm. Although transport of both PHAL and CTb is observed to overlap in the substantia innominata, supramammillary nucleus, and ventral tegmental area, the observed patterns appear strikingly distinct. These data contribute to a better understanding of the neural architecture of the LHA, shedding light on structural heterogeneity potentially underlying its diverse functional roles and paving the way for targeted therapeutic interventions for conditions such as obesity, addiction, and sleep disorders.

Disclosures: V.I. Navarro: None. **I. Delgado:** None. **M. Guillen:** None. **A.M. Khan:** None.

Poster

PSTR179: Hypothalamus and Whole Brain Networks

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR179.02/L11

Topic: F.08. Food and Water Intake and Energy Balance

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COURI MERITUS Award to ID
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UTEP Research Incentive Fund (AMK)

Title: Neural cartography of feeding control: Fine-scale spatial distributions of AgRP and alpha-MSH in the adult male rat brain

Authors: *I. DELGADO[1], O. KELLY[2], V. I. NAVARRO[3], A. M. KHAN[3];
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Abstract: The central melanocortin system plays a pivotal role in regulating energy balance and food intake homeostasis within the central nervous system. At its core are Agouti-related protein (AgRP) neurons situated in the ventromedial region of the arcuate hypothalamic nucleus. AgRP neurons stimulate food intake through projections to various brain regions. Conversely, alpha-melanocyte stimulating hormone (α-MSH), an endogenous peptide hormone, acts as an appetite suppressant within this system by targeting melanocortin 3/4 receptors in the hypothalamus. AgRP inhibits these receptors, counteracting the effects of α-MSH and leading to increased food intake and body weight. While the distribution of AgRP and α-MSH has been studied, detailed mapping of their exact locations throughout the brain is lacking. To address this gap, we employed diaminobenzidine reactions in separate series of tissues labeled with antibodies against these neuropeptides. An adjacent tissue series was Nissl-stained to delineate cytoarchitectonic boundaries and assign levels according to Brain Maps 4.0 rat brain atlas. Chemoarchitectural distributions for each neuropeptide were mapped at high-spatial resolution within the atlas templates to analyze where these regions differ and overlap, providing valuable insights into their neural circuitry. Initial mapping highlights their projections to key appetite-regulating brain regions such as the paraventricular nucleus, arcuate nucleus, lateral hypothalamus, and nucleus accumbens. AgRP exhibits concentrated fiber expression in the bed nuclei stria terminalis anteromedial subregion and the medial preoptic area. In more posterior brain regions, both AgRP and α-MSH fiber expression remains largely restricted to the hypothalamic and mammillary recesses of the third ventricle. One key difference between the two neuropeptide distributions is their fiber density patterns. AgRP appeared to display a decreased fiber density at more mediolateral levels compared to α-MSH fibers. This was evident in several subregions of the lateral hypothalamic area. Also, AgRP showed more projecting fibers at more posterior levels that were more numerous than the shorter fibers displayed by α-MSH. Overall, these differences in fiber density are striking, and mapping their distributions affords us with a finer level of detail for their fiber systems. Understanding the spatial distributions of AgRP and α-MSH in the brain is likely critical for deciphering the neural mechanisms underlying metabolism and appetite control.

Disclosures: I. Delgado: None. **O. Kelly:** None. **V.I. Navarro:** None. **A.M. Khan:** None.

Poster

PSTR179: Hypothalamus and Whole Brain Networks

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR179.03/L12

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH Grant GM127251 to AMK
NIH Grant MH114961 (AMK co-I)
NIH Grant OD030148 (MJK PI, AMK co-I)
UTEP College of Science (SB)
UTEP Research Incentive Fund (AMK)

Title: Further elaboration and kernel density estimation of glucagon-like peptide 1 (GLP-1)-immunoreactive neuronal populations in the caudal brainstem of the adult male rat

Authors: G. P. TAPIA[1], A. R. ARVIZU[2], J. V. SALCIDO[1], S. BALIVADA[1], ***A. M. KHAN**[1]; [1]Biol. Sci., [2]The Univ. of Texas at El Paso, El Paso, TX

Abstract: Glucagon-like peptide 1 (GLP-1) is a versatile molecule that is produced peripherally in the L-cells of the intestine and centrally in a population of neurons located in the caudal brainstem. A growing body of literature suggests that GLP-1 signaling is associated with feeding control, and has led to emerging new treatments for Type 2 diabetes and obesity, making this hindbrain cell group a translationally-significant target for feeding-related experiments. Thus, a coordinate system for stereotaxic targeting of these cells is of high importance. To address this, we performed a series of optimization experiments for antibodies against GLP-1, and immunolabeled brain sections of adult male Sprague-Dawley rats with these antibodies using multiple detection methods. Photomicrographs of these sections were aligned with those of an adjacent series of Nissl-stained tissue that served as a cytoarchitectural reference, enabling identification of sections that most closely corresponded to Brain Maps 4.0 (BM4.0); (Swanson LW, 2018, J Comp Neurol) atlas templates, and accurate mapping of GLP-1-immunoreactive (ir) perikarya profiles. We performed kernel density estimation of the annotated cells and generated isopleth maps of the GLP-1-immunoreactivity, which revealed that the territory of spatially-distributed GLP-1-ir cells is wider than we previously understood. In addition to the GLP-1-immunopositive cells observed in the medial (NTSm), commissural (NTSco), and lateral NTS (NTSl), a large number of labeled perikarya fell within the cytoarchitecturally-defined medullary reticular nucleus. Overall, these atlas assignments place the GLP-1-expressing cell group in better registration within stereotaxic space and will be valuable in guiding our future experiments geared towards stereotaxically-targeting this cell group.

Disclosures: G.P. Tapia: None. **A.R. Arvizu:** None. **J.V. Salcido:** None. **S. Balivada:** None. **A.M. Khan:** None.

Poster

PSTR179: Hypothalamus and Whole Brain Networks

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR179.04/L13

Topic: F.08. Food and Water Intake and Energy Balance

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NIH Grant OD030148 (MJK PI, AMK co-I)
UTEP College of Science (SB)
LSAMP PRELS Fellowship Program (MLQ)
UTEP Research Incentive Fund (AMK)

Title: Architecture of the putative adrenergic system in the medulla oblongata: Mesoscale comparison of the phenylethanolamine N-methyltransferase-immunoreactive morphological features between 2-D high-resolution transverse maps and 3-D volume visualizations

Authors: *S. BALIVADA, G. P. TAPIA, J. V. SALCIDO, M. L. QUINTANA, M. J. KENNEY, A. M. KHAN;
Biol. Sci., The Univ. of Texas at El Paso, El Paso, TX

Abstract: Putative adrenergic C1, C2, and C3 neurons in the medulla oblongata have been implicated in nutrient sensing, respiration, and cardiovascular and sympathetic nervous system regulation. Structural features of the somata and neurites of these neurons have been identified through phenylethanolamine *N*-methyltransferase (PNMT) immunohistochemistry in medulla oblongata slices as 2-D maps but have not yet been finely scrutinized with 3-D volumetric visualization methods. Here, we used light sheet fluorescence microscopy (LSFM) to visualize mesoscale PNMT-immunoreactive cellular morphology and compared it to that visualized using 2-D high-resolution transverse maps. Fixed, hydrogel-protected lower brainstems from Sprague-Dawley rats underwent aqueous delipidation methods before being immunostained for PNMT and imaged using a SmartSPIM LSFM. Images were converted to volume renders using IMARIS software. Separately, PNMT-immunopositive structural features from lower brainstem sections detected by Ni^{2+}-enhanced DAB immunohistochemistry were mapped onto *Brain Maps 4.0* (*BM4.0, J. Comp. Neurol., 2018*) atlas templates using standardized mapping methods. 3-D spatial distribution of PNMT immunopositive soma revealed C1 and C2 neuron groups to be bilateral rostrocaudal columns and the C3 group as a midline longitudinal structure of the medulla. Five structural features of the PNMT neurites were observed on the maps and visualized as volumes: (1) Bundles of short neurite segments in the dorsal reticular nucleus, with the rostrocaudal orientation of these bundles confirmed through volume visualization; (2) a very fine 3-D meshwork of short PNMT+ neurites in the *nucleus of solitary tract (>1840)*; (3) dorsoventrally- and (4) mediolaterally-oriented long neurites in the medulla; and (5) a lattice-like pattern for these long neurites formed from their overlap. These results extend the architectural description of the putative adrenergic system in 3-D and underscore how 2-D maps can be used as ground truth for describing 3-D features.

Disclosures: S. Balivada: None. **G.P. Tapia:** None. **J.V. Salcido:** None. **M.L. Quintana:** None. **M.J. Kenney:** None. **A.M. Khan:** None.

Poster

PSTR179: Hypothalamus and Whole Brain Networks

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR179.05/L14

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH Grant GM127251 to AMK
NIH Grant MH114961 (AMK co-I)

Title: Identification of neuropeptide distributions in the parabrachial nucleus of the adult male rat: atlas-based chemoarchitectural analysis in 2-D and 3-D

Authors: *G. P. TAPIA, L. LUCERO, A. M. BLAKE, A. A. MIN, V. A. RAMOS, A. D. TERRAZAS, S. BALIVADA, A. M. KHAN;
Biol. Sci., The Univ. of Texas at El Paso, El Paso, TX

Abstract: The parabrachial nucleus (PB) is understood to play a significant role in the control of food intake and has previously been demonstrated to be activated after refeeding (G Zséli et al., *J Comp Neurol*, 2016). However, the structural arrangement of the chemically-diverse cell populations and fiber systems that may be associated with feeding in the PB has not been mapped using an atlas-based approach. Here, we present our progress towards systematically mapping the chemical anatomy of the PB to an atlas of the rat brain (L W Swanson, *Brain Maps 4.0*, *J Comp Neurol*, 2018)(*BM4.0*) and extending our visualization of these systems into 3-D. We processed brain sections containing the PB for immunolabeling using antibodies against calcitonin gene-related peptide (CGRP), oxytocin-associated neurophysin (PS38), or choline acetyltransferase (ChAT), and binding was identified using Ni^{2+}-enhanced peroxidase immunohistochemistry. Photomicrographs of these sections were aligned with those of an adjacent series of Nissl-stained tissue that served as a cytoarchitectural reference, enabling identification of sections that most closely adhered to *BM4.0* atlas templates (Levels 47-52). Immunoreactive density was plotted across animals to generate consensus maps for each peptide. CGRP-labeled perikarya were most dense within a small zone of the external lateral PB (PBle), with fibers spreading across the lateral ventral (PBlv) and Kölliker-Fuse (KF) subregions. ChAT-labeled neurons of varying morphology were observed to cluster at several levels of the PB, and OT-containing fibers were more widely distributed than previously understood, representing a “sweeping” pattern across the pontine central gray (PCG), traveling through the medial and lateral PB subnuclei. The maps generated served as ground truth for whole-tissue immunolabeling and guided our light sheet microscopy-based optical slicing of the 3-D dorsolateral pons at comparable planes to the *BM4.0* atlas levels. Overall, the consensus maps shown here are a first step towards anatomically locating PB areas with dense expression of feeding-associated neuropeptides and will facilitate precise targeting of these pathways in future experiments.

Disclosures: G.P. Tapia: None. **L. Lucero:** None. **A.M. Blake:** None. **A.A. Min:** None. **V.A. Ramos:** None. **A.D. Terrazas:** None. **S. Balivada:** None. **A.M. Khan:** None.

Poster

PSTR179: Hypothalamus and Whole Brain Networks

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR179.06/L15

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH Grant GM127251 to AMK
NIH Grant MH114961 (AMK co-I)
NIH Grant OD030148 (MJK PI, AMK co-I)
UTEP College of Science (SB)
LSAMP PRELS Fellowship Program (MLQ)
UTEP Research Incentive Fund (AMK)

Title: Evaluating suitable proxies for Nissl staining to identify rat hypothalamic cytoarchitecture in light sheet fluorescent microscopy: the region-defining ability of Neuronal Nuclei (NeuN) in 3-D

Authors: *M. L. QUINTANA, S. BALIVADA, V. I. NAVARRO, A. M. KHAN;
Biol. Sci., The Univ. of Texas at El Paso, El Paso, TX

Abstract: Nissl staining is commonly used to define regions of the brain based on cytoarchitecture and is the foundation for Swanson's rat brain atlas (2018; Brain Maps 4.0 (BM4.0) J. Comp. Neurol.), the rat brain atlas we use for our mapping efforts. However, traditional Nissl staining is not yet compatible with the tissue-clearing process that allows for 3-D imaging of the brain by light sheet fluorescence microscopy (LSFM). A lack of three-dimensionality in our current rat brain atlases can obscure connectional relationships in tissues, potentially clouding our understanding of the overall architecture of the brain. Since the rat brain-atlas community stands to benefit from this new technology, we explored alternatives to Nissl staining as a way to identify brain regions in 3-D volumes. Here, we used Neuronal Nuclei (NeuN), a pan-neuronal marker, in sectioned and whole diencephalon tissues to assess its ability to differentiate brain regions in the rat hypothalamus. To validate the potential region-defining properties of NeuN in the hypothalamus, optical sections from an immunostained whole brain and thin sections of a separate brain were parcellated and compared against the parcellations determined separately for the adjacent Nissl-stained tissue. We found general agreement in the boundaries for mainly the denser cellular regions of the hypothalamus between the two methods. While less conspicuous, regions of low intensity lying alongside regions of high intensity also aided in boundary determinations. In our study, we found distinct morphological patterns in each brain region revealed by NeuN immunostaining, underscoring its potential as a Nissl stain proxy in LSFM. Moreover, our findings underscore the importance of incorporating a combination of 2-D and 3-D approaches in neuroanatomical research, highlighting their synergistic potential. Establishing a fiducial marker-defining global stain compatible with LSFM not only provides the foundation for a comprehensive 3-D brain atlas but also suggests a method for voxel-based coordinates for stereotaxic targets, expanding the volumetric visualization of brain regions.

Disclosures: M.L. Quintana: None. **S. Balivada:** None. **V.I. Navarro:** None. **A.M. Khan:** None.

Poster

PSTR179: Hypothalamus and Whole Brain Networks

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR179.07/L16

Topic: F.08. Food and Water Intake and Energy Balance

Support: the National Science and Technology Innovation 2030 Major Program (No. STI2030-2021ZD0200100)
Shanghai Municipal Science and Technology Major Project (No. 2018SHZDZX05)

Title: Comparative analysis of hypothalamic cells across species

Authors: Z. CHEN[1], J. MA[2], X. LIU[3], J. YANG[4], L. GU[3], C. CHEN[3], C. LI[3], H.-T. XU[5], X. XU[3], Z. SHEN[3], W. WEI[5], Y. LEI[6], Q.-F. WU[1], ***S. XU**[3];
[1]Inst. of Genet. and Developmental Biol., Chinese Acad. of Sci., Beijing, China; [2]Inst. of Nutr. and Hlth., Chinese Acad. of Sci., Shanghai, China; [3]Inst. of Neurosci., Chinese Acad. of Sci., Shanghai, China; [4]BGI-Research, Hangzhou, China; [5]Lingang Lab., Shanghai, China; [6]BGI-Research, HH, China

Abstract: The hypothalamus, a small yet mighty brain region, plays a pivotal role in maintaining physiological homeostasis and survival by harboring a tremendous diversity of cell types. Despite the consensus on its evolutionary conservation, comparative analyses across species have been notably lacking. In this study, we bridged this gap by creating a comprehensive dataset using single-nucleus RNA sequencing (snRNA-seq) and spatial transcriptomics (stereo-seq), examining the hypothalamus in mice, marmosets, and macaques. Our systematic comparative analyses uncovered several species-specific cell types, both neuronal and non-neuronal. Notably, we identified a unique cell subtype in the marmoset MnPO region, which might play a crucial role in stress regulation. Moreover, we discovered a novel transcriptomic-defined region in non-human primates, situated in the dorsal lateral part of the hypothalamus. This region encompasses the traditional LH, ZI, and AHN regions and aligns closely with the cortico-hypothalamic projection target area identified through circuit-tracing datasets. This suggests its potential function as a critical cortico-hypothalamic interface. Additionally, this newly identified region is characterized by a high concentration of oligodendrocytes. Overall, our findings provide unprecedented insights into the spatial distribution of hypothalamic cells, laying the groundwork for future research in this vital brain area.

Disclosures: Z. Chen: None. **J. Ma:** None. **X. Liu:** None. **J. Yang:** None. **L. Gu:** None. **C. chen:** None. **C. li:** None. **H. Xu:** None. **X. Xu:** None. **Z. Shen:** None. **W. Wei:** None. **Y. Lei:** None. **Q. Wu:** None. **S. Xu:** None.

Poster

PSTR179: Hypothalamus and Whole Brain Networks

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR179.08/L17

Topic: F.08. Food and Water Intake and Energy Balance

Support: EC H2020 WIDESPREAD-03-2017: ERA Chairs - 810425
NCN GRIEG 2019/34/H/NZ3/00733

Title: Hypothalamic and midbrain neuropeptide mRNA tails are elongated by cytoplasmic polyA polymerase

Authors: *B. TARKOWSKI[1], H. GRZESIK[2], P. S. KRAWCZYK[2], O. GEWARTOWSKA[2], A. DZIEMBOWSKI[2,3];
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Abstract: Neuropeptides were originally considered as hypothalamus-derived regulators of hormonal homeostasis controlling pituitary. Currently, they are commonly seen as transmitters of the “wireless” connectome of the nervous system.
Neuropeptide biogenesis involves proteolytic trimming of pre-propeptides that are often encoded conjointly by a single transcript. Surprisingly, little research has been devoted to the modification mechanisms of their mRNAs, even though it was observed in 1980s that some polyA tails are exceptionally long and are dynamically regulated upon physiological stimulation, like for AVP mRNA and dehydration. The length of the polyA tail determines transcript stability, shielding it from digestion by exonucleases, enhancing translation.
The non-canonical mRNA polyA polymerase Tent5A is a member of a family of proteins that elongate polyA tails of secreted protein mRNAs in the cytoplasm. Interestingly UK Biobank data indicates TENT5A polymorphisms in humans associated with height and sleep disorders. Mutations in TENT5A lead to osteogenesis imperfecta, for which a patient with hypotrophic pituitary has recently been reported.
We discovered Tent5A expression in specific nuclei of the mouse hypothalamus and the midbrain, including PVH, SO, LHA, TU, PMv, with the highest expression in the Edinger-Westphal nucleus. IHC for tagged Tent5A and neural cell type markers reveals neurosecretory identities producing AVP, OXT, galanin, hypocretin, SST, PMCH, QRFP, NPVF, GHRH, urocortin and CART.
Brain biopsies RNA-seq from WT and Tent5a KO mice showed among downregulated transcripts significant enrichment in those encoding neuropeptides. The decreased neuropeptide stability correlates with pituitary hypothrophy in neural-restricted Tent5A KO mice. Notably, direct RNA sequencing using Oxford Nanopore Technology revealed neuropeptide mRNA polyA tail shortening by half in Tent5A KO, and specific induction of polyA tail elongation of

AVP and OXT mRNAs in water-deprived WT mice, but not the KO. This correlates with reduced urine osmolarity as well as decreased growth, upon neural Tent5a ablation, indicating impaired AVP-regulated water resorption, and GHRH secretion, respectively.
Here we describe a role of cytoplasmic mRNA polyA tail elongation in the efficient production of numerous neuropeptides, with physiological role unravelled in animal models and human relevance indicated by genomic data.

Disclosures: B. Tarkowski: None. **H. Grzesik:** None. **P.S. Krawczyk:** None. **O. Gewartowska:** None. **A. Dziembowski:** None.

Poster

PSTR179: Hypothalamus and Whole Brain Networks

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR179.09/L18

Topic: F.08. Food and Water Intake and Energy Balance

Support: Jane Coffin Childs Postdoctoral Fellowship
Harvard University startup funds to PI

Title: A new neuroscience model for distributed computation, brain regeneration, and evolution

Authors: *V. CHANDRA;
Dept. of Organismic and Evolutionary Biol., Harvard Univ., Cambridge, MA

Abstract: Our understanding of brain development and function derives primarily from the study of a few model organisms. These animals share features of brain organization that are not universal; many marine invertebrates have fundamentally different brains. Understanding these unfamiliar forms may reveal new principles for neuroscience, and will help us understand how our brains first evolved. In my postdoctoral work, I am developing as a neuroscience model a marine invertebrate with such a brain: the acoel worm *Hofstenia miamia*.
My postdoctoral lab has developed *Hofstenia* as a model for whole-body regeneration. *Hofstenia* can rebuild a complete brain from virtually any initial tissue configuration. Our anatomical studies of its brain show that it is a cylindrical network of densely-packed neurites in the worm's head, extending posteriorly into a sparse network through the body. We find that it lacks major landmarks and internal regionalization. Light and electron microscopy reveal thousands of multipolar neurons with complex arborization. We find that exact brain shape varies enormously across worms and through development. Molecular labeling finds that most neural cell types are not regionalized, suggesting that the *Hofstenia* brain has organization different from that of typical model organisms.
Hofstenia is an aggressive predator, and its foraging requires rapid integration of internal state and external stimuli. We find that its foraging behavior is not affected by amputation of large fractions of the brain, or while it reorganizes and regenerates. This suggests that most or all foraging computations occur in a distributed, decentralized manner. How such a brain could be

organized is not understood.
To study how such distributed computations could be implemented, we are generating an electron microscope connectome of the worm brain, engineering tools to see and perturb neural activity in behaving animals, and developing high-resolution postural analysis methods to understand the dynamical structure of foraging behavior. Together, this enables a research program studying the organizing principles of this diffuse brain, how its neural circuits compute and reassemble after damage, and will inform our understanding of early brain evolution.

Disclosures: V. Chandra: None.

Poster

PSTR179: Hypothalamus and Whole Brain Networks

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR179.10/L19

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH grant K08 DK132493
NY Obesity and Nutrition Research Center (5P30DK026687-42)
BBRF-NARSAD Young Investigator Award

Title: Whole-brain network topologies of hunger and satiety can be modulated by cues in the sensory environment

Authors: *A. RAMIREZ[1], L. ROGERSON[2], C. D. SALZMAN[3];
[1]Columbia Univ., New York City, NY; [2]Columbia Univ., Brooklyn, NY; [3]Zuckerman Inst., Columbia Univ., New York, NY

Abstract: Internal states such as hunger, fear, or satiety can be inferred based on an animal's behavioral responses to environmental conditions, but the latent network dynamics responsible for these behavioral states, and state changes, remain poorly understood. A major obstacle to understanding global brain states is the challenge to develop approaches that can identify functional activity in neurons across a whole brain. Currently, the only way to measure evoked activity across an entire mouse brain is by combining whole-brain microscopy with labeling of immediate early genes, such as cfos. However, variability in cfos activity and presence of noise pose significant statistical challenges in distinguishing meaningful signals from background fluctuations, limiting the utility of these approaches. To overcome this challenge, we devised a method for whole-brain two-time-point labeling and statistical inference in mice, allowing us to robustly identify regions that are significantly activated by different internal states, even with a modest sample size. Here we present our novel methodological approach to whole-brain activity screening and validate the ability of our pipeline to detect brain regions activated during internal states induced by fasting and refeeding. We identify areas exclusively activated by fasting, refeeding, as well as brain areas activated by both fasting and refeeding in non-overlapping cell populations. Correlations across brain regions are then visualized using graph-theoretical tools,

allowing us to examine the network topologies underlying hunger and satiety. We exploit this approach to ask whether cues in the sensory-environment associated with rewarding-foods can induce network states that resemble those of a fasted animal - i.e. “cue-induced hunger state”. This novel approach to whole-brain activity screening and network analysis has wide applicability and promise for uncovering mechanisms of internal states and internal-state changes.

Disclosures: A. Ramirez: None. **L. Rogerson:** None. **C.D. Salzman:** None.

Poster

PSTR179: Hypothalamus and Whole Brain Networks

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR179.11/L20

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIDDK K08 DK132493 (PI - Ramirez)
NY Obesity and Nutrition Research Center - 5P30DK026687-42 (PI - Ramirez)

Title: Comparing whole-brain networks of hunger and satiety states induced by environmental context vs. gastrointestinal signaling

Authors: *L. ROGERSON[1], A. RAMIREZ[2], C. D. SALZMAN[3];
[1]Neurosci., Columbia Univ., New York, NY; [2]Psychiatry, Columbia Univ., New York City, NY; [3]Zuckerman Inst., Columbia Univ., New York, NY

Abstract: Hunger and satiety states can be modulated by controlling levels of food-intake or by administering naturally occurring gastrointestinal hormones. It remains unknown if these different means of controlling hunger and satiety exert their effects through activation of common networks in the brain. To examine this question, we compare whole-brain network topologies of hunger and satiety states induced by environmental context (fasting and refeeding) to similar internal states induced by gastro-intestinal signals for hunger (ghrelin) and satiety (GLP-1). We examine similarities and differences in network modularity across different states and identify brain areas with characteristic features of hub nodes that are likely to function as key regulators of state transitions between hunger and satiety. Our findings promise to facilitate future research aimed at understanding the contributions of individual hormonal signals to coordinating internal state changes via changes in the identified brain networks. The insights gained may help identify candidates for targeted therapeutic interventions for obesity and metabolic disorders.

Disclosures: L. Rogerson: None. **A. Ramirez:** None. **C.D. Salzman:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.01/L21

Topic: G.01. Fear and Aversive Learning and Memory

Support: Department of Psychological Science, University of Vermont
R01MH118734 (TT)

Title: Estrogen infused into the dorsolateral striatum modulates fear extinction in female rats

Authors: Z. MOHAMMED[1], D. POWERS[2], T. P. TODD[3], *D. TOUFEXIS[1];
[1]Univ. of Vermont, Burlington, VT; [2]Biol., Boston Univ., Boston, MA; [3]Psychological Sci., Univ. of Vermont, Burlington, VT

Abstract: Estrogen enhances fear extinction in both women and female animal models. Although stimulus-response learning likely plays a role in fear expression, the dorsolateral striatum (DLS), which mediates stimulus-response learning, has not been investigated with respect to estrogen's facilitation of fear extinction. In the present study we initially examined the effect of subcutaneous estradiol (E2) injection on female rats in which stimulus-response learning in an operant training paradigm had produced habitual responding. Results showed that E2 significantly enhanced sensitivity to reward devaluation, thus increasing behavioral flexibility. A follow-up study examined the effect of E2 infused directly into the DLS during the extinction of fear-conditioning in female rats. Data showed a significant interaction between E2 and control-infused rats wherein E2 initially enhanced fear responding early in the extinction test, then rapidly reduced fear responding by the end of the test. These data indicate that E2 acting within the DLS may contribute to E2's facilitation of fear extinction in female rats.

Disclosures: Z. Mohammed: None. **D. Powers:** None. **T.P. Todd:** None. **D. Toufexis:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.02/L22

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIMH R15068283

Title: Exploring the relationship between the dorsolateral striatum and the hippocampus in contextual gating of fear extinction

Authors: *J. FREUND;
Univ. of Colorado Denver, Golden, CO

Abstract: Fear relapse phenomena limit the long-term efficacy of extinction-based exposure therapy. For example, context impacts fear extinction; whereby exposure to previously extinguished fear cues in a context different from where fear extinction was learned elicits a return of fear, termed renewal. Increasing our understanding of the neural mechanisms underlying fear extinction and renewal is critical to improve the long-term efficacy of exposure therapy. We have observed that increasing DA D1R signaling in the dorsolateral striatum (DLS)during fear extinction impairs the contextual gating of fear extinction and reduces fear renewal. However, the mechanism through which the DLS impacts this contextual processing is unknown. The hippocampus has been historically implicated in contextual processing and its activity during fear extinction and renewal is necessary for fear renewal. It is therefore possible that the DLS is involved in the contextual gating of fear extinction memories via modulating hippocampal activity during fear extinction or renewal. Here we examined whether the DLS directly projects to the hippocampus. Adeno-associated viral vector (AAV) expressing mCherry was injected into the DLS of adult, female Long Evans rats. Immunohistochemistry was used to amplify mCherry signal in terminal regions for imaging. mCherry was observed in DLS terminals throughout the hippocampus. Current experiments are verifying this projection with retrograde tracing from the hippocampus to the DLS. The findings of this experiment support the possibility that a DLS-hippo pathway is involved in contextual gating during fear extinction. Further research is required to better understand the functional role of this pathway in fear extinction and renewal.

Disclosures: J. Freund: None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.03/L23

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant R01 DA052108

Title: Sex Differences in Infralimbic Cortex and Nucleus Accumbens Shell Signaling Dynamics During Extinction of Conditioned Taste Aversion

Authors: *J. E. DOUTON[1], R. M. CARELLI[2];
[1]Univ. of North Carolina, Chapel Hill, NC; [2]Psychology and Neurosci., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Dysfunctional hedonic processing is associated with many psychiatric disorders and can lead to the development of negative affect, which is particularly prevalent in diseases such as depression and substance use disorders. Understanding how the brain tracks negative affect can

help develop treatments to prevent its development or facilitate its extinction. Projections from the infralimbic cortex (IL) to the nucleus accumbens shell (NAcSh) have been linked to learned negative affect, as 20 Hz optogenetic stimulation of this circuit reduces conditioned taste aversion (CTA) in male rats. However, the same stimulation did not produce an effect in female rats. We have previously characterized the changes in oscillatory activity within these regions that underlie such behavioral differences, showing differential engagement of the IL-NAcSh circuit across sex. The goal of this study was to extend our understanding of how these regions track negative affect by studying its response during extinction of CTA. To do so, we used taste reactivity to assess affective states and Lithium Chloride (LiCl) CTA to study the development and extinction of learned negative affect. We simultaneously recorded local field potential (LFP) activity in the IL and NAcSh in response to 30 intraoral infusions (3.5 sec infusion, VT30 sec schedule) of commonly rewarding (0.15% saccharin) tastant, following induction of CTA (LiCl, 127 mg/kg ip), and across 4 days of CTA extinction in male (n=9) and female (n=6) Sprague-Dawley rats. Here, we showed that the reduction in LFP activity at beta frequency elicited by CTA in males, gradually recovered across extinction trials in both the IL and NAcSh. Also, disrupted IL-NAcSh functional connectivity (coherence) observed during CTA test was completely recovered on the first extinction day following testing. In females, no clear LFP activity effects were observed during CTA or extinction trials. However, while IL-NAcSh functional connectivity was not disturbed during CTA, we observed overall reduced IL-NAcSh coherence in later extinction trials. Collectively, our data reveals clear sex-specific differences in the processing of extinction of CTA. Particularly, while activity at beta frequency in both the IL and the NAcSh can track the recovery in behavioral responding in male rats, it is not the case for females. On the contrary, although IL-NAcSh coherence is disrupted during CTA expression and immediately recovered in males, the opposite pattern is observed in females, as IL-NAcSh functional connectivity gets disrupted during later extinction trials independent of behavioral recovery.

Disclosures: J.E. Douton: None. **R.M. Carelli:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.04/L24

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH R01MH065961

Title: Hippocampus and medial prefrontal cortex engage in distinct yet synergistic mechanisms during retrieval of extinguished fear memories.

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Abstract: Extinction learning is a highly context-dependent mechanism and results in the gradual decline of conditioned fear responses. Several studies have unveiled the pivotal role of the hippocampus (HPC) in providing contextual cues that activate several neuronal networks to modulate the fear extinction. In general, within a range of mnemonic processes, contextual information flows from the HPC to the medial prefrontal cortex (mPFC). Conversely, at the onset of event memory, the direction of information flow reverses, with the mPFC controlling the HPC activity to establish connections among past events. To test the hypothesis that contextual information provided by the HPC can trigger the retrieval of an extinction memory, and in turn, mPFC can exert control over HPC, we recorded local field potentials (LFP) from both brain areas during the acquisition and expression of extinguished fear. Male and female adult Long-Evans rats underwent surgery for the implantation of two 16-channel tungsten microwire arrays targeting the prelimbic (PL) and infralimbic (IL) regions of the mPFC and the dorsal CA1 area of the HPC. Rats underwent an auditory fear conditioning with a 3-min stimulus-free baseline followed by 5 auditory conditioned stimuli (CS; 10 s, 80 dB, 8 kHz) paired with a foot-shock unconditioned stimulus (US; 1.0 mA; last 2s of the CS). Over the days that followed, LFPs were recorded during fear extinction (3-min baseline; 45 CS; no US) and extinction retrieval sessions (3-min baseline; 5 CS; no US), both of which occurred in a context distinct. At the onset of extinction training, all rats showed high levels of conditioned freezing during the early trials (fear retrieval) and freezing decreased over successive CS presentations. During extinction retrieval, freezing behavior remained low. Spectral power analysis revealed that both areas exhibited distinct, well-defined frequency peaks during fear and extinction retrieval. During fear retrieval, both IL and PL showed a significant peak ~4 Hz, whereas during extinction retrieval, the peak was ~7 Hz and ~8 Hz. The HPC exhibited peaks ~7 Hz in both sessions, but was more prominent during extinction retrieval, alongside a peak ~8 Hz. The dynamical interaction between the two substrates was analyzed through Granger causality. This analysis revealed that the HPC led the mPFC in the 7 Hz band in both sessions, but with a greater effect ~7-9 Hz for extinction retrieval. In turn, during extinction retrieval, the mPFC leads the HPC ~2-5 Hz. Collectively, bidirectional theta dynamics contributes to both contextual information processing and context-dependent memory retrieval.

Disclosures: F. Mourão: None. **M. Totty:** None. **T. Tuna:** None. **S. Maren:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.05/L25

Topic: G.01. Fear and Aversive Learning and Memory

Support: R 01 MH 065961

Title: Role for the dorsal hippocampus and bed nucleus of stria terminalis in circuit-induced relapse of extinguished fear

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Abstract: Extinction learning is a core component of behavioral interventions for trauma- and stressor-related disorders, such as post-traumatic stress disorder (PTSD). Recent work from our laboratory reveals that the nucleus reuniens (RE), a midline thalamic hub interconnecting the hippocampus (HPC) and medial prefrontal cortex (mPFC) is critical for learning and retrieving extinction memories. We hypothesize that the RE is critical for suppressing contextual fear memories that drive fear relapse. The dorsal hippocampus (dHPC) and bed nucleus of the stria terminalis (BNST) are two brain areas critical for the expression of contextual fear memories. Here we examined whether pharmacologically silencing the dHPC or BNST prevents the circuit-induced relapse of extinguished fear that occurs after RE inhibition. We implanted cannula targeting both the RE and either the dHPC or BNST in adult male and female Long-Evans rats. After recovery from surgery, rats underwent auditory fear conditioning followed by extinction 24 hours later. On the subsequent day, animals received either intra-HPC or intra-BNST infusions of either saline or the AMPA receptor antagonist, NBQX (10 ug/ul, 0.3 µl per side) (n = 8-16 per group), along with intra-RE infusions of either vehicle saline or muscimol, in the extinction context using a factorial design; the dHPC and BNST infusions were conducted in independent experiments. Consistent with prior results, RE inactivation increased freezing and resulted in a relapse of fear to the extinguished conditioned stimulus (CS). Pharmacological inactivation of the dHPC, but not the BNST, mitigated circuit-induced relapse. We conclude that HPC-dependent contextual fear memories are critical for the relapse of extinguished fear. Further research aims to explore the neural pathways by which the RE modulates hippocampal circuits to regulate extinguished fear.

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Poster

PSTR180: Aversive Memory: Extinction

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Program #/Poster #: PSTR180.06/L26

Topic: G.01. Fear and Aversive Learning and Memory

Support: JST-FOREST(JPMJFR2243)
AMED-PRIME

KAKENHI 20K21458
KAKENHI 21H05176
KAKENHI 22H02942

Title: Prediction error signals of the ventral hippocampus in aversive memory extinction

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Abstract: Adaptive regulation of aversive memory is important to control defensive behavior such as freezing, and balance the risk with animals' needs for primary resources. Recent studies have demonstrated that a prediction error (PE) signal is important to update aversive memory when a predicted aversive outcome is omitted. The hippocampus is thought to compare predicted events with current sensory input, and the ventral hippocampus (vHPC) plays a pivotal role in extinction of aversive memory. However, it remains unclear if and how the vHPC represents a PE signal during aversive memory extinction. To unveil these, we first performed multi-fiber photometry to detect Ca^{2+} transients at the axon terminals of vHPC neurons in the ventromedial prefrontal cortex (vmPFC), nucleus accumbens (NAc), and basal amygdala (BA) of C57BL/6 mice. We found that Ca^{2+} activities of all vHPC terminals dropped below the baseline at the post conditioned stimulus (CS), omission period of a predicted foot shock, during early phase of extinction and that this shock omission response was attenuated during late phase. We next examined the causal relationship between the observed PE signal and extinction using optogenetics. We found that optogenetic activation of the vHPC-vmPFC or vHPC-BA axon terminal suppressed freezing behavior during the CS period of extinction whereas optogenetic inhibition of the vHPC-vmPFC or vHPC-BA terminal tended to increase freezing behavior during the CS period of extinction. Together, our results so far suggest that the vHPC represents a PE signal at the shock omission period, which is then transmitted to downstream regions to hinder extinction of aversive memory. We thus propose that the PE signal in the vHPC represents potential threats to maintain the expression of aversive memory, rather than instantaneous changes in aversive value to drive extinction.

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Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

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Program #/Poster #: PSTR180.07/L27

Topic: G.01. Fear and Aversive Learning and Memory

Support: MHR01107435
NIH grant T32 MH064913.

Title: Role of dorsal hippocampal neurons during learning and extinction of contextual fear

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Abstract: Acquisition and extinction of associative fear memories are critical for guiding adaptive behavioral responses to environmental threats, and dysregulation of these processes is thought to represent important neurobehavioral substrates of trauma and stress-related disorders. We have previously shown that pharmacological inhibition of 2-arachidonoylglycerol (2-AG) synthesis, a major endogenous cannabinoid regulating synaptic suppression, enhances fear learning and impairs within-session extinction of contextual fear. It is not well understood, however, how the neural activity of the dorsal hippocampus, a crucial region for spatial associative learning, changes across time with the presentation of aversive stimuli in a context and subsequent extinction learning during basal and 2-AG deficient states. To address this question, we used in-vivo calcium imaging coupled with pharmacological manipulation, which allowed us to monitor hundreds of cells (n=5 mice) from area CA1 of the dorsal hippocampus across days. We found that CA1 neurons exhibit responses to the aversive stimuli (electric foot shock), with a significant increase in activity by late conditioning (day 4). Interestingly, the number of neurons recruited to the ensemble during context exposure decreases across conditioning days, while the number of shock-responsive neurons remains constant. This suggests that the neural ensemble representing the context refines throughout learning, while the one for aversion remains stable. Surprisingly, the shock-responsive neurons between early (day 1) and late (day 4) phases of conditioning are distinct, with only around 8% of them responding both days. Graph Theory analysis revealed that the activity of day 1 shock-responsive cells does not significantly correlate with those responding to the shock on day 4. Taken together, these results suggest a turnover or representational drift in dorsal hippocampus neurons, with cells "going in and out of the ensemble" rather frequently. Moreover, these results support the idea of a "trade-off" between specificity and flexibility of memory engrams to just not persist in time, but also to accommodate new information and update learning. Further analysis during the extinction stage of our paradigm will be added to these data, in order to fully characterize the neural activity in the dorsal hippocampus across both conditioning and extinction stages.

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Poster

PSTR180: Aversive Memory: Extinction

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Topic: G.01. Fear and Aversive Learning and Memory

Support: This work is part of the SFB 1280 projects A03 and was supported by the Deutsche Forschungsgemeinschaft (DFG) (project number 31680338)

Title: Multimodal dynamics of neuronal processes underlying fear extinction learning assessed by simultaneous EEG and fMRI in humans

Authors: *A. GABDULKHAKOV[1], M. F. SPERL[2], L.-I. KLATT[1], C. FRAENZ[1], N. AXMACHER[3], E. GENC[1];
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Abstract: Anxiety and comorbid disorders affect a significant portion of the global population. While extensive research in fear extinction learning could explain mechanisms of fear and anxiety formation, there remains a gap in bridging findings from animal models to humans as well as between electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) studies. To date, there is just a single study by Sperl and colleagues (2019) that attempted to close this gap by conducting simultaneously recorded EEG and fMRI in the renewal/recall phase only. Thus, mechanisms of EEG-fMRI co-activations in fear acquisition and extinction remain underinvestigated. Similarly, there is a notable gap in the literature regarding findings on the dynamics of fear acquisition and extinction mechanisms in humans. To address these gaps, we conducted a simultaneous EEG-fMRI study with healthy adults (N=50, age 18-25) during fear acquisition and extinction. To analyze the dynamics of learning, each phase of the experiment was divided into halves of trials. First, we confirmed known results with EEG and fMRI data individually, showing significant differential CS+/CS- frontal theta (4-8 Hz) effects, and differential CS+/CS- blood-oxygenation level-dependent (BOLD) effects in the visual and anterior cingulate cortices (ACC), and cerebellum. By using trial-by-trial variations of frontomedial theta power as a parametric modulation regressor in GLM, we found a significant differential CS+/CS- increase in frontal-theta driven EEG-BOLD co-activation in the dorsal anterior cingulate cortex and somatosensory areas in the first half of trials in the acquisition phase and visual cortex and hippocampus in the second half of trials. Within the CS, we found a significant increase in EEG-BOLD co-activation in the second half of CS+ in the hippocampus, as well as the thalamus and cerebellum. We observed a reverse pattern in the extinction phase, namely that medial temporal lobe structures were active for CS- in the first half of trials of extinction, but in the second half, only the visual areas were involved, which suggests that during the extinction the old association of conditioned fear is being replaced. Our findings provide a novel perspective into fear learning dynamics across brain regions, highlighting experimental phase-specific changes in EEG theta power and BOLD co-activation. Respective findings have implications for future research aimed at developing individualized brain stimulation protocols to advance novel treatment approaches for anxiety and comorbid disorders.

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Poster

PSTR180: Aversive Memory: Extinction

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Program #/Poster #: PSTR180.09/L29

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant R01MH122561

Title: Central amygdala-dependent modulation of defensive behavior during complex fear extinction

Authors: *Q.-S. LE[1], T. ALAM[2], P. H. STOLIN[2], J. KLAR[2], D. S. HEREFORD[2], C. D. BORKAR[3], K. EVANS[4], J. P. FADOK[5];
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Abstract: Traumatic experiences can have long-term negative effects on physical and emotional well-being, and recovery from trauma-related fear disorders, like post-traumatic stress disorder, is impeded by persistent fear responses that resist extinction. The central amygdala (CEA) contains somatostatin-positive (SOM+) and corticotropin-releasing-hormone-positive (CRH+) cells that are involved in fear response modulation and post-extinction fear renewal. SOM+ and CRH+ neurons in the CEA are involved in driving conditioned freezing and flight respectively, and they mutually inhibit each other to bias specific defensive behaviors. Thus, it is critical to understand how these CEA populations contribute to maladaptive defensive responses. To investigate these populations' roles in directing defensive behavior, we used optogenetics to transiently activate/inhibit SOM+ and CRH+ CEA neurons in male and female adult SOM-Cre and CRH-Cre mice within a modified Pavlovian conditioning paradigm, in which we paired footshock with a serial compound stimulus (SCS) consisting of distinct tone and white noise (WN) periods. Previously, we found that animals conditioned in this SCS paradigm would transition between freezing during tone to explosive jumping and darting behaviors during WN. During extinction learning, tone-evoked freezing diminishes, and WN-evoked jumping behaviors are replaced with freezing and darting over extinction sessions. With these findings, we manipulated SOM+/CRH+ CEA neurons during WN presentations within extinction to investigate how they influence defensive responses. We found that inhibition of CRH+ neurons reduced flight and eliminated jumping responses to WN, while SOM+ neuron activation promoted freezing over flight during WN and facilitated reduction of tone-evoked freezing. Overall, we find that SOM+ and CRH+ CEA neurons significantly mediate changes in defensive behaviors across extinction. Further, to better understand the role of context in defensive responding following extinction we are currently investigating the role of connections between the ventral hippocampus (vHPC) and the CEA in extinction-dependent defensive ethograms by using optogenetics to activate/inhibit the vHPC-CEA pathway during WN presentation in post-extinction recall sessions.

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Poster

PSTR180: Aversive Memory: Extinction

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Program #/Poster #: PSTR180.10/L30

Topic: G.01. Fear and Aversive Learning and Memory

Support: Basic Science Research Program (339-20230013)

Title: Regulation of trace fear extinction via prefrontal neuron type-specific mitochondrial calcium release

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Abstract: The sustained increase activity in the prefrontal cortex (PFC), referred to as persistent activity (PA), during the trace interval in trace fear conditioning (TFC) plays a crucial role in the acquisition of trace fear memory. However, the neurophysiological mechanisms underlying PA are poorly understood. Post-tetanic potentiation (PTP) was proposed as a form of short-term plasticity that might mediate the generation of PA during working memory. PTP in the PFC was induced in a manner dependent upon the synaptic type, and its modulation was differentially mediated by the mitochondrial calcium regulation. However, whether neuron type-specific mitochondrial calcium regulation underlies the PA during trace interval in the TFC has not been elucidated. To understand role of mitochondria calcium regulation, I used tetraphenylphosphonium (TPP), a specific mitochondrial NCX blocker, and performed TFC with in vivo calcium imaging and electrophysiological recording. Studying the effect of TPP infusion into the PL area on TFC, I found that TPP did not affect the trace memory formation but reduced the maintenance of the fear memory during fear memory extinction test. Furthermore, inactivating corticopontine neurons (CPn), a specific postsynaptic cell type in which mitochondrial-dependent PTP is induced, accelerated trace fear memory extinction, whereas trace fear memory formation was not abolished as in the TPP infusion experiment. As a result of in vivo electrophysiological recording, the TPP infusion into the PL area abolished PA during both trace fear conditioning and extinction training. In vivo calcium imaging of L5 commissural (COM) and CPn neurons during the tone test revealed that the CS input is represented by a larger number of COM cells rather than CPn cells. However, early CS responsive cells within CPn neurons exhibited significantly higher PA during the post-CS period compared to COM neurons. These results imply that mitochondrial and postsynaptic type dependent PTP at the PFC is required for post-CS PA during trace fear conditioning and plays a role in maintenance of trace fear memory.

Disclosures: H. Lee: None. **S. Lee:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.11/Web Only

Topic: G.01. Fear and Aversive Learning and Memory

Support: Defence Research and Development Organisation, Ministry of Defence

Title: Sleep deprivation affects neuronal oscillations in the basolateral amygdala and medial prefrontal cortex during fear extinction memory recall in rats

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Abstract: Background: Neuronal oscillations, rhythmic patterns of neural activity, coordinate communication between brain regions by regulating the timing of synaptic transmission. Sleep deprivation (SD) disrupts memory consolidation, but its specific impact on neuronal oscillations during extinction memory recall is not well understood. Our hypothesis suggests that sleep deprivation following extinction training alters the power and synchronization of gamma oscillations (high-frequency oscillations associated with cognitive processes) between the Basolateral Amygdala (BLA) and medial prefrontal cortex (mPFC) during extinction memory recall, potentially affecting memory retrieval processes.**Materials and Methods:**Animals were randomized into Controls(AC), SD groups. Teflon coated Silver electrodes implanted into BLA and mPFC, animals recovered for 10 days. We sleep deprived male rats for 48h after extinction training on day 2. Local Field potentials (LFP) were recorded during extinction recall on day4 and were analyzed using Brainstorm, MATLAB. **Results:**Power spectral density analysis revealed gamma power increased after extinction training ($p<0.05$). However, SD rats exhibited sustained higher gamma power ($p<0.01$) during extinction recall in BLA and mPFC compared to AC rats. Synchronization of gamma oscillations was significantly reduced in SD comparable to AC rats. Bivariate Granger causality analysis revealed directionality of synaptic transmission from mPFC to the BLA was impaired in SD rats. This suggests that SD disrupts the power, synchronization, and directionality of gamma oscillations. **Discussion:**Current results strongly suggest that gamma oscillations play a vital role in BLA-mPFC communication during extinction recall, which is disrupted by sleep deprivation. Our investigation elucidates the intricate gamma band neuronal oscillations underlying the retrieval of competing memories, particularly the interplay between fear and safety memory systems. These findings hold potential for novel insights into the etiology and therapeutic approaches for anxiety disorders, including post-traumatic stress disorder (PTSD).

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Poster

PSTR180: Aversive Memory: Extinction

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Topic: G.01. Fear and Aversive Learning and Memory

Support: Italian Ministry of Health (GR-2018-12365733)
Ricerca Corrente Fondazione Santa Lucia
KAUST BAS 01/01

Title: Optogenetic stimulation of infralimbic pyramidal neurons rescues inefficient fear extinction and modulates synaptic and transcriptomic features of amygdala pyramidal neurons

Authors: *A. PANUCCIO[1], J. GIMENEZ[1], G. SCIAMANNA[2], A. TERMINE[1], C. FABRIZIO[1], M. DE BARDI[1], F. BALSAMO[3], V. ORLANDO[4], L. PETROSINI[1], D. LARICCHIUTA[5];
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Abstract: Fear associative learning increases the chances of survival by allowing individuals to anticipate threatening events and respond pre-emptively. However, the suppression of fear memory when the danger is removed (the so-called extinction process) is crucial to permit other survival functions, and impairment in such a coping mechanism may lead to maladaptive behaviors, as in the case of trauma-related disorders. These highly debilitating conditions have immeasurable social and economic costs and affect more than 4% of the population who have witnessed traumatic events. It is classically considered that traumatized individuals with fear symptomatology show responses that probably activate the neurobiological processes of inadequate fear inhibition (a failure in the extinction process). The ground-breaking hypothesis of this research is that, in susceptible individuals, the fearful event reactivates a footprint already existing before the event itself. Hence, trauma-related disorders might reflect not only post-trauma consequences, but also neural and molecular risk factors present already before the trauma. In this framework, we characterized mouse phenotypes that spontaneously exhibit individual differences in approach and avoidance behaviors toward conflicting emotional stimuli. Their behavioral differences represent robust predictors of vulnerability or resilience to impaired fear extinction. This model enables the identification of specific morphological, electrophysiological, and transcriptomic patterns in amygdala-prefrontal cortex pyramidal neurons predisposing to impaired fear extinction before exposure to fearful experiences. Finally, utilizing an optogenetic approach, we showed the possibility to rescue the inefficient fear extinction activating infralimbic pyramidal neurons and to impair fear extinction by activating prelimbic pyramidal neurons. These behavioral findings are supported by electrophysiological and transcriptomic data, particularly highlighting that optogenetic stimulation in pyramidal neurons of the infralimbic cortex in animals exhibiting deficits in fear memory extinction led to decreased activity in the prelimbic cortex and amygdala, while enhancing glutamatergic transmission in the infralimbic cortex. These findings are consistent with transcriptomic results, which revealed an overexpression of genes related to trauma recovery in amygdala cortex pyramidal neurons.

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Poster

PSTR180: Aversive Memory: Extinction

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Program #/Poster #: PSTR180.13/L32

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant MH127835

Title: Role of prelimbic-infralimbic medial prefrontal cortex connection in fear extinction and reinstatement in female rats

Authors: *C. C. CRESTANI[1], L. A. DE OLIVEIRA[2], J. B. CHAMBERS[3], J. P. HERMAN[4]; [1]Pharmacol. and Systems Physiol., Univ. of Cincinnati, Cincinnati, OH; [2]Drugs and Medicines, São Paulo State Univ. (UNESP), Araraquara, Brazil; [3]Pharmacol. & Systems Physiol., Univ. of Cincinnati, Cincinnati, OH; [4]Dept Pharmacol. and Systems Physiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: An intrinsic circuit within the medial prefrontal cortex (mPFC) connects pyramidal neurons of the prelimbic (PL) cortex with the infralimbic (IL) cortex. Studies in male rats describe a role of this intrinsic mPFC pathway in fear extinction. However, the presence and involvement of a PL-IL connection in modulation of fear responses in females has yet to be determined. The present study explored the role of PL neurons projecting to IL in fear expression, extinction and reinstatment in female rats. We used an intersectional DREADD approach to specifically activate or inhibit IL-projecting PL neurons. Adult female Sprague-Dawley rats received bilateral stereotactic injections (200nL) of a retrograde adeno-associated virus (AAV2)-packaged construct expressing Cre (pENN.AAV.hSyn.HI.eGFP-Cre.WPRE.SV40) into the IL, followed by bilateral PL injections (200nL) of Cre-dependent AAV2-hsyn-DIO-hM4D(Gi)-mCherry (inhibitory), AAV2-hsyn-DIO-hM3D(Gq)-mCherry (excitatory) or AAV2-hsyn-DIO-mCherry (control). Seven weeks after stereotaxic procedures, animals were subjected to auditory fear conditioning procedures [6x (120s intertrial interval (ITI), 20s auditory tone co-terminating with a 0.5s/0.45mA shock]. The day following acquisition animals were subjected to three extinction sessions on successive days [10 tone repetitions/session (120s ITI)]. Forty-eight hours after the third extinction session, animals were exposed to a reinstatement session [0.5s/0.45 mA shock followed by 5 repetitions of the tone (120s ITI)]. To test efficacy DREADD actuation on retrieval (day 1 of extinction), extinction and reinstatement, animals received clozapine-N-oxide (CNO) (0.5 mg/kg) one hour prior to each extinction session and the reinstatement session. Evidence of retrograde transport (expression of Cre-dependent mCherry) was observed in the PL, indicating the presence of IL-projecting neurons in the PL. Chemogenetic activation or inhibition of the PL-IL circuit did not affect

freezing behavior during tone presentation in first and second extinction sessions. However, activation of this circuit increased freezing behavior during the third extinction session in relation to DREADD control rats (P=0.017), whereas inhibition of PL-IL connection decreased the freezing (P=0.026). Chemogenetic activation of the intrinsic mPFC circuitry also increased the freezing during the reinstatement session (P=0.048). Taken together, these findings suggest the presence of a PL-IL conection within the mPFC of female rats, and this circuitry seems to play an inhibitory role in fear extiction and facilitates the fear reinstatment.

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Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.14/L33

Topic: G.01. Fear and Aversive Learning and Memory

Support: R01MH086507
R01DA056447

Title: Chemogenetic modulation of prefrontal GABAergic and pyramidal neurons in fear extinction behavior

Authors: *A. M. M. MIGUELEZ FERNÁNDEZ, A. CABALLERO, K.-Y. TSENG;
Anat. and Cell Biol., Univ. of Illinois Chicago - Col. of Med., Chicago, IL

Abstract: The prefrontal cortex integrates information from cortical and subcortical structures to regulate complex behaviors within the cognitive and affective domains. Of particular interest is the recruitment of excitatory and inhibitory synaptic transmission by long-range inputs, which in turn is expected to shift the functional state of the prefrontal output and its impact on behavior. In fact, disruption of prefrontal GABAergic maturation (PMID: 32403119, PMID: 32430298) or limiting the recruitment of local GABAergic activity by glutamatergic inputs (PMID: 33478990) resulted in similar impairments in trace fear extinction. Here we asked whether acute chemogenetic modulation (hM3D-Gq vs. hM4D-Gi) of prefrontal GABAergic interneurons (AAV-DLX) impacts fear extinction behavior. We found that the pattern of freezing response during fear extinction is differentially affected by prefrontal inhibition or stimulation of local GABAergic activity in a task-dependent manner (trace fear vs. delay fear extinction). Surprisingly, similar chemogenetic modulation of prefrontal pyramidal activity (AAV-CaMKII) does not fully mirror the resulting effects obtained following GABAergic inhibition or stimulation. Together, these results suggest that distinct cell-type specific postsynaptic mechanisms are recruited during fear extinction behavior, which are dissociable from the feed-forward inhibitory impact on prefrontal output activity.

Disclosures: A.M.M. Miguelez Fernández: None. **A. Caballero:** None. **K. Tseng:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.15/L34

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant R01MH065961
NIH Grant R01MH1178

Title: Pharmacological stimulation of the infralimbic cortex during fear memory consolidation facilitates extinction

Authors: *H. BAYER, J. E. HASSELL, Jr., C. R. OLEKSIAK, G. GARCIA, S. MAREN; Texas A&M Univ., College Station, TX

Abstract: There is a large body of evidence implicating the infralimbic cortex (IL) in extinction learning. Recent work suggests that the IL may be recruited during or soon after fear conditioning to mediate subsequent extinction learning. To test this hypothesis, we examined whether post-conditioning pharmacological stimulation of the IL would facilitate fear extinction in male and female rats. Rats were surgically implanted with bilateral cannulas targeting the IL. One week later they underwent a standard auditory pavlovian fear conditioning protocol (5 CS-US pairings) in context A. Extinction learning and retrieval (both consisting of 45 CS presentations) were conducted in context B on subsequent days. In experiment 1, rats received intra-IL infusions of picrotoxin (PIC) or vehicle (VEH) immediately after auditory fear conditioning. During the drug-free extinction session, PIC-treated animals exhibited less freezing and this effect resulted in greater extinction retrieval the following day. In experiment 2, we observed a similar effect when intra-IL PIC infusions were performed 24 hours after fear conditioning and a third experiment revealed that this effect was long-lasting (at least two weeks). This suggests that stimulation of the IL outside of the classical consolidation window facilitates extinction learning and retrieval. We hypothesized that these effects depend on conditioning-induced plasticity in the IL. To test this in experiment 4, rats received intra-IL VEH or anisomycin (ANI, a protein synthesis inhibitor) immediately after conditioning and VEH or PIC was infused into the IL 24h later. Interestingly, ANI prevented the effects of PIC (animals that received ANI and PIC did not exhibit lower levels of freezing during extinction). Together, these results indicate that stimulation of the IL after learning (up to two weeks after consolidation is over) leads to a "pharmacological extinction" of the fear memory. This effect is long lasting and relies on protein synthesis during early stages of memory consolidation in the IL. This work supports the counterintuitive notion that role for IL in fear memory extinction is established during the consolidation of the fear memory.

Disclosures: H. Bayer: None. **J.E. Hassell:** None. **C.R. Oleksiak:** None. **G. Garcia:** None. **S. Maren:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.16/L35

Topic: G.01. Fear and Aversive Learning and Memory

Support: R01MH117852

Title: Chemogenetic activation of the locus coeruleus suppresses neuronal activity in the prefrontal cortex: Role for noradrenergic receptors in the basolateral amygdala.

Authors: *S. O. SWECK[1], H. BAYER[2], A. BINETTE[2], S. MAREN[2];
[1]Texas A&M Chapter, College Station, TX; [2]Psychological and Brain Sci., Texas A&M Univ., College Station, TX

Abstract: Extinction learning is central to cognitive-behavioral interventions for a variety of trauma- and stressor-related disorders. The locus coeruleus (LC), a major source of norepinephrine (NE) in the brain, modulates extinction learning via projections to the basolateral amygdala (BLA) and infralimbic (IL) cortex. We have recently shown that the LC-NE in the BLA contributes to stress-induced extinction impairments. For example, both footshock and LC activation drive sustained increases in BLA spike firing, and this is mediated by β-noradrenergic receptors in the BLA. Here we test the hypothesis that β-noradrenergic receptors in the BLA mediate LC-induced reductions in IL firing that contribute to stress-induced extinction impairments. To test this idea, adult male and female Long-Evans rats (n = 5) were unilaterally infused with AAV-CaMKII-GCaMP6m and implanted with a GRIN lens into the IL to allow for recording of single-cell calcium activity. Additionally, these animals were bilaterally infused with AAV-PRSx8-hM3Dq-HA, an excitatory DREADD, in the LC and implanted with bilateral cannula into the BLA. After recovery from surgery, rats were subjected to four 20-minute recording sessions after systemic injections of either vehicle (VEH) or the DREADD ligand clozapine-*N*-oxide (CNO; 3 mg/kg, i.p). Prior to the VEH/CNO administrations, animals received local infusions of either VEH or propranolol (PROP; 5 µg/µl, 0.5 µl/hemisphere) into the BLA. We found that LC-NE chemogenetic activation in the absence of BLA β-adrenergic antagonism drives sustained increases in freezing behavior and attenuates spontaneous calcium transients in IL neurons (n = 460). This behavioral effect was transiently rescued by propranolol in the BLA. Importantly, propranolol infusions in the BLA attenuated the inhibition of IL activity produced by chemogenetic activation of the LC. These results reveal that the inhibition of IL principal cell activity observed under stress or noradrenergic hyperarousal is mediated by noradrenergic release in the BLA, which promotes stress-induced extinction impairments by recruiting inhibitory networks in the IL. Beta blockers may be a useful therapeutic adjunct to promote extinction learning under stress.

Disclosures: S.O. Sweck: None. **H. Bayer:** None. **A. Binette:** None. **S. Maren:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.17/L36

Topic: G.01. Fear and Aversive Learning and Memory

Support: R56MH126516
R01MH126516

Title: Inhibition of Fear Extinction Circuitry During Natural and Vagus Nerve Stimulation-Enhanced Fear Extinction

Authors: *D. CALDERÓN-WILLIAMS[1], S. RAZA[1], C. DRISKILL[2], J. E. PLOSKI[3], C. A. THORN[4], C. K. MCINTYRE[4];
[1]Univ. of Texas at Dallas, Richardson, TX; [2]Sch. of Behavioral and Brain Sci., Univ. of Texas at Dallas, Dallas, TX; [3]Neural and Behavioral Sci., Pennsylvania State Univ., Hershey, PA; [4]Sch. of Behavioral and Brain Sci., Univ. of Texas at Dallas, Richardson, TX

Abstract: Vagus nerve stimulation (VNS) is FDA approved for the treatment of epilepsy, depression, and stroke rehabilitation and is currently being tested as an adjuvant for exposure therapy in the treatment of post-traumatic stress disorder (PTSD) in humans. While VNS has been shown to both accelerate and enhance extinction of conditioned fear, the mechanisms behind this process remain unclear. VNS drives neuronal activity in the locus coeruleus (LC), leading to downstream elevations in norepinephrine in fear extinction pathways. For example, the basolateral amygdala receives direct inputs from the LC and plays a role in fear conditioning and extinction. Here, we sought to investigate the role of these LC and its' projections to the BLA in VNS enhanced extinction. An ArchT-expressing, cre-dependent opsin was infused bilaterally into the LC of adult, Th-Cre+, Long-Evans rats. Three weeks later, bilateral optic fibers were implanted over the LC and a stimulating cuff electrode was implanted on the vagus nerve. Fear conditioned rats were given extinction training paired with VNS or sham stimulation and optogenetic inhibition of LC cells or control treatment (viral infusion and fiber illumination without expression of the ArchT opsin). As hypothesized, VNS enhanced extinction and LC inhibition blocked this effect. However, in sham animals, inhibition of the LC *also* enhanced extinction. No significant difference was found between sham and sham-ArchT animals on anxiety tests, indicating that natural enhancement of fear is not due to anxiolytic properties of LC inhibition. A second experiment is in progress, inhibiting only LC-BLA projecting neurons in both VNS and sham treated animals. We hypothesize that inhibition of these noradrenergic LC-BLA cells during VNS paired extinction training will attenuate the extinction enhancing effects of VNS. In a separate cohort, BLA-projecting LC neurons were tagged with an infusion of a retrograde virus expressing tdTomato into the BLA. A VNS cuff electrode was implanted four weeks later, and rats were given either sham/VNS, then perfused 90 minutes later. Immunohistochemistry was carried out to label cells expressing the immediate early gene cfos, dopamine beta hydroxylase, and the tdTomato fluorophore in the BLA and LC. Colocalization of

activity (cfos) and fluorescence were measured. Preliminary results show an increased number of activated LC cells following VNS, including those projecting to the BLA as compared to animals given SHAM stimulation.

Disclosures: D. Calderón-Williams: None. **S. Raza:** None. **C. Driskill:** None. **J.E. Ploski:** None. **C.A. Thorn:** None. **C.K. McIntyre:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.18/L37

Topic: G.01. Fear and Aversive Learning and Memory

Support: R 01 MH 065961

Title: Optogenetic theta-paced stimulation of the thalamic nucleus reuniens facilitates the acquisition of extinction memories

Authors: *T. TUNA[1], F. MOURÃO[1], S. MAREN[2];
[2]Psychological and Brain Sci., [1]Texas A&M Univ., College Station, TX

Abstract: The nucleus reuniens (RE) is a midline thalamic nucleus interconnecting the medial prefrontal cortex (mPFC) and the hippocampus (HPC), structures known to be involved in fear and extinction memory processes. Recent work indicates that the RE is crucial for the acquisition and retrieval of fear extinction memories: the RE synchronizes mPFC and HPC theta oscillations (4 – 12 Hz) during extinction retrieval and pharmacological inactivation of the RE impairs both extinction retrieval and mPFC-HPC theta coherence. Furthermore, theta-paced (8-Hz) optogenetic stimulation of RE neurons prevents fear renewal after extinction, a common form of fear relapse. In this study, we examined if theta-paced RE stimulation would facilitate the acquisition of extinction memories. We injected an AAV encoding the blue light-shifted excitatory opsin ChR2 (AAV9-CaMKII-ChR2-mCherry) or a blank control AAV (AAV9-CaMKII-mCherry) and implanted a fiber optic in the RE in adult male and female Long-Evans rats (n = 12/sex). Animals underwent auditory fear conditioning with five tone (CS)-footshock (US) pairings in Context A and extinction with 45 CS-alone trials in Context B, separated by 24h. Two groups of animals (ChR2-Ext and mCherry-Ext) received 8-Hz blue light stimulation during extinction CSs. A third group (ChR2-NoExt) received laser stimulation but was not extinguished. The next day, animals were tested for extinction retrieval in Context B with 5 CS-alone trials without any stimulation. All groups exhibited similar levels of conditioned freezing after fear conditioning. During extinction, the ChR2-Ext group exhibited significantly lower freezing compared to the mCherry-Ext group. The suppression of conditoned freezing was also evident in the ChR2-Ext during the stimulation-free retention test. These results indicate that RE theta-paced stimulation suppresses conditioned freezing and facilitates the acquisition of long-

term extinction memories. This work suggests that oscillatory activity in the RE is essential to the retrieval of extinction memories by hippocampal-prefrontal networks.

Disclosures: T. Tuna: None. **F. Mourão:** None. **S. Maren:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

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Program #/Poster #: PSTR180.19/M1

Topic: G.01. Fear and Aversive Learning and Memory

Support: n°956414
C.E.N
European Union

Title: A cerebello-thalamic pathway regulating fear extinction learning and thalamo-prefrontal cortex synchronization

Authors: *A. AYYAZ[1], R. SALA[1], C. LÉNA[1], D. POPA[2];
[1]Inst. de Biologie, Ecole Normale Supérieure, Paris, France; [2]Neurosciences; CNRS UMR 8197 / INSERM U 1024, Ecole Normale Superieure, Paris, France

Abstract: Fear extinction is a form of inhibitory learning that suppresses the expression of aversive memories and plays a key role in the recovery of anxiety and trauma-related disorders. Here, we identify a cerebello-thalamo-cortical pathway for fear extinction. The cerebellar fastigial nucleus (FN) projects to the lateral subregion of the mediodorsal thalamic nucleus (MD), which is reciprocally connected with the dorsomedial prefrontal cortex (dmPFC). The inhibition of FN inputs to MD impairs fear extinction and increases the bursting of MD neurons, a firing pattern known to prevent extinction learning. Indeed, this MD bursting is followed by high levels of the dmPFC 4Hz oscillations causally associated with fear responses during fear extinction, and inhibition of FN-MD neurons increases the coherence of MD bursts and oscillations with dmPFC 4Hz oscillations. Overall, these findings reveal the regulation of fear-related thalamo-cortical dynamics by the cerebellum and its contribution to fear extinction.

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Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

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Topic: G.01. Fear and Aversive Learning and Memory

Support: NSFC/RGC/JRS (N_HKU735/21)
RGC/GRF (17102120, 17108821, 17103922)
RGC/CRF (C1024-22GF, C7074-21G)
HMRF (09200966)

Title: The role of serotonin in fear conditioning and fear extinction

Authors: *X. LIU;
The Univ. of HongKong, Hong Kong, China

Abstract: Table of Contents

The role of serotonin in fear conditioning and fear extinction Post-traumatic stress disorder (PTSD) is a psychiatric disorder characterized by excessive physiological arousal and dysregulated fear responses. Serotonin (5-HT) is a neurotransmitter that regulates the higher brain functions, especially in the sensory processing, decision making, and emotion regulation. Selective serotonin reuptake inhibitor (SSRI) is the first line treatment for PTSD. Previous animal studies showed that SSRI can reduce the acquisition and expression of conditioned fear, which is used as an animal model for PTSD. However, the role of serotonergic system in fear learning remain elusive. In this study, we investigated the activity of serotonergic neurons in the mouse dorsal raphe nucleus (DRN) during auditory-cued fear conditioning and fear recall by using genetically encode calcium sensor and fiber photometry. We found that the 5-HT neuron activity showed a rapid increase in respond to the fear conditioned stimulus, suggesting DRN serotonergic neuron involve in fear memory recall and threat detection. Our work implicates the key role of 5-HT system in fear learning.

Disclosures: X. liu: None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.21/M3

Topic: G.01. Fear and Aversive Learning and Memory

Title: Psilocybin impairs the extinction of contextual fear in adult female, but not male, rats

Authors: *L. P. PUPPEL, A. K. AL-OLIMAT, K. M. KING, M. J. SAVINI, C. E. MILLER, C. R. DEL VALLE, H. R. SPARKMAN, B. E. BRAMLAGE, P. R. ZOLADZ;
Psychology and Neurosci., Ohio Northern Univ., Ada, OH

Abstract: Fear-related disorders are often treated with exposure therapy, a technique based on the concept of extinction. However, this type of therapy is ineffective for many individuals, so finding a pharmacological adjunct that augments the extinction of fear could lead to better treatment outcomes. In recent preclinical work, investigators have shown that psychedelics can accelerate the extinction of fear, potentially through their impact on neurotrophic signaling. We previously found that psilocybin exerted sex-dependent effects on the extinction of conditioned fear to an isolated cue (i.e., a tone). Specifically, psilocybin enhanced the extinction of cue fear in male rats but impaired it in female rats. In the present study, we extended this work by examining the dose-dependent effects of psilocybin on the extinction of contextual fear, as well as the expression of brain-derived neurotrophic factor (BDNF) in several brain regions. On Day 1, adult male and female Sprague-Dawley rats were placed in a fear conditioning chamber, and following a 3-min acclimation period, were exposed to 5 unsignaled footshocks (2-sec, 1.5 mA), each separated by 60 sec. On Day 2, the rats were injected intraperitoneally with psilocybin (0.1, 0.3, 0.6, or 1 mg/kg) or vehicle (0.9% saline), and thirty minutes later, they underwent fear extinction by being placed in the same context as Day 1 for 10 min. On Day 3, the rats underwent extinction recall by being placed in the same context as Days 1 and 2 for 10 min. Freezing behavior was quantified by the FreezeFrame software (Actimetrics, Inc.). One hour after extinction recall on Day 3, rat brains were collected; the hippocampus, amygdala, and prefrontal cortex (PFC) were dissected and subsequently processed for BDNF expression via Western blotting. Analyses of freezing behavior during training and early extinction demonstrated that all rats developed strong fear for the context. Although psilocybin had no significant impact on the extinction of contextual fear in males, it significantly impaired the extinction of contextual fear in females. Specifically, female rats treated with psilocybin, particularly doses above 0.3 mg/kg, exhibited greater freezing than controls during extinction recall on Day 3. Psilocybin had no significant impact on BDNF expression in female rats. However, a low dose of psilocybin (0.3 mg/kg), one that we previously reported to enhance the extinction of cue fear in males, led to reduced BDNF expression in the PFC of male rats. The observed behavioral findings are consistent with our previous work in females and, once again, suggest that psilocybin may exert sex-dependent effects on the extinction of conditioned fear.

Disclosures: L.P. Puppel: None. **A.K. Al-Olimat:** None. **K.M. King:** None. **M.J. Savini:** None. **C.E. Miller:** None. **C.R. Del Valle:** None. **H.R. Sparkman:** None. **B.E. Bramlage:** None. **P.R. Zoladz:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.22/M4

Topic: G.01. Fear and Aversive Learning and Memory

Title: Evaluation of an MDMA Intervention for Cued Fear Extinction in a Preclinical Model of Blast TBI

Authors: *M. MCGUIER[1], L. COUGHLIN[1], L. DWIEL[1], M. COMPANY[1], K. DISANO[2], C. E. NOLLER[3], P. E. HOLTZHEIMER[2], W. DOUCETTE[1];
[1]Psychiatry, Dartmouth Col., Lebanon, NH; [2]VA Med. Ctr., White River Junction, VT; [3]Dartmouth Col., Lebanon, NH

Abstract: Nearly 70 million people suffer from traumatic brain injury (TBI) worldwide, with military personnel at particularly high risk of experiencing a blast-related TBI (bTBI). bTBI produces a wide variety of symptoms as well as an increased risk of post-traumatic stress disorder (PTSD). Current treatment options for these consequences of bTBI provide some relief but remain ineffective for many individuals. Growing evidence supports MDMA-assisted therapy as a treatment for PTSD, including fear expression and extinction related symptomology. In a preclinical model of bTBI in rats, we have previously found that bTBI rats show enhanced fear response following fear conditioning. We have also used this model to assess a reverse translation of clinical MDMA outcomes by determining the efficacy of MDMA or saline (SAL) to reduce freezing behavior during re-exposure to the conditioned cue. We hypothesized that bTBI rats would freeze more following conditioning during cue re-exposures and that MDMA treatment would cause a decrease in freezing behavior during cue re-exposure over SAL (enhanced extinction) and relate to changes in brain activity. Thirty-eight (18M, 20F) Sprague-Dawley rats were exposed to a blast overpressure injury at 12 weeks old using a blast tube (ORA, Inc) (three events ~126kPa [18 psi] with a ~3 minute interblast interval) or were naïve controls (N=16M, N=8F). Rats were implanted with custom electrode arrays targeting the bilateral infralimbic cortex, orbitofrontal cortex, nucleus accumbens core, and the central nucleus of the amygdala for LFP recording. LFPs were collected at baseline and during all non-conditioning days of behavior. Rats were conditioned to repeated 20 second(s) tone cues (80db) co-terminating with a 2s footshock (1mA) with a 120s inter-shock interval. Freezing responses to cue re-exposures were recorded by video and assessed following extinction of context-related freezing. Following conditioning, bTBI rats exhibited significantly higher cue induced freezing (two-way ANOVA p=0.0148) compared to controls. A subset of rats (N=14 bTBI and N=10 Ctrl) were conditioned and extinguished to context before receiving intervention (3mg/kg MDMA or SAL by i.p. injection) daily just prior to three cue extinction sessions followed by a fourth extinction session without intervention. Our initial findings align with previous studies reporting increases in cue induced freezing post-bTBI and ongoing analyses assessing the hypothesis that MDMA will ameliorate impaired fear extinction will be presented. Additionally, machine learning derived LFP-based biomarkers of cue induced freezing will be compared between MDMA and SAL groups.

Disclosures: M. McGuier: None. **L. Coughlin:** None. **L. Dwiel:** None. **M. Company:** None. **K. Disano:** None. **C.E. Noller:** None. **P.E. Holtzheimer:** None. **W. Doucette:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.23/M5

Topic: G.01. Fear and Aversive Learning and Memory

Support: MOST 111-2410-H-006-111
NSTC 112-2410-H-006-096

Title: Extinction of Aversive Memory under Dexmedetomidine-induced Anesthesia in Rats

Authors: *D.-Y. CHEN, C.-H. HUANG, P. CHEN, H.-Y. HSIAO;
Dept Psychology, Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: Previous research indicates that humans and rodents can form memories even while in a partially conscious state, such as under anesthesia. Our prior studies have demonstrated that memory acquisition is possible under dexmedetomidine (DEX) anesthesia using various paradigms. However, it remains unclear whether rats can undergo extinction learning under the same anesthesia. In the present study, we used both inhibitory avoidance task and conditioned freezing to explore the possibility. In the first experiment, rats were trained in the inhibitory avoidance task while awake. They received footshock when they entered into the dark component of the inhibitory avoidance apparatus. In the following days, one group of rats received extinction training under anesthesia, but the other group did not. During each extinction session, rats were anesthetized by DEX (50 μg/kg, s.c.) and placed into the dark component of the inhibitory avoidance apparatus for 200 s. Rats were woken up by atipamezole (0.25 mg/kg, s.c.) that reversed the effect of DEX, and returned to their home cage. On the last day, rats were subjected to the memory retention test while awake. The results indicated that extinction training under anesthesia for three days significantly reduced the step-through latency, just like another group of rats received extinction training while awake. On the other hand, rats received extinction training under anesthesia for only two days did not show reduced step-through latency. However, systematic epinephrine injection (1.0 mg/kg, i.p.) immediately after each extinction session could facilitate the extinction effect. In addition, microinjection of adrenergic β-receptor antagonist, propranolol, into infralimbic cortex (IL) can block the extinction facilitation effect induced by systematic epinephrine injection. In the second experiment, rats were trained to associate light and shock (20 pairs/day). One group of rats received extinction training under anesthesia for three days, but the other group did not. The memory retention test indicated that the extinction group showed significant reduced conditioned freezing to light stimulus. Throughout these findings, the present study has revealed the possibility of establishing memory extinction under anesthesia. It can be enhanced by systemic injection of epinephrine, and this effect is mediated by adrenergic β-receptor in IL. Memory extinction under anesthesia provides a new approach for clinical therapy development to treat anxiety-based disorders. By reducing the discomfort patients experience during treatment, this approach can encourage them to be more willing to undergo therapy.

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Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.24/M6

Topic: G.01. Fear and Aversive Learning and Memory

Title: Neuromodulation in the visceral insular cortex gates fear extinction balance

Authors: *A. D. ZYCH[1,2], M. MALEZIEUX[1], E. CHO[1], R.-E. HUETTL[1], A. RESSLE[1], B. SCHMID[1], N. GOGOLLA[1];
[1]Emotion Res. Dept., Max Planck Inst. of Psychiatry, Munich, Germany; [2]International Max Planck Research School for Translational Psychiatry, Munich, Germany

Abstract: Maintaining emotions in an adaptive range is important for survival. Our group has previously shown, that the posterior insular cortex (pInsCtx) plays a crucial role in keeping fear in balance upon extinction learning. Surprisingly, we found that the pInsCtx exerts both, a fear extinction impeding, but also facilitating roles depending on the strength of the acquired fear levels. We hypothesize that the pInsCtx is a key regulator in keeping fear extinction within an adaptive range. While we previously found a role for the integration of bodily feedback with the learned aversive sensory cues (Klein et al., 2021, Science), we do not mechanistically understand, which factors may enable the dual role of the pInsCtx on fear extinction learning. The neuromodulators acetylcholine (ACh) and noradrenaline (NA), are known to be released in response to sensory stimuli, as well as upon changes in affective states. Furthermore, these neuromodulators have been previously implicated in fear and extinction learning, as well as the regulation of bodily functions. Given these roles, we here aimed at investigating a potential contribution of these neuromodulators to the role of the pInsCtx in keeping fear in balance. To understand the potential recruitment of ACh and NA within the pInsCtx during fear learning and extinction, we characterized the release of ACh and NA using GRAB sensors during classical auditory fear conditioning in freely moving mice, as well as in head-fixed animals, where we obtained physiological readouts of heart rate, pupil and diverse fear expressions. Our results reveal specific differences in the dynamics of NA and ACh release in response to different fear-related sensory stimuli, changes in autonomic readouts and behaviors. Strikingly, interference with neuromodulatory release in the pInsCtx during fear extinction via optogenetic terminal inhibition resulted in bidirectional effects. While inhibition of NA terminals impaired fear extinction, inhibition of ACh terminals facilitated fear extinction. Together, our findings suggest that NA and ACh in the pInsCtx are crucial in regulating the adaptive balance of fear maintenance and extinction learning. Ongoing work aims at further elucidating how these two neuromodulators may act in concert and how they react to bodily signals in order to maintain fear homeostasis.

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Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

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Program #/Poster #: PSTR180.25/M7

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIMH R01MH126516

Title: Effects of propranolol on long-term extinction of conditioned fear in male and female rats

Authors: C. EDOSOMWAN[1], P. PRASANNA[1], D. CALDERON-WILLIAMS[1], ***C. MCINTYRE**[2];
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Abstract: Extinction of conditioned fear requires consolidation of new memories, and norepinephrine (NE) is necessary for consolidation of many kinds of memories. We hypothesized that NE release from the locus coeruleus (LC) would be necessary for extinction of conditioned fear. However, we found that optogenetic inhibition of the LC during extinction training had no effect on conditioned fear expression 24 hours later, but significantly reduced fear expression 2 weeks later (delayed enhancement) when compared with control rats. However, it is still unclear what mechanisms mediated these findings. To examine the role of NE signaling at β adrenergic receptors in this delayed enhancement of extinction memory, rats were subjected to 2 days of auditory fear conditioning (days 1 and 2) followed, 24 hours later (day 3), by a pre-extinction retention test to quantify fear (freezing behavior) of the auditory conditioned stimulus (9 kHz tone, 30 sec) in the absence of a foot-shock. On the next day (day 4), an extinction session was given. Propranolol (10 mg/kg) or saline was administered (IP) 20 minutes before the session that consisted of 4 exposures to the conditioned stimulus alone. A post-extinction retention test (day 5) was given 24 hours, and 2 weeks later. Preliminary results indicate that propranolol administration prior to extinction training enhances extinction of conditioned fear in female rats but impairs extinction of conditioned fear in male rats at both timepoints. To determine whether this observed reduction in fear at the 2-week timepoint was due to forgetting over time or incubation of the extinction memory, we ran a separate cohort of male and female rats. Rats underwent conditioning and testing (days 1-3). On day 4, propranolol or saline was administered, however, no subsequent extinction session was given. A retention test was given 2 weeks later. Preliminary results indicate that conditioned fear expression is not significantly different between the first and second retention tests in propranolol or saline-treated rats, suggesting that the previously observed reduction in freezing at the delayed timepoint was not due to forgetting over time. These findings suggest that the β adrenergic receptor blocker propranolol may have different effects on extinction of conditioned fear in male and female animals and these effects appear to be specific to extinction memory and not forgetting. This research provides important information about the mechanisms by which long-term fear memories are maintained in male and female animals and has potential to be applied to treatment approaches in clinical populations, such as in patients with post-traumatic stress disorder.

Disclosures: C. Edosomwan: None. **P. Prasanna:** None. **D. Calderon-Williams:** None. **C. McIntyre:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.26/M8

Topic: G.01. Fear and Aversive Learning and Memory

Support: Endowed Faculty Career Award
UMass Boston Undergraduate Research Funds
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UMass Boston Internal Project Funds

Title: Estradiol effects on gut microbiome and ERK signaling mechanisms during fear extinction memory consolidation

Authors: *C. HARTSGROVE, G.-M. WALKER, K. D. SILVA, K. NUNEZ, M. JOSEPH, K. ALEJOS, L. Y. MAENG;
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Abstract: Neural circuits regulating fear extinction are altered in individuals affected by trauma- and anxiety-related disorders. Fear extinction, learning to reduce fear after a threat is no longer present, is dependent on the fear circuit and can be influenced by gonadal hormones, such as estradiol (E2). Prior research has demonstrated that higher E2 levels during fear extinction learning improves fear extinction recall tested 24h later. The current study examined how long-lasting this effect is and the potential mechanisms through which E2 enhances extinction memory, particularly the extracellular signal-regulated kinase (ERK) pathway and gut microbiome. Past research has found that inactivation of the ERK pathway or alterations to gut microbiome can impair fear extinction recall in a sex-dependent way. Naturally cycling adult female Sprague-Dawley rats underwent estrous cycle tracking and a 3-day auditory cued fear conditioning/extinction paradigm. On day 1, all rats completed habituation and conditioning in estrus, a low-E2 phase of the estrous cycle. On day 2, 21 rats received a SC injection of 17β-estradiol in a sesame oil vehicle (15 µg/kg), and 22 rats received vehicle alone 30 minutes prior to extinction training. All rats underwent a recent fear extinction recall test on day 3, and 25 animals received a remote fear extinction recall test 7-15 days after extinction training. All freezing behavior was scored using ANY-maze software then averaged as two-trial blocks for analysis. A one-sided independent samples t-test revealed that the administration of E2 30 min prior to extinction training significantly decreased freezing behavior 24h later at the recent fear extinction recall test ($t(41) = 1.680$, $p=0.05$), but not remote ($p > 0.05$). Brain tissue was collected at recent and remote recall and processed for ERK immunohistochemistry. E2 administration did not seem to alter ERK expression in the amygdala, medial prefrontal cortex, or hippocampus at either recall timepoint. In addition, 16S rRNA sequencing of gut microbiota was used to examine differences in abundance and diversity within groups and across behavioral phases. LEfSe biomarker analysis of bacterial abundance revealed significant enrichment of several estrogen-responsive bacterial families (Lactobacillaceae, Clostridiaceae, Streptococcaceae) in the Firmicutes phylum in the E2-treated group at recent and remote fear extinction recall. Together, these findings suggest a link between the gut microbiome and E2 effects on fear extinction.

Disclosures: C. Hartsgrove: None. **G. Walker:** None. **K.D. Silva:** None. **K. Nunez:** None. **M. Joseph:** None. **K. Alejos:** None. **L.Y. Maeng:** None.

Poster

PSTR180: Aversive Memory: Extinction

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR180.27/M9

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH

Title: A substantia nigra to dorsolateral striatum pathway mediates the effects of female estrous cycle on fear extinction and is a novel target for the prevention of fear relapse.

Authors: *A. A. HOHORST[1], M. K. TANNER[2], R. HAN[3], J. D. WESTERMAN[4], C. SANCHEZ[5], L. M. ALVAREZ[2], E. C. LOETZ[4], E. B. OLESON[4], B. N. GREENWOOD[4]; [1]Integrative and Systems Biol., Univ. of Colorado Denver, Denver, CO; [2]Univ. of Colorado Denver, Denver, CO; [3]Skaggs Sch. of Pharmacol. and Pharmaceut. Sci., Univ. of Colorado Anschutz, Aurora, CO; [4]Psychology, Univ. of Colorado Denver, Denver, CO; [5]Biol., Univ. of Colorado Denver, Denver, CO

Abstract: Extinction-based exposure therapy is an effective strategy for treating stress-related disorders; however, fear extinction remains vulnerable to fear relapse. Women are more susceptible to developing these stress-related disorders than men, yet sex differences in fear extinction and relapse are understudied. Fear extinction is sex divergent; whereby female rodents and humans exposed to fear extinction during times of high estrogen, either via estrogen administration or during phases of the cycle during which estrogen is elevated (proestrus and estrus; Pro/Est), have enhanced extinction memory and less relapse compared to males and females with low ovarian hormones (metestrus and diestrus; Met/Di). Ovarian hormone-modulation of fear extinction is clinically relevant, but the underlying mechanisms have yet to be fully explored. Here, we investigated the role of a substantia nigra-to-dorsolateral striatum (SN-DLS) dopamine (DA) pathway in mediating the effects of the estrous cycle on fear extinction and relapse, given the emerging role of SN and DLS DA in relapse-resistant fear extinction. Chemogenetic inhibition of the SN-DLS pathway during fear extinction restored fear relapse in females exposed to fear extinction during Pro/Est. Furthermore, chemogenetic activation of the SN-DLS pathway during fear extinction reduced fear relapse in males. These results suggest that a SN-DLS pathway mediates the effects of female ovarian hormones during fear extinction on later fear relapse and is a novel target for the reduction of fear relapse following fear extinction. Future studies will focus on the role of the SN-DLS pathway in the retrieval of extinction memory during fear renewal.

Disclosures: A.A. Hohorst: None. **M.K. Tanner:** None. **R. Han:** None. **J.D. Westerman:** None. **C. Sanchez:** None. **L.M. Alvarez:** None. **E.C. Loetz:** None. **E.B. Oleson:** None. **B.N. Greenwood:** None.

Poster

PSTR180: Aversive Memory: Extinction

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Program #/Poster #: PSTR180.28/M10

Topic: G.01. Fear and Aversive Learning and Memory

Support: VA Merit Grant 1I01BX005367
NIH NIAAA 7R01AA024526

Title: Unveiling the Cellular Underpinnings of Impaired Extinction Memory in Comorbid PTSD/AUD: A TRAP Approach

Authors: *L. WILLS[1], J. T. GASS[2];
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Abstract: Background: Comorbidity of PTSD & AUD disrupt emotional learning, hindering the ability to extinguish fear memories. This translates to a heightened vulnerability to the relapse of fearful behaviors upon encountering trauma reminders. To elucidate the cellular basis for this phenomenon, we investigated the infralimbic cortex (IfL), a brain region critical for fear extinction. Employing a novel Targeted Recombination in Active Populations (TRAP) technique, we genetically tagged extinction engrams (activated neuronal ensembles) to assess their reactivation during extinction recall in a model of PTSD/AUD. **Methods:** Adult Wistar rats were randomly assigned to four groups: Control (CTRL), Stress & Alcohol (RS+CIE), TRAP tagging (4-OHT), and Vehicle tagging control. All groups received bilateral intracranial injections in the IfL with a dual-virus cocktail. The 1st virus expresses an inducible Cre recombinase enzyme (CreERT2) under the control of the Fos promoter, specifically targeting recently activated neurons. The 2nd virus carried a Cre-dependent red fluorescent protein (oScarlet). The RS+CIE model involved a 2h restraint stress followed by 2 weeks of chronic intermittent ethanol vapor exposure (CIE). Following a withdrawal period, all groups underwent fear conditioning using a Contextual Fear Conditioning (CFC) paradigm. Subsequently, extinction training aimed to suppress the conditioned fear response. Approximately 2h after the final extinction session, rats in the tagging groups received either 4-OHT (10mpk, i.p.) to permanently tag TRAP+ (Fos-activated) neurons with oScarlet, or vehicle solution. One month later, all rats underwent fear extinction re-training to consolidate extinction memory. Finally, a remote extinction recall test was conducted 24h later to assess extinction memory persistence. Tissue containing the IfL was collected for analysis of TRAP expression (oScarlet) and neuronal activity marker cFos. Colocalization of TRAP and cFos within IfL neurons was assessed using confocal microscopy. **Results:** Our findings revealed a significant reduction in colocalized (TRAP+ & cFos+) neurons in the IfL of RS+CIE rats compared to controls following remote

extinction recall. This suggests that prior stress and alcohol exposure disrupts the reactivation of extinction engrams during memory retrieval. **Conclusion:** This study provides novel insights into the cellular mechanisms underlying extinction memory deficits in individuals with PTSD/AUD. By demonstrating impaired reactivation of extinction engrams in the IfL, our findings shed light on the potential neural basis of relapse vulnerability in these populations.

Disclosures: L. Wills: None. **J.T. Gass:** None.

Poster

PSTR180: Aversive Memory: Extinction

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Program #/Poster #: PSTR180.29/M11

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIMH Grant R15MH127534
NSF Grant 2320195

Title: Individual Differences in Fear Generalization Predict Extinction Performance and Anxiety-like Behavior in Male, but not Female, Mice

Authors: *T. TSUKUDA, A. DAM, N. FITZGERALD, R. LIU, T. PASERMAN, R. SUBRAMANIAN, S. ZWEIG, H. C. BERGSTROM;
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Abstract: Individual differences in fear memory generalization have been linked with anxiety-disorders and PTSD (Dunsmoor and Paz, 2015). It has been proposed that preclinical models of trauma-related and anxiety disorders should include the study of individual differences. In this series of experiments, we screened a large cohort of ArcCreER^{T2} X eYFP mice (C57BL/6J background) on unconditioned defensive behavioral tests (elevated zero maze and the novel open field) and conditioned defensive behavioral tests (generalization and extinction). Results revealed a wide spectrum of cued fear generalization. A k-means clustering algorithm separated mice into high and low generalization groups. Results showed in males, high generalizers exhibited greater freezing to the original CS compared with low generalizers. In females, low and high generalizers exhibited equivalently high freezing in response to the CS. In males, there were more correlated variables among conditioned and unconditioned behavioral indices, as compared with females. These data indicate a sex-dependent relationship between fear generalization performance and both unconditioned and conditioned defensive behaviors. Efforts to expand quantification of alternate defensive states related to pre-encounter threat (scanning behavior) is underway. We also plan to take advantage of the ArcCreER^{T2} X eYFP mice to visualize neuronal ensembles associated with both conditioned and unconditioned defensive behaviors.

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Poster

PSTR181: Acquisition and Memory Modification

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR181.01/M12

Topic: G.02. Reward and Appetitive Learning and Memory

Support: K01AA027832
P50AA012870

Title: Physiological mechanisms of alcohol-related stimulus generalization across the life span

Authors: *Z. BAI[1], J. ZIMMERMAN[1], S. NAIR[1], J. PRATT[1], S. KANG[2], G. LARRABEE[1], E. V. GOLDFARB[1];
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Abstract: Spreading past associations to novel situations is critical for adaptive behavior, yet can also lead to maladaptive responses (e.g., experiencing fear in a situation that is actually safe). Based on past findings that individuals with anxiety disorders overgeneralize fear, we recently found that individuals engaging in risky alcohol drinking also overgeneralize alcohol associations, in part due to less precise memory for alcohol-related events. Here we tested the hypothesis that alcohol overgeneralization would also characterize a clinical population with alcohol use disorder (AUD). We also tested two novel mechanisms: first, that generalization would be modulated by dopaminergic (indexed by blink rate) and noradrenergic (indexed by pupil dilation) signaling, and second, that generalization would increase in older adults. In our ongoing eye-tracking study, we compared individuals with AUD (current N=8/50) and light social drinkers (current N=28/50).
Participants completed tasks designed to assess the degree to which they generalize alcohol-related associations. They first learned associations between cards depicting shapes (CS) and trial-unique tokens (US). All tokens were associated with reward, but importantly one CS was paired with alcohol tokens (CSalc) while the other was paired with object tokens (CSobj). All participants successfully learned these associations, with social drinkers demonstrating increased pupil dilation for rewarding CSs and a robust tendency to gaze toward the anticipated token location. To examine the generalization of these learned associations, participants then chose which cards they wanted to play with next. Critically, the cards vary in perceptual similarity to the original CSs, allowing us to quantify generalization gradients. As hypothesized, while both groups generalized learned associations to perceptually similar options, the AUD group generalized alcohol associations more broadly. Strikingly, older social drinkers generalized all reward associations more broadly. Preliminary pupil analyses revealed that dilation patterns continued to track learned associations along this generalization gradient. Finally, we probed participants' memory for trial-unique US tokens. Consistent with prior findings, older adults displayed less precise memory for US tokens. Notably, less precise memory for US tokens was associated with a broader tendency to generalize. Together, these findings demonstrate that both

AUD and aging can lead to broader generalization, and suggest that proxies for attention and noradrenergic signaling are related to the generalization of learned associations.

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Poster

PSTR181: Acquisition and Memory Modification

Location: MCP Hall A

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Program #/Poster #: PSTR181.02/M13

Topic: G.02. Reward and Appetitive Learning and Memory

Support: DFG Grant 316803389 – SFB 1280

Title: Appetitive and aversive classical conditioning: Self-reported ratings and physiological responses

Authors: *M. PETRENKO[1], G. BATSIKADZE[2], E. NIO[3], C. J. MERZ[4], D. TIMMANN[2], T. M. ERNST[6], L. COENEN[6], S. CHENG[5], N. DIEKMANN[4];
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Abstract: Understanding the neural mechanisms underlying appetitive and aversive conditioning has important clinical implications because maladaptive associative learning processes are thought to contribute to various mental disorders, including anxiety, mood and eating disorders, as well as addiction and chronic pain. Since brain areas related to appetitive and aversive conditioning overlap with one another, but are probably also distinct, it is of interest to directly compare appetitive and aversive conditioning in behavioural and imaging studies. To what extent do multimodal outcome recordings in appetitive and aversive conditioning tasks match? We compared self-reported ratings and physiological responses (skin conductance responses, pupil size, and heart rate) using commonly applied appetitive and aversive unconditioned stimuli (US) in 40 healthy participants (20 women, mean age 24,6 years, range 19 -35 years). The first experiment compared self-reported and physiological assessments that were elicited by electric shock and three monetary rewards (one Euro, two Euros and five Euros). In the second experiment, differential aversive and appetitive conditioning was performed on two consecutive days with order being randomized between participants. Since outcome measures of electric shock best matched the one Euro reward, one Euro was used as US in the appetitive conditioning paradigm. In both experiments physiological responses were significantly lower towards appetitive conditioned stimuli (CS) and US compared to aversive CS and US. Self-reported ratings, on the other hand, showed much fewer differences in response magnitude and differential CS responding comparing appetitive and aversive CS and US. Overall, self-reported (absolute) valence ratings were higher towards monetary rewards compared to the electrical

stimulus considering both responses to the US in experiment 1 and CS in experiment 2. Our findings show that full comparability between multimodal outcomes can probably not be achieved in appetitive and aversive conditioning paradigms since outcomes might easily diverge . Studies comparing the neural responses in processing of aversive and appetitive stimuli using brain imaging, electroencephalography or other neurobiological methods, need to control for differences in response magnitudes and learning rates.

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Poster

PSTR181: Acquisition and Memory Modification

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR181.03/M14

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01MH108623
R01DC019813
R00MH118422
R01MH129582

Title: Temporal specific hippocampal computations during associative learning with delays

Authors: *M. ZHOU[1], B. WU[1], M. KHEIRBEK[2], V. K. NAMBOODIRI[3];
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Abstract: The hippocampus is crucial for associative learning, yet the specific neural computations supporting this role remain elusive. One clue comes from the fact that an intact hippocampus is specifically required for trace conditioning when there is a temporal gap between the conditioned stimulus (CS) and the unconditioned stimulus (US). What computations might explain such a selective role in trace conditioning? One potential hypothesis is that hippocampal computations maintain a memory trace between the discontinuous CS and US and help bind the CS memory with the subsequent US. An alternative mechanism is that computations immediately after the US (e.g., CS replays) bind the US with preceding CSs. In this project, we aim to investigate whether hippocampal subregions, particularly dorsal CA1 (dCA1) and the dentate gyrus (DG), support these mechanisms separately and whether activities in these regions contribute to the stimulus representation formation and stimulus-outcome binding differently. Using temporally precise optogenetic manipulation during an appetitive trace conditioning task, we found that DG activity is essential specifically during the CS period within the CS-US interval. Conversely, dCA1 activity is crucial during both the CS-US period and after the US, suggesting its involvement in supporting both mechanisms. Notably, dCA1 inhibition produced a generalized learning impairment for a non-inhibited CS predicting the same reward type, hinting

at an overlapping representation in dCA1 for these cues with shared rewarded outcomes. In our next step, we hope to measure stimulus representations and stimulus-outcome binding within these areas across appetitive trace learning with two-photon calcium imaging. Ultimately, we believe this study will significantly advance our understanding of the associative learning processes supported by the hippocampus.

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Poster

PSTR181: Acquisition and Memory Modification

Location: MCP Hall A

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Program #/Poster #: PSTR181.04/M15

Topic: G.02. Reward and Appetitive Learning and Memory

Support: F31-AA030936
R01-MH125951
R01-AA029386

Title: Carbon dioxide reactivity predicts extinction memory to fear, food, and alcohol cues in rats

Authors: *M. RASKIN[1], M. OLVERA[2], C. MALONE[3], E. HILZ[4], N. KELLER[1], L. AGEE[5], J. D. SHUMAKE[2], R. GONZALES[4], H. J. LEE[2], M. H. MONFILS[2];
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Abstract: Maladaptive associations underlie persistent responding to previously neutral stimuli. For example, cues present during a traumatic event may result in fear responses, and cues that precede rewards lead to seeking behavior (e.g., in addiction). These responses can be attenuated through extinction learning, where cues are repeatedly presented without the previously learned outcome. Extinction learning underlies exposure therapy, which is one of the best available treatments for anxiety-related disorders and addiction; however, not everyone responds to this approach, and our ability to predict whether an individual will or not remains limited. We recently demonstrated that behavioral CO_2 reactivity in rats predicts fear extinction memory and orexin activation, and that orexin activation predicts fear extinction memory, suggesting that a CO_2 challenge may enable identifying whether an individual is a good candidate for an extinction-based approach. The purpose of the present study was to determine whether the predictive power of CO_2 reactivity can be replicated in fear cues and whether it generalizes to food and alcohol cues. In experiment 1, male rats (n = 28) underwent fear conditioning, extinction and a long-term memory (LTM) test. In experiment 2, male rats (n = 48) underwent food conditioning, extinction, and LTM. In experiment 3, male and female rats with a history of alcohol dependence (n = 35) underwent alcohol conditioning, extinction and LTM. All rats then

received a CO_2 challenge, were euthanized, and their brains harvested for immunohistochemical analysis of cFos and orexin in the lateral hypothalamus. We used the best subset approach to linear regression (Monfils et al., 2019) to determine whether CO_2 reactivity would predict extinction memory separately for each experiment, and found that CO_2 reactivity explained 32% of the variability in extinction for fear, 23% for food, and 17% for alcohol. We thus find that the predictive power of CO_2 reactivity is replicated for fear cues, and generalizes to food and alcohol cues. Next, we will determine whether the number of CO2-activated orexin neurons predicts extinction phenotype across these reinforcers.

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Poster

PSTR181: Acquisition and Memory Modification

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Program #/Poster #: PSTR181.05/M16

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01 1R01AA029386-01A1 (HJL)

Title: Patterns of Ultrasonic Vocalizations in Rats Exposed to Chronic Ethanol Vapor

Authors: *T. A. SIMMONS[1], C. CERVANTES[1], R. GONZALES[2,3], M. H. MONFILS[1,3], H. J. LEE[1,3];
[1]Dept. of Psychology, [2]Pharmacol., [3]Dept. of Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: Rats exhibit ultrasonic vocalizations (USVs) that are indicative of positive emotional states during heightened motivational states, such as the anticipation or consumption of food or drugs of abuse. Conversely, during withdrawal from chronic exposure to substances of abuse, rats emit USVs in the 20 kHz range, reflecting adverse effects. Increases in 20 kHz USVs have been reported during alcohol withdrawal; however, the impact of chronic ethanol vapor exposure on USVs remain understudied. This study aimed to investigate the effects of ethanol vapor vs. air exposure on both 20 kHz and 50 kHz USVs. Male Long-Evans rats underwent a 10-min USV recording session before vapor (or air) exposure to establish baseline USVs. The vapor group was exposed to chronic intermittent ethanol vapor (14 hr/day x 10 days) in inhalation chambers. The remaining rats (air group) were treated the same, but exposed to air in control inhalation chambers or on a shelf. Then, USVs were recorded approximately 7-8 hrs after the last vapor (or air) exposure—a time at which the ethanol group experienced withdrawal. Ultrasonic vocalizations (USVs) were recorded during two phases: initially, rats were left undisturbed for one minute in a clean cage, followed by the limb flexion test where each rat was gently lifted by the loose skin at the back of its neck three times for measuring limb flexion, a sign of alcohol withdrawal. The rats were then left undisturbed for an additional minute. USVs were recorded

using Avisoft software, counted manually by an experimenter, and categorized on the basis of their vapor exposure status. The acoustic characteristics of USVs were classified based on the bandwidth of negative affect (20-29 kHz) and positive affect (>30 kHz), as well as the patterns between flats and frequency-modulated calls. Results showed no differences in the number of 20 kHz and 50 kHz calls at baseline, as expected. During the withdrawal period, rats displayed minimal USV calls when undisturbed; however, after the limb flexion test, vapor-exposed rats displayed significantly more 50 kHz frequency-modulated calls compared to air-exposed rats ($p=0.028$). These results suggest that vapor exposure may selectively enhance positive emotional vocalizations in rats when subjected to physical provocation during withdrawal. We plan to expand our sample size and include females to understand the complex influence of vapor exposure and withdrawal on emotional states in rats.

Disclosures: T.A. Simmons: A. Employment/Salary (full or part-time):; The University of Texas at Austin. 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.; Hongjoo J Lee, Marie-H Monfils, Rueben A Gonzales, Catalina Cervantes. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIH R01 1R01AA029386-01A1 (HJL) . D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); N/A. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); N/A. F. Consulting Fees (e.g., advisory boards); N/A. Other; N/A. **C. Cervantes:** A. Employment/Salary (full or part-time):; The University of Texas at Austin. 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.; Hongjoo J Lee. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIH R01 1R01AA029386-01A1 (HJL) . D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); N/A. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); N/A. F. Consulting Fees (e.g., advisory boards); N/A. Other; N/A. **R. Gonzales:** A. Employment/Salary (full or part-time):; The University of Texas at Austin. 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.; Rueben A Gonzales. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIH R01 1R01AA029386-01A1 (HJL) . D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); N/A. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); N/A. F. Consulting Fees (e.g., advisory boards); N/A. Other; N/A. **M.H. Monfils:** A. Employment/Salary (full or part-time):; The University of Texas at Austin. 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.; Marie-H Monfils. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIH R01 1R01AA029386-01A1 (HJL) . D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); N/A. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property

rights/patent holder, excluding diversified mutual funds); N/A. F. Consulting Fees (e.g., advisory boards); N/A. Other; N/A. **H.J. Lee:** A. Employment/Salary (full or part-time):; The University of Texas at Austin. 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.; Hongjoo J Lee. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIH R01 1R01AA029386-01A1 (HJL) . D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); N/A. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); N/A. F. Consulting Fees (e.g., advisory boards); N/A. Other; N/A.

Poster

PSTR181: Acquisition and Memory Modification

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR181.06/M17

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Associative Structures Underlying Pavlovian-Instrumental Transfer in an Operant Biconditional Discrimination Task with Rats

Authors: *J. ABRAMS[1], N. TU[1], A. R. DELAMATER[2];
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Abstract: The neural activations produced by the presentation of reward versus a cue-evoked expectation of reward are presumed to differ, but this assumption requires more investigation. We explored this question using an incongruent operant biconditional discrimination task, shown by others to recruit prefrontal mechanisms. In this task, the choice of one response lever, R1, was reinforced on trials that began with a noncontingent presentation of one of two qualitatively distinct reinforcing outcomes, O1, but another response lever, R2, was reinforced on trials beginning with the other outcome, O2. Liquid sucrose and grain pellets served as the distinct reinforcing outcomes, and the response levers were simultaneously presented to the left and right sides of the food magazine on each discrete trial. Critically, the reinforcers on each trial type were opposite to those that began the trial to create O1-R1-O2 and O2-R2-O1 relations. Thus, each reinforcer served the dual roles of trial-initiating discriminative stimulus and trial-terminating reinforcer. Responses to the incorrect R on a given trial were not reinforced. In separate off-baseline sessions, two Pavlovian auditory cues were each paired differentially with these Os (CS1-O1, CS2-O2) and we then explored the ability of these CSs to selectively transfer to the instrumental responses in Pavlovian-to-instrumental Transfer (PIT) tests. At issue was whether PIT would be based on R-O (e.g., CS1 elevates R2) or O-R (e.g., CS1 elevates R1) associative relations. During PIT tests conducted with both response levers freely available throughout the test, we confirmed an earlier result reported by de Wit et al (2009) that the Pavlovian cues selectively elevated instrumental responses ($p = 0.008$) based on the R-O, not O-

R, relations. Following these PIT tests, the rats were retrained on the operant and Pavlovian tasks, and then given "CS substitution" probe trials in which brief presentation of the Pavlovian cues were inserted into the beginning of the biconditional discrimination trial to determine if they might substitute for the noncontingent outcomes, themselves, in directing choice according to the acquired discriminative O-R relations. When presented with the reinforcing outcome itself to start the trial, rats showed a strong bias toward the lever reinforced with the opposite outcome ($p < 0.001$), but when presented with a trial-initiating CS they more often selected the lever reinforced by the same outcome with which the CS had been paired ($p = 0.018$) - meaning that the reward itself and the reward's expectation cause opposite response choices, and these continue to be mediated by R-O, not O-R, associative relations.

Disclosures: J. Abrams: None. **N. Tu:** None. **A.R. Delamater:** None.

Poster

PSTR181: Acquisition and Memory Modification

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR181.07/M18

Topic: G.02. Reward and Appetitive Learning and Memory

Support: JSPS KAKENHI Grant Number JP18K03182

Title: A progressive delay-discounting task in Spontaneously hypertensive rats (SHRs): A preliminary study of impulsive choise in SHR/Izm

Authors: *T. SATO[1], H. AMBO[2];
[1]Psychology, Edogawa Univ., Nagareyama, Japan; [2]Sch. of Educ., Tohoku Univ., Sendai, Japan

Abstract: The SHR/Izm is a substrain of Spontaneously Hypertensive Rat (SHR). In addition to high blood pressure, various behavioral characteristics have been reported. Recently, reports have emerged regarding delay discounting in another SHR substrain, along with high impulsivity. Therefore, we experimentally investigated whether SHR/Izm rats exhibited a similarly high impulsivity level using an impulsive choice procedure (Mar & Robbins, 2007). Six male SHRs and Wistar-Kyoto strain rats (WKYs) served as controls for normal blood pressure, with each session of 60 trials classified into five blocks of 12 trials each. Each 12-trial block began with two forced-choice trials, randomly presenting either the left or right lever, followed by 10 free-choice trials with both levers available. Each rat had one lever designated as the "immediate" lever and the other as the "delay" lever. Pressing the "immediate" lever resulted in an immediate reward pellet, while pressing the "delay" lever led to four reward pellets after a specific delay (0, 10, 20, 40, and 60 sec, for each of the five 12-trial blocks). Trials were presented 100 s apart (i.e., 60 trials in 100 min). Although this study was preliminary and the number of subjects was relatively small, the results were not statistically significant; however, they were marginal for some indices. The choice ratio, i.e., the ratio of pressing the "delay" lever as compared to the "immediate" lever in the SHRs, was lower in the fifth blocks (the delays were

60 sec) than in the WKYs, with the difference being marginally significant (p = 0.078). This result suggests that SHR/Izm, like another substrain, may exhibit higher impulsivity than WKY/Izm.

Disclosures: T. Sato: None. **H. Ambo:** None.

Poster

PSTR181: Acquisition and Memory Modification

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR181.08/M19

Topic: G.02. Reward and Appetitive Learning and Memory

Title: What Constitutes Successful Observational Learning? Understanding Social and Behavioral Factors

Authors: *T. WRENN[1], E. J. MARKUS[2];
[1]Psychological Sci., Univ. of Connecticut, Storrs, CT; [2]Psychology, Univ. of Connecticut, Storrs Mansfield, CT

Abstract: Observational learning is a change in behavior following the observation of another animal performing a task rather than personally performing the task. Our lab studies observational learning in a food location task. In our task, student (observer) rats must learn to watch a teacher (demonstrator) rat in an adjacent chamber respond at one nose poke and choose the corresponding nose poke in their own chamber. The behaviors of both the teacher, student and their interactions are tracked and related to the student's success or failure on a trial. There at up to 80 observation trials per session, allowing an in-depth analysis of the multitude of behaviors that can occur throughout the session. With this type of data collection, performance of the teacher was measured, as well as distance and heading orientation of the student to the teacher throughout the task. Precise behavioral assessment of both the learner and teacher rats is possible through a machine learning assisted program, Social LEAP Estimates Animal Poses (SLEAP), that can track their movements (Pereira et al., Nature Methods, 2022). There are crucial intervals when the observation must take place in order to succeed on a given trial. The teacher rat has up to 4 sec from when it is cued regarding the current/cued goal to nose-poke the goal hole. It must remain in the goal hole for 4 sec to get rewarded. The student must be attending to the teacher during this 4-8 sec interval to know which of its two nose-poke holes is the correct goal for that trial. Focusing on this 4-8 sec period we can determine what variables in the teacher's behavior are related to successful and unsuccessful observation. Student rats differ in their success on this task and this maybe the result of an interaction with the identity of the teacher. Social factors, such as dominance, may influence the behaviors of both teacher and student rats in this task. To quantify social hierarchies and dominance we are using a battery of tasks requiring competition between pairs of rats. These include a water maze competition task, a tube test, and a food competition task.Taken together these studies will help determine what

behavioral characteristics of the teacher and student rats facilitated successful observational learning.

Disclosures: T. Wrenn: None. **E.J. Markus:** None.

Poster

PSTR181: Acquisition and Memory Modification

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR181.09/M20

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Discovery Grant from Natural Sciences and Engineering Research Council of Canada

Title: A study ofthe pharmacology of memory modulation by glucose

Authors: F. LERI;
Univ. of Guelph, Guelph, ON, Canada

Abstract: Reinforcing stimuli such as glucose impact behaviour in part because of their action on memory consolidation. In fact, when administered post-training, glucose improves learning of a variety of tasks in humans and animals. Here we show in male Sprague-Dawley rats that post-training injections (SC) of 100 and 2000 mg/kg glucose improve consolidation of memory if delivered immediately, but not 6 hours, following the sample phase of the object recognition memory task. To study the pharmacology of this effect, rats were pretreated with 3 mg/kg naltrexone (a non-selective opioid antagonist), 30 mg/kg bupropion (a monoamine agonist), or a combination of the two. Only naltrexone blocked modulation of memory by glucose, possibly by antagonism of both central and peripheral receptors because it had the same effect of 10 mg/kg naloxone methiotide, which does not readily cross the blood-brain barrier. Taken together, these experiments suggest that the reinforcing action of glucose on memory consolidation is critically dependent on central and peripheral endogenous opioid receptors.

Disclosures: F. Leri: None.

Poster

PSTR181: Acquisition and Memory Modification

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR181.10/M22

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Littlefield and Ransom Fellows Grant
T32 AA007471

Title: Effects of high gonadal hormone status and hormonal contraceptives on female rat extinction learning

Authors: *A. VASQUEZ[1], G. KIM[1], P. E. ANTONACCI[1], J. M. DOMINGUEZ[2], M. H. MONFILS[3], H. J. LEE[4];
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Abstract: Evidence suggests that gonadal hormones contribute to the maintenance of substance use disorders (SUD) in females. One common treatment for SUD is exposure therapy, which is based on extinction training, and changes in gonadal hormone levels across the menstrual cycle, as well as those induced by hormonal contraceptives (HC), affect extinction learning. The rat estrous cycle is comprised of four stages, which are often clustered into "low" and "high" hormonal stages based on estradiol (E2) and progesterone (P4) levels. E2 and P4 are low during metestrus and diestrus stages. In proestrus, E2 and P4 levels gradually rise, reaching their peak, and begin to decline as estrus commences, where females are sexually receptive and the genomic effects of E2 are present. Previous work in our lab showed that levonorgestrel (LNG), a synthetic progestin commonly found in HCs, led to decreased amphetamine (AMPH)-preference across extinction training compared to naturally cycling rats in a high hormonal state (i.e., proestrus and estrus). However, whether AMPH preference differs by proestrus or estrus stages AND whether this preference is reduced by LNG exposure during extinction training is unknown. In the present study, female rats first underwent AMPH-conditioned place preference and were tested for their AMPH-preference for three sessions (i.e., extinction learning) after receiving either oral administration of LNG (250μg/rat, 500μg/rat, or 2mg/rat). Serum hormone levels and weights were obtained to assess their relationship to AMPH-preference across extinction sessions. All groups initially displayed AMPH-preference; however, only the females that were first injected with AMPH during estrus and subsequently administered with 500μg of LNG during extinction showed significantly reduced AMPH-preference. The proestrus rats and those that received 250μg and 2mg of LNG rats did not. A negative relationship between estradiol levels and weight suggested that, regardless of treatment, lower estradiol levels are associated with increased weight. Interestingly, a positive correlation between progesterone and the first extinction session was observed, proposing that increased levels of progesterone lead to increased AMPH-preference. These results are the first to show differential behavioral effects between proestrus and estrus stages that are further discerned after HC manipulation in a dose-dependent manner. Future work will investigate whether these effects extend to AMPH-primed reinstatement to assess conclusive effects of LNG on drug-seeking behavior in female rats.

Disclosures: A. Vasquez: None. **G. Kim:** None. **P.E. Antonacci:** None. **J.M. Dominguez:** None. **M.H. Monfils:** None. **H.J. Lee:** None.

Poster

PSTR181: Acquisition and Memory Modification

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR181.11/M23

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01AA029386

Title: Sex differences in Pavlovian cue-induced alcohol-seeking relapse are dependent on the light-dark phase of the day

Authors: *P. E. ANTONACCI[1], A. VASQUEZ[1], H. KERRIGAN[2], W. S. PETERS[3], R. GONZALES[4], M. H. MONFILS[1], H. J. LEE[1];
[1]Dept. of Psychology, [2]Dept. of Mathematics, [3]Dept. of Computer Sci., [4]Dept. of Pharmacol., Univ. of Texas at Austin, Austin, TX

Abstract: Excessive alcohol use is driven by biological, psychological, and social factors. The degree to which these factors contribute to problematic drinking and subsequent relapse after recovery varies greatly among individuals. One common factor present in all alcohol drinkers is the set of cues closely linked to alcohol consumption. Environmental cues that reliably precede alcohol availability and subsequent consumption elicit conditioned responses, such as alcohol-seeking behavior, and ultimately promote problematic drinking. The current experiment aimed to better understand how a Pavlovian cue associated with alcohol influences relapse-like behavior in male and female rats, and examine whether effects differ across the rats' day-light cycle. Groups of male and female Long-Evans rats were housed in a reverse light cycle (lights off at 9am) and a standard light cycle (lights on at 9am), respectively. A subset of male and female rats received Pavlovian alcohol conditioning and extinction either at the beginning of the dark phase or light phase. Our conditioning paradigm consisted of a 20-sec light presentation paired with 10-sec access to 15% alcohol. Rats developed similar conditioned alcohol-seeking behavior across housing and light conditions, with the females showing greater alcohol consumption per body weight compared to males. The rats also showed comparable extinction of alcohol-seeking behavior when the 15% alcohol was no longer available during the light presentation. When rats were tested for spontaneous recovery of alcohol-seeking, an interaction effect of sex by housing light condition was seen. Female rats showed a return of alcohol-seeking behavior, with those tested in the light-phase displaying greater spontaneous recovery. Male rats tested in the light-phase, but not in the dark-phase, showed spontaneous recovery of alcohol-seeking. In males, the degree of spontaneous recovery correlated with alcohol consumption when the rats were given another opportunity to drink. Our data suggest that sex-specific effects might be mediated by the timing of when alcohol-seeking is acquired and extinguished. Our results further suggest that the contribution of environmental cues to relapse and consequent drinking differs between males and females. Our study has important implications for understanding sex and circadian rhythm as biological variables in animal models, and translational relevance for understanding and treating humans with alcohol use disorder.

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Poster

PSTR181: Acquisition and Memory Modification

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR181.12/M24

Topic: G.02. Reward and Appetitive Learning and Memory

Support: T32AA007471
R01AA029386

Title: Cue reactivity predicts relapse-like behavior after extinction but not after retrieval+extinction in rats with a history of alcohol dependence

Authors: *M. OLVERA[1], R. A. GONZALES[2], M. H. MONFILS[3], H. J. LEE[4];
[1]Univ. of Texas at Austin, Austin, TX; [2]Pharmacol., The Univ. of Texas at Austin, Austin, TX; [3]Dept. of Psychology, Univ. of Texas At Austin, Austin, TX; [4]Psychology Dept, Univ. of Texas at Austin Dept. of Psychology, Austin, TX

Abstract: Discrete cues precede alcohol availability during consumption. Over time, this pairing results in a conditioned response to the cue alone, or cue-reactivity, which can promote alcohol consumption and relapse in recovering individuals. Subsequently, treatments that reduce cue-reactivity can prove beneficial in preventing relapse.
Using Pavlovian conditioning, we created a paradigm to model the acquired association between environmental cues and alcohol use. A house light precedes alcohol availability via a sipper. This task allows us to measure appetitive approach behavior during cue presentation and consummatory licking behavior during sipper presentation. Additionally, we can measure cue-directed behavior (i.e., orienting/rearing to the light) associated with attention and cue salience. Extinction based therapy reduces cue-reactivity, but conditioned behavior often returns, likely due to the creation of a new competing extinction memory rather than modifying the original memory. However, retrieval+extinction (R+E), where an extinction session is conducted after the presentation of a retrieval cue, can update the original memory and be more effective at preventing relapse.
In this study, we examined acquisition and extinction of conditioned responses (i.e., approach, licking, orienting) and tested whether any of these behaviors might predict relapse-like behavior. We applied extinction and R+E approaches and asked if relapse might be predicted by the same set of behaviors.
Male Long-Evans rats first went through alcohol induction via a two-bottle choice paradigm and received chronic intermittent ethanol vapor (or control air) to induce physical dependence. Then, rats were conditioned to associate a light cue to unsweetened 15% alcohol delivery over 12 sessions. Rats were divided into two groups, extinction (n=20) and R+E (n=22) and underwent their respective extinction training over 14 sessions. Rats were then tested for long-term memory after 48hr.
Orienting response during extinction was moderately correlated with sipper interaction during the long-term memory testing in both the R+E ($r(20)=-0.41$, $p=0.06$) and extinction paradigm

(r(18)=-0.44, p=0.055). Anticipatory approach behavior during light presentation (prior to sipper insertion) during the extinction session was also negatively correlated with sipper interaction during the long-term memory test, but only in the extinction group (r(18)=-0.47, p=0.035). Our results suggest that different behavioral outcomes of the R+E paradigm and standard extinction predict alcohol-seeking behavior.

Disclosures: M. Olvera: None. **R.A. Gonzales:** None. **M.H. Monfils:** None. **H.J. Lee:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.01/M25

Topic: G.05. Mood Disorders

Title: Exploring the therapeutic potential of psilocybin in treatment-resistant depression: preclinical investigations in mice.

Authors: *K. MARSZALEK[1], M. DOMZALSKA[1], J. KWIATKOWSKA[1], J. R. HUXTER[2], E. SOKOLOWSKA[1];
[1]Transpharmation Ltd., Olsztyn, Poland; [2]Transpharmation, Ltd., Sawston, United Kingdom

Abstract: Treatment-resistant depression is a widespread global health concern, with urgent need for novel approaches. Recent research indicates that psilocybin and its derivatives hold promise as substances with potential therapeutic benefits. Psilocybin, a non-selective 5-HT2AR agonist, has been found to alleviate depressive mood in individuals with treatment resistant depression (Carhart-Harris et al., 2017). Moreover, there is renewed interest in studying non-hallucinogenic LSD derivatives for their potential efficacy against depression. In this study we conducted various preclinical tests (commonly used in depression and anxiety research) to assess the effects of psilocybin on C57Bl/6 mouse behaviour. Wireless electroencephalography (EEG) was used to monitor vigilance states and brain activity following psilocybin administration. Automatic sleep-wake scoring was performed, and the hourly distribution of wakefulness, NREM-sleep and REM-sleep was analyzed using proprietary Transpharmation software. Psilocybin's hallucinogenic effect was assessed using Head Twitch Response (HTR) assay, measuring the number of rapid head shakes in mice immediately after treatment. The Forced Swim Test (FST) focused on immobility time of mice subjected to various psilocybin doses. Social Preference test (SP) was used to evaluate social avoidance behaviour in mice following chronic social defeat procedure (CSD) and psilocybin treatment. Psilocybin was administered intraperitoneally (i.p.) at 1, 3, 5 and 10 mg/kg. Psilocybin briefly reduced wakefulness while increasing non-REM sleep, but caused longer-lasting reductions in REM sleep for up to 4 hours. Psilocybin, particularly at 10mg/kg, produced a robust reduction in sigma-alpha oscillations during non-REM sleep.As expected psilocybin at all tested doses (1 & 5 mg/kg) evoked head twitches, confirming its hallucinogenic effect ($p<0.0001$). Two highest doses of psilocybin (5 & 10 mg/kg) presented antidepressant efficacy in the FST 24 hr post-administration ($p<0.01$,

p<0.05 respectively). The CSD procedure resulted in subgroups of stress-resilient (49%) and stress-susceptible (51%) C57BL/6J mice. Psilocybin (10 mg/kg) showed prolonged antidepressant efficacy 1, 7 and 14 days after a single administration (p<0.01, p<0.05, p<0.01 respectively). Our data demonstrated the effectiveness of psilocybin in alleviating depression/anxiety-like phenotypes in mice. However, due to widespread concerns in the therapeutic use of psychedelics, research on non-hallucinogenic LSD analogues is resuming.

Disclosures: K. Marszalek: None. **M. Domzalska:** None. **J. Kwiatkowska:** None. **J.R. Huxter:** None. **E. Sokolowska:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.02/M26

Topic: G.05. Mood Disorders

Support: G. Harold & Leila Y. Mathers Charitable Foundation
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Title: Parsing the therapeutic and psychedelic effects of psychedelic compounds mediated by the 5-HT_{1A} and 5-HT_{2A} receptors

Authors: *I. C. SERRANO[1], D. LANKRI[1], M. CUNNINGHAM[2], L. F. PARISE[3], V. D J[4], P. DUGGAN[5], V. HAVEL[1], S. J. RUSSO[6], S. S. CHANDRA[7], D. WACKER[8], D. SAMES[1]; [1]Chem., Columbia Univ., New York, NY; [2]Gilgamesh Pharmaceuticals, New York, NY; [3]Neurosci., Icahn Sch. of Med. at Mt.Sinai, New York, NY; [4]Neurosci. and Neurol., Yale Univ., New Haven, CT; [5]Columbia Univ., New York, NY; [6]Neurosci., Icahn Sch. of Med. at mount sinai, New York, NY; [7]Neurosci. & Neurol., Yale Univ., New Haven, CT; [8]Pharmacol. Sci., Icahn Sch. of Med. at Mt Sinai, New York, NY

Abstract: Interest in psychedelics like lysergic acid diethylamide (LSD), psilocybin, and 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) is surging due to their potential in treating neuropsychiatric and neurological disorders. Whether the psychedelic experience is essential for therapeutic outcomes and the mechanisms behind serotonergic psychedelics' effects remain elusive. To address this, we conducted behavioral experiments using male C57BL/6J mice, randomly assigned to treatment groups by observers blinded to drug and dose. To investigate the

necessity of the psychedelic experience, we explored the therapeutic potential of Ariadne, a non-hallucinogenic serotonin (5-HT) receptor 2A agonist. Despite clinical trials at Bristol-Myers Company suggesting remarkable therapeutic effects without hallucinogenic properties, Ariadne was abandoned as a drug candidate with unknown molecular pharmacology. Compared to a phenethylamine positive control, Ariadne shows markedly attenuated head twitch response (HTR, n = 5/group) – a proxy for psychedelic activity *in vivo* – as well as lower signaling potency and efficacy in multiple signaling pathways examined ($G_{q/11}$, and β-arrestin2) coupled to 5-HT_{2A} *in vitro*. The novelty suppressed feeding paradigm demonstrated Ariadne's anxiolytic-like effects 7 days post-administration (n = 10/group). In a pilot auxilin knockout Parkinson's Disease (PD) model study (n = 4-6/group), Ariadne acutely rescued severe motor deficits akin to levodopa, suggesting promising therapeutic potential. Our findings propose the lower 5-HT_{2A} receptor signaling efficacy of Ariadne as an explanatory model for its lack of hallucinogenic effects, which did not diminish its therapeutic effect. To elucidate the polypharmacology of psychedelics, we explored therapeutic effects associated with substances engaging 5-HT_{1A} receptors, as LSD and 5-MeO-DMT are effectively equipotent at $5HT_{1A}$ and $5HT_{2A}$. A newly synthesized 5-HT1A-selective 5-MeO-DMT analogue, (4-fluoro, 5-methoxy-N,N-pyrrolidinyl-tryptamine, 4-F, 5-MeO-PyrT) lacked any HTRs (n = 5/group), confirming lack of 5HT2A activity, while exhibiting anxiolytic and antidepressant-like effects in social interaction and sucrose preference assays in chronic social defeat with CD-1 aggressor mice (n = 9-18/group). This was further
validated with the negation of positive effects with pre-treatment of a 5HT1A antagonist, WAY-100635 (n = 5-10/group). Moreover, it alleviated haloperidol and tetrabenazine-induced catalepsy, suggesting potential therapeutic efficacy in PD. Ariadne and 4-F, 5-MeO-PyrT may facilitate development of new neuropsychiatric therapeutics.

Disclosures: I.C. Serrano: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on a patent application related to the compounds. **D. Lankri:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on a patent application related to the compounds. **M. Cunningham:** A. Employment/Salary (full or part-time):; Cofounder of Gilgamesh Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on a patent application related to the compounds. **L.F. Parise:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on a patent application related to the compounds. **V. D j:** None. **P. Duggan:** None. **V. Havel:** None. **S.J. Russo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on a patent application related to the compounds. **S.S. Chandra:** None. **D. Wacker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on a patent application related to the compounds. F. Consulting Fees (e.g., advisory boards); Consults for Otsuka Pharmaceutical, Longboard Pharmaceuticals and Ocean Bio on the design of psychedelic-based therapeutics. **D. Sames:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on a patent application related to the compounds. Other; Cofounder of Gilgamesh Pharmaceuticals and Kures.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.03/M27

Topic: G.05. Mood Disorders

Title: Discovery of novel 5-HT2A receptor agonists with non-hallucinogenic potential and translational antidepressant drug-like profiles

Authors: *C. A. BOWEN[1], T. A. KHAN[1], R. B. PERNI[2], T. C. ROBERTSON[1], H. B. JANSSENS[1], J. D. S. HOLT[1], P. KLEINE[1], A. L. HALBERSTADT[3], S. G. RAO[1], G. F. SHORT, III[1];
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Abstract: Mood disorders, particularly treatment-resistant depression, remain a significant unmet medical need. Clinical and preclinical research suggests the potential for psychedelic 5-HT2A receptor (5-HT2AR) agonists, such as psilocybin's active metabolite psilocin, to produce rapid and lasting antidepressant activity after a single dose. Preclinical neuroplasticity and behavioral studies also support antidepressant-like effects of non-hallucinogenic 5-HT2AR agonists, which may offer more convenient dosing to a broader patient population. Thus, research was focused on discovering novel 5-HT2AR agonists with CNS drug-like properties, non-hallucinogenic potential, and in vivo antidepressant drug-like profiles. Novel agonists with 5-HT2AR selectivity over 5-HT2B receptor (5-HT2BR) were prioritized to reduce potential risks associated with prolonged 5-HT2BR stimulation (e.g., valvulopathy). Artificial intelligence-driven *de novo* drug design followed by medicinal chemistry structure-activity relationship (SAR) development identified a series of small-molecules that exhibited nanomolar potencies for in vitro activation of human and rodent 5-HT2AR, with selectivity over 5-HT2BR agonism, and favorable physicochemical properties. Hits showed high brain penetrance in rodents following multiple routes of administration. Unlike known psychedelic 5-HT2AR agonists, hits did not induce the head-twitch response (HTR) in male mice, a behavioral proxy for human hallucinogenic effects. In addition, novel compounds dose-dependently attenuated the HTR induced by the hallucinogen DOI, indicative of in vivo 5-HT2AR interactions. Antidepressant-like activity was observed in a forced swim test (FST) 24h post dose in male mice, consistent with a psilocybin reference control. Translational antidepressant drug-like effects were demonstrated using electroencephalography (EEG)-based measures of rapid eye movement (REM) sleep in male Wistar Kyoto (WKY) rats, which exhibit increased REM sleep consistent with symptoms observed in clinical depression. Over the first 6h post dose, novel hits suppressed REM sleep, indicative of antidepressant-like responses and similar to the effects of psilocybin in this model. Hits also altered EEG spectral power across frequencies during wakefulness, similar to the effects of psilocybin. Current SAR efforts focus on increasing 5-HT2AR potency and oral bioavailability, while maintaining 5-HT2AR selectivity over 5-HT2BR agonism, non-hallucinogenic potential and translational antidepressant drug-like profiles.

Disclosures: C.A. Bowen: A. Employment/Salary (full or part-time):; Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **T.A. Khan:** A. Employment/Salary (full or part-time):; Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **R.B. Perni:** F. Consulting Fees (e.g., advisory boards); Consultant for atai Life Sciences; Atai Therapeutics, Inc., is an atai platform company. **T.C. Robertson:** A. Employment/Salary (full or part-time):; Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **H.B. Janssens:** A. Employment/Salary (full or part-time):; Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **J.D.S. Holt:** A. Employment/Salary (full or part-time):; Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **P. Kleine:** A. Employment/Salary (full or part-time):; Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **A.L. Halberstadt:** 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.; Contracted researcher for atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **S.G. Rao:** A. Employment/Salary (full or part-time):; Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **G.F. Short:** A. Employment/Salary (full or part-time):; Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.04/M28

Topic: G.05. Mood Disorders

Support: R01-MH084894
T32-MH020030
F31-DA057818
VCU Breakthroughs Fund

Title: Characterization of the psychedelic quipazine analog VCU-1012 in mice

Authors: *J. L. MALTMAN[1], J. YOUNKIN[2], A. GHORPADE[3], M. FIORILLO[3], A. JASTER[4], H. I. AKBARALI[5], I. S. RAMSEY[6], M. DUKAT[3], J. GONZÁLEZ-MAESO[2]; [1]Neurosci., [2]Physiol., [4]Pharmacol. and Toxicology, [3]Virginia Commonwealth Univ., Richmond, VA; [5]Virginia Commonwealth Univ., Richmond, VA, ; [6]Sch. of Medicine, Virginia Commonwealth Univ., Richmond, VA.

Abstract: Background: Psychedelics are generating extensive research interest due to their recent success in clinical trials for mood disorders, such as depression and post-traumatic stress disorder. Classical, or serotonergic, psychedelics exert their hallucinogenic effects via agonism of the serotonin 2A receptor (5-HT_{2A}R), which is a G protein-coupled receptor, and are generally divided into two categories based on their chemical structures: tryptamine and phenethylamine

psychedelics. Like other receptors, the 5-HT_{2A}R experiences biased agonism due to how different structures affect the conformation and therefore downstream signaling of the receptor. One way to better understand the underlying mechanisms and separability of the hallucinogenic and therapeutic effects of psychedelics is by characterizing novel psychedelic compounds with unique binding modalities. We hypothesized that quipazine may represent a new class of psychedelics given its differing chemical structure from classical psychedelics and its agonist activity at the 5-HT_{2A}R, although its one drawback is agonism of the serotonin 3 receptor (5-HT_3R), which is a pentameric ion channel that produces adverse gastrointestinal effects. Here, we synthesized and characterized a quipazine analog VCU-1012 that retains 5-HT_{2A}R agonist activity, while removing its action at the 5-HT_3R.
Methods: •Binding displacement assays with [^{3}H]ketanserin in HEK-293 cells stably expressing h5HT_{2A}R cDNA.•Agonist-induced Ca^{2+} mobilization assay in HEK-293 cells stably expressing h5HT_{2A}R cDNA.•Head-twitch response in adult male C57BL/6 mice.•Whole-cell voltage clamp electrophysiology in a tetracycline-inducible Flp-In-293 T-REx cell line.•Gut motility assay in adult male C57BL/6 mice.•Forced swim test in adult male C57BL/6 mice.•Frontal cortex dendritic spine density in adult male C57BL/6 mice.
Results: Our data suggest that VCU-1012 is an agonist of the 5-HT_{2A}R, but not the 5-HT_3R. It also dose-dependently increases head-twitch behavior, which is a behavioral readout of hallucinogenic activity in rodents. We show that VCU-1012 can reduce immobility time in the forced swim test post-acutely, indicating it may have therapeutic value. Lastly, we expect our dendritic spine analysis to reveal similar neuroplastic effects to psilocybin, a classical tryptamine psychedelic.
Conclusions: These findings suggest that our quipazine analog VCU-1012 represents a new structural class of psychedelics and that it has therapeutic potential pre-clinically.

Disclosures: J.L. Maltman: None. **J. Younkin:** None. **A. Ghorpade:** None. **M. Fiorillo:** None. **A. Jaster:** None. **H.I. Akbarali:** None. **I.S. Ramsey:** None. **M. Dukat:** None. **J. González-Maeso:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.05/M29

Topic: G.05. Mood Disorders

Support: ANR AIS in Dep, ANR 22 CE37 001

Title: The prototypical hallucinogen, lysergic acid diethylamide, produces rapid antidepressant-like effects via 5-HT2B receptor activation in rats

Authors: *A. BOULOUFA, Jr[1], S. DELCOURTE[2], B. P. GUIARD[3], N. HADDJERI[1]; [1]Stem-cell and Brain Res. Inst., Bron, France; [2]Brain Hlth. Inst., Rutgers Univ. RBHS, Piscataway, NJ; [3]Ctr. De Recherche Sur La Cognition Animale, Toulouse, France

Abstract: Recent clinical trials reveal that serotoninergic psychedelics such as lysergic acid diethylamide (LSD) present a promising potential to treat psychiatric disorders, including depression. LSD is a potent serotonin (5-HT) receptor ligand and is regularly used as a pharmacological tool to characterize 5-HT1A/2A receptors mediation. However, a crystal structure of LSD in complex with the human 5-HT2B receptor has been recently described.The present work was aimed to evaluate the role of the 5-HT2B receptor in the action of LSD in male rats, firstly in the forced swim test (FST, despair-like response), ultrasonic vocalizations (USV, anxious-like response) and head twitch response (HTR, hallucinatory-like response) and secondly on the spontaneous firing activity of dorsal raphe (DRN) 5-HT neurons.For the FST, rats experienced a pre-test session followed 24h later by a test session (5 min). Vehicle or RS-127445 (0,16mg/kg, i.p.) were injected acutely before vehicle or LSD (50µg/kg, i.p.) that were administered 5 days before the test (n=6-9).Rats were exposed to sessions of 1 to 4 randomly distributed electric shocks until 22-kHz USV emissions. After 24 hours, they received a single shock after vehicle administration. 24 hours later, they received a single shock after acute LSD (50µg/kg, i.p.) injection with or without RS-127445 (0,16mg/kg, i.p.)(n=4-5).For the HTR, rats were placed in a cage and the number of head twitches were counted during a 30-min period. LSD (50µg/kg, i.p.) was injected immediately before the observation while vehicle or RS-127445 (0,16mg/kg, i.p.) was injected prior to LSD administration (n=7).Finally, extracellular unitary recordings of rat DRN 5-HT neurons were performed. LSD (10µg/kg, i.v.) was injected until cell firing was completely suppressed after injection of vehicle or the selective 5-HT2B antagonist RS-127445 (5µg/kg, i.v.)(n=5-6).Data were analyzed using a student t-test or a two-way analysis of variance (ANOVA), followed by a Tuckey post-hoc comparison, when multiple comparison was needed.Findings indicate that LSD induced i) a decrease of immobility time in the FST (*p<0,05, two-way ANOVA), ii) a suppression of the duration of USV (****p<0,0001, two-way ANOVA) and iii) an increase of the HTR (****p<0,0001, two-way ANOVA). Notably, the latter actions of LSD were significantly counteracted by a prior injection of RS-127445. Moreover, LSD suppressed totally DRN 5-HT neurons firing rate. Importantly, RS-127445 prevented the suppressant effect of LSD (**p<0,01, t-test).Collectively, the present results suggest for the first time that 5-HT2B receptors present a permissive role in the antidepressant-like effects of LSD.

Disclosures: A. Bouloufa: None. **S. Delcourte:** None. **B.P. Guiard:** None. **N. Haddjeri:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.06/M30

Topic: G.05. Mood Disorders

Title: Exploring the time and concentration dependence of psychedelic exposure to neuronal cultures using a high-density microelectrode array system

Authors: L. AGHOLME[1], S. LARDELL[1], T. RAY[2], *P. KARILA[1], P. WREN[2];
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Abstract: Background: Functional imaging and neurophysiology technologies have provided a clinical link to neuroplasticity mechanisms of psychedelics[1, 2]. Since neuroplasticity changes are similar between humans and animals after psychedelic exposure[3], assessment of neuronal firing patterns in rodent cell cultures could increase throughput when investigating mechanisms of novel psychedelic compounds. As an exemplar tool molecule, we assessed the time and concentration dependence of 5-methoxy-N,N-dimethyltryptamine to see the potential sensitivity of neuronal functional dynamics after psychedelic exposure in cell cultures using a high-density microelectrode array (MEA) system. 5-MeO-DMT, has recently completed a Ph1 and Ph2a open label study with early evidence of potential durable signals in treatment resistant depression[4,5]. Methods: Cortical preparations from E18 rats were seeded on PDL and laminin coated MaxTwo 24-well MEA plates with 26,400 electrodes/well (Maxwell biosystems). At 14 DIV, cortical preparations were treated with 5-MeO-DMT or vehicle control (DMSO). Network scans were performed at 0.5, 1, 2, 3 and 24 h post compound addition. Analysis was performed with fixed settings for all timepoints. Results: Despite no apparent changes in mean firing rate or burst frequency, there was an acute (1-3 h) statistically evident concentration-dependent increase in mean burst firing rate which intriguingly was followed by a profound and statistically significant increase in mean spikes per burst as well as burst duration at 24 h for all concentrations tested. Conclusions: Using the high-density MEA system, we were able to detect psychedelic-induced changes in neuronal function in vitro. These initial findings suggest a dynamic and temporally regulated modulation of neuronal activity, with both acute and delayed effects on burst firing characteristics that may involve different molecular and cellular pathways involved in synaptic transmission, neuronal excitability, and network dynamics. More in-depth assessments of 5-MeO-DMT mechanisms of action coupled with a range of pharmacologically and qualitatively diverse psychedelic drugs may pinpoint common and diverse mechanisms of action of psychedelics and how they may link to the expression of diverse altered states of consciousness.
References: 1. McCulloch et al., 2022. Neurosci Biobehav Rev. 2022 Jul;138:104689. 2. Carhart-Harris RL, et al., 2012. Proc Natl Acad Sci U S A. 2012 Feb 7;109(6):2138-43. 3. Vejmola Č, et al., 2021. Transl Psychiatry. 2021 Oct 2;11(1):506. 4. Rucker JJ et al., 2024. J Psychopharmacol. 2024 Apr 14:2698811241246857. 5. https://www.beckleypsytech.com/

Disclosures: L. Agholme: A. Employment/Salary (full or part-time):; Cellectricon AB. **S. Lardell:** A. Employment/Salary (full or part-time):; Cellectricon AB. **T. Ray:** A. Employment/Salary (full or part-time):; Mindstate Design Labs. **P. Karila:** A. Employment/Salary (full or part-time):; Cellectricon AB. **P. Wren:** A. Employment/Salary (full or part-time):; Mindstate Design Labs.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.07/M31

Topic: G.05. Mood Disorders

Title: A circuit-based, neurobehavioral assay for preclinical antidepressant profiling

Authors: *J. NASSI, L. BELLIER, N. REN, S. HUANG, D. CHENG, L. GRAY, O. MILLER; Inscopix Discovery Lab., Inscopix, Inc., Mountain View, CA

Abstract: The current paradigm for evaluating the efficacy of new treatments for depression, primarily semi-anthropomorphic behavioral assessments, is severely underperforming as evidenced by the high rate of clinical trial failure for lack of efficacy. An alternative approach is to leverage our understanding of the neural circuitry compromised in psychiatric disease to develop preclinical assays that read out the instantaneous relationship between neural activity and behavior. Here we do so, using Inscopix miniscopes and genetically encoded calcium sensors to assess how pharmacological interventions impact depression-relevant neural circuits.
Male and female mice (n=10-20) were injected with GCaMP6f and implanted with a GRIN lens in the medial prefrontal cortex for pyramidal neuron calcium imaging. Mice were recorded at baseline and post vehicle/ drug administration. Data were analyzed using a linear mixed effects model (LMM) with condition (baseline, post-treatment) and dose (vehicle, low, moderate, high) as fixed effects, focusing on 15 metrics for drug profiling and comparative analysis.
We tested a panel of 12 compounds developed for Major Depressive Disorder (MDD) including Ketamine, Rapastinel, Traxoprodil, and Psilocin, all of which have “antidepressant” effects in canonical behavioral assays but vary in their clinical efficacy. In our assay, each drug has a distinct neurobehavioral “fingerprint”. Ketamine, for example, elicits a dose-dependent increase in locomotion, suppression of spike rate, and decorrelation of ensemble activity ($p<0.05$, $p<0.01$, $p<0.001$ at 3, 10 and 30 mg/kg, respectively). Using unsupervised modeling, we observe that drugs follow coherent, dose-dependent trajectories though PCA space. We find that Rapastinel only slightly differentiates from vehicle, suggesting a lack of target engagement and aligning with its failure in Phase 3 trials for MDD.
Our neurobehavioral assay offers a novel approach to preclinical antidepressant profiling, identifying unique drug fingerprints that align more closely with clinical outcomes than traditional assays. This approach can enhance clinical trial predictability and reduce failure rates in antidepressant development. Our results emphasize the importance of incorporating detailed neurobehavioral investigation into preclinical research programs, paving the way for more effective and targeted MDD treatments.

Disclosures: J. Nassi: A. Employment/Salary (full or part-time):; Inscopix, Inc. **L. Bellier:** A. Employment/Salary (full or part-time):; Inscopix, Inc. **N. Ren:** A. Employment/Salary (full or part-time):; Inscopix, Inc. **S. Huang:** A. Employment/Salary (full or part-time):; Inscopix, Inc. **D. Cheng:** A. Employment/Salary (full or part-time):; Inscopix, Inc. **L. Gray:** A. Employment/Salary (full or part-time):; Inscopix, Inc. **O. Miller:** A. Employment/Salary (full or part-time):; Inscopix, Inc..

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.08/M32

Topic: G.05. Mood Disorders

Title: Characterization of the anti-anxiety and antidepressant properties of ITI-1549, a novel serotonin 5-HT2a agonist, non-hallucinogenic psychedelic, for the treatment of neuropsychiatric disorders

Authors: *V. LEHMANN, S. DUTHEIL, N. AWADALLAH, L. ZHANG, G. L. SNYDER, R. E. DAVIS;
Intra-Cellular Therapies, New York, NY

Abstract: Dysregulation of serotonergic signaling in the brain has been implicated in pathogenesis of several neuropsychiatric disorders. Psychedelic hallucinogens which interact with serotonin 5-hydroxytryptamine 2 (5-HT2) receptors are powerful psychoactive substances that alter perception and mood. The use of these agents can relieve symptoms in mood, anxiety, and other CNS disorders, and potentially restore signaling in discrete brain networks that are dysregulated. However, serotonergic psychedelics are subject to unique adverse risks that may ultimately limit their widespread use; this encompasses hallucinogen persisting perception disorder, abuse liability/dependence, harm to self/others, and heart valvulopathy (associated with 5-HT_{2B} receptor agonism). At Intra-Cellular Therapies, we designed, synthesized and selected for development multiple novel 5-HT_{2A} agonists, exemplified by ITI-1549, which is devoid of agonist effects at 5-HT_{2B} receptors and does not induce hallucinogen-like behaviors in rodent models. Male and female rodents received acute or daily administration of ITI-1549 (N=8-17 / group) and were subjected to behavioral testing for the assessment of anxiety-like behavior, locomotion, social behavior, and anhedonia. All statistical analyses were performed using GraphPad Software. In mice, we found that ITI-1549 did not elicit hallucinogen-like behaviors at less than or equal to the highest tested dose of 10 mg/kg. In contrast, ITI-1549 promoted prosocial behavior at doses of <1.0 mg/kg and decreased anxiety-like behavior with no effects on locomotion. Additionally, ITI-1549 reversed anhedonic-like behavior induced by LPS in rats, as seen in the female urine sniffing test (FUST) in males and the sucrose preference test (SPT) in both sexes. In sum, these data show that ITI-1549 is capable of attenuating anxiety and anhedonic- and depression-like behavior in male and female rodent models while not being associated with risks for adversities possessed by hallucinogenic psychedelics. To the extent that these data translate to humans, ITI-1549 may be useful for the acute and chronic treatment of certain neuropsychiatric diseases.

Disclosures: V. Lehmann: A. Employment/Salary (full or part-time):; Intra-Cellular Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intra-Cellular Therapies. **S. Dutheil:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intra-Cellular Therapies. **N. Awadallah:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intra-Cellular Therapies. **L. Zhang:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intra-Cellular Therapies. **G.L. Snyder:** A.

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Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.09/M33

Topic: G.05. Mood Disorders

Title: Discovery and biochemical characterization of ITI-1549, a novel serotonin 5-HT2a agonist, non-hallucinogenic psychedelic, for the treatment of neuropsychiatric disorders

Authors: *N. AWADALLAH, E. LEHMANN, L. ZHANG, S. DUTHEIL, W. YAO, P. LI, G. L. SNYDER, R. DAVIS;
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Abstract: Serotonergic hallucinogenic psychedelics like psilocybin, tryptamines, ibogaine, lysergic acid diethylamide, and their analogues exert their effects, at least in part, through direct agonist activity at brain 5-HT_{2A} receptors. While these agents may be useful in alleviating symptoms associated with certain neuropsychiatric disorders, and could participate in the rewiring of dysregulated discrete brain networks; their hallucinogenic properties pose unique adverse risks that ultimately limit their use. To circumvent these issues, we designed and synthesized a chemically novel series of 5-HT_{2A} receptor agonists. These novel agonists lack hallucinogenic potential and other aversities associated with psychedelic compounds but retain beneficial effects in rodent models predictive of enduring antidepressant and anxiolytic activity. Here, we describe the discovery and biochemical characterization of this class of compounds as exemplified by ITI-1549, our lead compound in this series. ITI-1549 binds with high affinity to the $5HT_{2A}$ receptor (Ki = 10.2 nM). It acts as a biased agonist activating the β-arrestin pathway, and lacks significant activation of Gq pathway, unlike the hallucinogenic psychedelics that signal at the $5HT_{2A}$ receptors through both β-arrestin and the Gq pathways. Importantly, ITI-1549 does not have agonist activity at $5HT_{2B}$ receptors, mitigating the risk of cardiac adversities. In addition, ITI-1549 does not elicit head twitch responses at across different doses and time-points, in both male and female mice, suggesting it lacks hallucinogenic potential. The beneficial effects of hallucinogenic psychedelic-like drugs may be mediated by neuroplastic changes in select brain regions biochemically associated with increased activation of the mTORC1 pathway. Twenty-four hrs after a single administration to mice, ITI-1549 causes a significant increase in expression of phosphorylated proteins in the mTOR signaling pathway (e.g., AKT, ERK, P70) in the prefrontal cortex. These data indicate that ITI-1549 can impact brain plasticity and possesses enduring effects that outlast initial drug exposure. Thus, ITI-1549 may be a safe, non-

hallucinogenic psychedelic lacking the potential to induce hallucinations and cardiac pathologies but retaining the potential as an acute or chronic treatment of mood, anxiety, and other neuropsychiatric disorders.

Disclosures: N. Awadallah: A. Employment/Salary (full or part-time):; Intra-Cellular Therapies, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intra-Cellular Therapies, Inc. **E. Lehmann:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intra-Cellular Therapies,. **L. Zhang:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intra-Cellular Therapies. **S. Dutheil:** A. Employment/Salary (full or part-time):; IntraCellular Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IntraCellular Therapies. **W. Yao:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intra-Cellular Therapies. **P. Li:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intra-Cellular Therapies. **G.L. Snyder:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intra-Cellular Therapies. **R. Davis:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intra-Cellular Therapies.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.10/M34

Topic: G.05. Mood Disorders

Title: Network pharmacology study on similar anti-depression mechanism of "Quercetin" and "Kaempferol" in Bupleurum Chinense De Candolle

Authors: *Q. WU[1], J.-X. CHEN[2], T. CAO[3], J. LI[4];
[1]Beijing Univ. of Chinese Med., Beijing, China; [2]Beijing Univ. Chinese Med., Beijing, China; [3]Johns Hopkins Univ., Baltimore, MD; [4]Johns Hopkins Med. Institutions, Beijing, China

Abstract: Clinical studies and basic science experiments have widely demonstrated the antidepressant effects of the Chinese herbal *Bupleurum Chinense* De Candolle (BR). However, the system mechanism of these effects has not been fully characterized. The present study

conducted a comprehensive network pharmacological analysis of BR and sorted all pharmacologically active components through the TCMSP webserver. Then, all potential molecular targets were predicted, of which there were 40 genes clearly related to depression. We found there are 49 common targets in "Quercetin" and "Kaempferol", which owns the most common targets among these active components. To further investigate the mechanism of antidepressant effects of BR's active components, a compound-depression targets (C-DTs) network was constructed, and Gene Ontology (GO) functional analyses were performed for the 24 common targets of "Quercetin" and "Kaempferol" and "Depression". Enrichment results revealed that BR could regulate multiple biology processes such as reactive oxygen species metabolic process, extrinsic apoptotic signaling pathway, ect. Therefore, we investigated the effects of "Quercetin" and "Kaempferol" in HT22 cell with oxidative stress model. The results suggest that "Quercetin" and "Kaempferol" alone or in combination administration have obvious protective effects on glutamate-induced oxidative stress damage of HT-22 cells, significantly reducing the content of reactive oxygen species and malondialdehyde, and increasing the content of Superoxidase Dismutase (SOD). The mechanism of action may be achieved by regulating the AKT phosphorylation signaling pathway. Overall, our research revealed the complicated antidepressant mechanism of BR, and also provided a rational strategy for revealing the complex composition and function of Chinese herb.

Disclosures: Q. Wu: A. Employment/Salary (full or part-time):; Johns Hopkins University. **J. Chen:** None. **T. Cao:** None. **J. Li:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.11/M35

Topic: G.05. Mood Disorders

Support: CIHR (FDN148374 and ENP161427 (ERA-NET ERA PerMed)).
FRQS (JC-339982)
NSERC Discovery grant DGECR-2019-00253

Title: Differential Chromatin Architecture and Risk Variants in Deep Layer Excitatory Neurons and Grey Matter Microglia Contribute to Major Depressive DisorderDifferential Chromatin Architecture and Risk Variants in Deep Layer Excitatory Neurons and Grey Matter Microglia Contribute to Major Depressive DisorderDifferential chromatin architecture and risk variants in deep layer excitatory neurons and grey matter microglia contribute to major depressive disorder.

Authors: *A. CHAWLA;
McGill Univ., Verdun, QC, Canada

Abstract: Major depressive disorder (MDD) associated genetic variants reside primarily in the non-coding, regulatory genome. Here we investigate genome-wide regulatory differences and

putative gene-regulatory effects of disease risk-variants by examining chromatin accessibility combined with single-cell gene-expression profiles in over 200,000 cells from the dorsolateral prefrontal cortex (DLPFC) of 84 individuals with MDD and neurotypical controls. MDD-associated accessibility alterations were prominent in deep-layer excitatory neurons characterized by transcription factor (TF) motif accessibility and binding of nuclear receptor (NR)4A2, an activity-dependent TF responsive to pathological stress. The same neurons were significantly enriched for MDD-associated genetic variation disrupting cis-regulatory sites and TF binding associated with genes involved in synaptic communication. Furthermore, a grey matter microglial cluster exhibited differentially closed chromatin in MDD affecting binding sites bound by TFs known to regulate immune homeostasis. In summary, our study points to specific cell types and regulatory mechanisms whereby genetic variation may increase predisposition to MDD.

Disclosures: A. Chawla: None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.12/M36

Topic: G.05. Mood Disorders

Title: Ropanicant (SUVN-911), an α4β2 nAChR Antagonist: A Phase-2 Study Evaluating the Safety and Efficacy in Participants with Moderate to Severe Major Depressive Disorder

Authors: *V. PALACHARLA, R. NIROGI, V. BENADE, J. RAVULA, S. JETTA, V. GOYAL, M. RASHEED, R. SUBRAMANIAN, S. PANDEY, P. JAYARAJAN, A. K. SHINDE; Suven Life Sci. Ltd, Hyderabad, India

Abstract: Ropanicant (SUVN-911) is a potent and selective α4β2 nAChR antagonist and has demonstrated more than 100-fold selectivity for α4β2 nAChR receptors (Caliper Life sciences selectivity panel and closely related α3β4 nAChR receptors). Ropanicant was well tolerated and safe up to the highest tested dose of 60 mg single dose and 45 mg once daily for 14 days in healthy humans. An open-label parallel group study to evaluate the safety and efficacy of ropanicant in participants with moderate to severe major depressive disorder (MDD) is currently recruiting patients (NCT06126497). The primary objective of the study is to evaluate the safety of ropanicant in participants with MDD. The secondary objectives include assessment of ropanicant treatment in reducing depressive symptoms and to evaluate pharmacokinetics. Subjects diagnosed by Mini International Neuropsychiatric Interview (MINI) meeting the DSM-5 criteria for MDD without psychotic features with current depressive episode of at least 4 weeks of duration prior to the screening, aged between 18 to 65 years (inclusive) with a Montgomery-Asberg Depression Rating Scale (MADRS) score of ≥25 and Clinical Global Impression - Severity (CGI-S) score ≥4 are considered eligible to participate in the study. Approximately 36 participants are planned to be randomized to receive ropanicant either 45 mg QD, 30 mg BID, or

45 mg BID in a ratio of 1:1:1. Following a screening period of up to 4 weeks, participants will be treated for 2 weeks with ropanicant. Safety will be measured by assessment of adverse events, physical examination, vital signs, ECG, clinical laboratory tests, and suicidal ideation/behavior evaluation by Columbia Suicidal Severity Rating Scale (C-SSRS). The efficacy assessments will include change from baseline to Day 14 in MADRS and CGI-S. Pharmacokinetics will be evaluated on day 1 and day 14 in subjects receiving BID dosing. Recruitment for the study is ongoing and results from the study are expected by Q3 2024. The results will be presented at the Society for Neuroscience conference. This open-label study will be a preface to a future double-blind placebo controlled study of ropanicant in participants with MDD.

Disclosures: V. Palacharla: A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Benade:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **J. Ravula:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **S. Jetta:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Goyal:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **M. Rasheed:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Subramanian:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **S. Pandey:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **P. Jayarajan:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **A.K. Shinde:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.13/M37

Topic: G.05. Mood Disorders

Title: Gender-based differences in gene expression of cannabinoid receptors in lymphocytes of depressive suicide attempters: a potential preventive biomarker for suicide?

Authors: *M. GARCÍA-GUTIÉRREZ[1], A. BAILEN TORREGROSA[2], M. CARRICAJO[3], O. BROTONS[3], J. MANZANARES[1];
[1]Inst. de Neurociencias, Univ. Miguel Hernandez, San Juan De Alicante, Spain; [2]Inst. de Neurociencias, Univ. Miguel Hernandez, Alicante, Spain; [3]Hosp. Gen. Universitario de Elda, Elda, Spain

Abstract: It is now evident that gender differences exist in suicidal behavior. A main gender difference is that women have more suicide attempts than men. Suffering from a psychiatric disease increases the risk of suicide, among which major depression (MDD) stands out. Despite these devastating data, the biological bases of depression and suicide remain incompletely understood, especially sex influence. Several lines of evidence support an association between the endocannabinoid system, depression and suicide. Postmortem studies found alterations in the

cannabinoid receptors 1 (CB1r) and 2 (CB2) gene expression in brain regions closely related to impulsivity and emotional reactivity of male suicide completers. Here, we aimed to further explore the potential utility of CB1r and CB2r as peripheral biomarkers for preventing suicide considering sex. Briefly, women and men newly hospitalized for suicide attempts have been recruited from the Psychiatric Service of General University Hospital of Elda (Alicante, Spain). The diagnosis of MDD has been made according to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-V criteria. Depressive symptoms were rated using the Beck Depression Inventory. Suicidality was assessed using the Suicide Assessment Scale. The gene expression of CB1r and CB2r were analyzed in lymphocytes of MDD suicide attempters (S) (13 women and 8 men) and controls (C) (12 women and 8 men, without psychiatric clinical history) by real-time PCR (qPCR). All samples were matched as much as possible in age (C: men 56±10.3 years, women 55.3±7.8 years); (S: men 51.6±6 years, women 45.8±14.6 years). Statistical analyses were performed using Student's t-test to compare two groups (SigmaPlot software, Systat Software Inc., Chicago, IL, USA). The results revealed a significant increase of CB1r (Student's t-test; t= -3.817, $p<0.001$, 23 df) and CB2r (Student's t-test; t= -3.128, p=0.005, 23 df) in lymphocytes of women depressive suicide attempters. In contrast, CB2r (Student's t-test; t= -5.457, $p<0.001$, 14 df) increased whereas CB1r (Student's t-test; t= 2,829, p=0.013, 14 df) was reduced in lymphocytes of men depressive suicide attempters. These preliminary results show gender-dependent alterations in CB1r and CB2r in the lymphocytes of depressive suicide attempters. Complementary large-scale studies are necessary to examine if these targets could serve as biomarkers for preventing suicide in a gender-differentiated manner.

Disclosures: M. García-Gutiérrez: None. **A. Bailen Torregrosa:** None. **M. Carricajo:** None. **O. Brotons:** None. **J. Manzanares:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.14/M38

Topic: G.05. Mood Disorders

Support: CONAHCYT GRANT CF-2023-G-112

Title: Relationship between inflammatory markers in human olfactory neural progenitor cells and antidepressant response

Authors: *M. FLORES-RAMOS[1], G. B. RAMIREZ-RODRIGUEZ[2];
[1]Clin. research, Inst. Nacional de Psiquiatría Ramón de la Fuente, Ciudad de México, Mexico;
[2]Natl. Inst. of Psychiatry Lab. of Neurogenesis, Mexico, D.F., Mexico

Abstract: Response to antidepressants is related to hippocampal neurogenesis integrity, a process mediated by neurotrophins, such as Brain Derived Neurotrophic Factor (BDNF). In turn, pro-inflammatory state appears to reduce neurogenesis, and has been associated with refractory

depressive states. We propose to analyze the human neural progenitor cells derived from the olfactory epithelium (HNPCs-OE) as an indicator of neurogenesis in humans. Therefore, we compared the number and content of HNPCs-OE in depressed patients taking antidepressants, according to response to treatment. Twenty depressed patients were followed during eight weeks after antidepressant treatment was prescribed. At the end evaluation they were divided in two groups according to Hamilton depression rating scale (HDRS) scores: responders and non-responders. We compared the number and components of HNPCs-OE between groups and observed an elevation of interleukine-8 in those patients who do not achieve response to treatment, BDNF levels were no related to antidepressant response.

Disclosures: M. Flores-Ramos: 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.; CONAHCYT GRANT CF-2023-G-112. **G.B. Ramirez-Rodriguez:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.15/M39

Topic: G.05. Mood Disorders

Support: Jaswa Innovator Grant to Laramie Duncan

Title: Genome-wide, brain-wide investigation of cell types in mood disorders and suicide

Authors: *M. SALEM, T. LI, N. SHAHVERDI, C. STAFFORD, W. J. GIARDINO, L. E. DUNCAN;
Psychiatry and Behavioral Sci., Stanford Univ., Palo Alto, CA

Abstract: Mood disorders including major depressive disorder (MDD) and bipolar disorder are leading causes of disease burden worldwide. Mood disorders are also strongly linked to risk of death by suicide. Due to challenges posed by their heterogeneous, polygenic, and environmentally-influenced nature, the etiologies of mood disorders remain poorly understood. In recent years, human genome-wide association studies (GWAS) have linked hundreds of genetic loci to mood disorder phenotypes; the current challenge is to uncover the biological mechanisms implicated by these loci. Here, we present a robust statistical approach that combines landmark GWAS and single-nucleus RNA sequencing (snRNAseq) gene expression datasets. Specifically, MAGMA v1.10 software was used to conduct gene property analysis on well-powered GWAS summary statistics for MDD (*n*=807,553; loci=102), bipolar disorder (*n*=413,466; loci=64), and suicide (*n*=815,178; loci=4) to identify significant associations with cell types as defined by the most comprehensive human brain snRNAseq dataset available to date (461 cell types, derived from 105 brain regions and 3,369,219 individual nuclei), published in 2023 by Siletti et al. Using this methodology, we mapped polygenic risk for MDD, bipolar

disorder, and suicide to specific human brain cell types and their anatomical locations. Results demonstrated distinct cellular profiles for the three phenotypes that not only identified cell types and brain areas concordant with prior findings, but also identified novel cell type associations such as inhibitory neurons in the superior and inferior colliculus for MDD (p=6.14x10^{-5}), excitatory neurons in the retrosplenial cortex for bipolar disorder (p=7.39x10^{-13}), and eccentric medium spiny neurons in the amygdala for suicide (p=1.35x10^{-5}). Further analyses conducted on cell type gene expression and gene specificity data with NeuronChat and WEBGESTALT drug over-representation analysis predicted complex molecular interactions between implicated cell types and suggested novel drug repurposing and development opportunities. In particular, genes with high relative expression in eccentric medium spiny neurons implicated in suicide were found to be targets of sympatholytics, atypical antipsychotics, and dopamine agonists after false discovery rate correction (FDR<0.05). This exploratory data-driven project is "unbiased" with respect to researcher-driven hypotheses about which cell types should be associated with mood disorders and reveals novel associations and a greater degree of cellular and molecular specificity regarding mood disorder and suicide etiology than ever previously available.

Disclosures: M. Salem: None. **T. Li:** None. **N. Shahverdi:** None. **C. Stafford:** None. **W.J. Giardino:** None. **L.E. Duncan:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.16/M40

Topic: G.05. Mood Disorders

Support: This research was supported by the Canadian Anesthesia Research Foundation (CTAB).
CTAB is supported by the Ministry of Health Clinician Investigator Program Salary Support Grant.
BAO is supported by a CIHR Foundation Grant.

Title: Modulation of $GABA_A$ receptors in the antidepressant effect of nitrous oxide

Authors: *C. T. A. BRENNA[1], D.-S. WANG[2], M. YU[2], L. KAUSTOV[3], B. A. ORSER[1]; [1]Dept. of Anesthesiol. & Pain Med., [2]Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada; [3]Dept. of Anesthesia, Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada

Abstract: Depression is among the most common illnesses worldwide, and current therapies are wanting. Recent trial data support nitrous oxide (N_2O) as a rapid, effective, and well-tolerated antidepressant. However, mechanisms underlying this effect remain poorly understood. Growing evidence implicates dysregulation of extrasynaptic α5 subunit-containing $GABA_A$ receptors (α5$GABA_A$Rs) in depression. We have previously shown that multiple common anesthetic drugs trigger long-lasting changes in α5$GABA_A$R function. Thus, we hypothesized that alterations to

the expression and function of α5$GABA_A$Rs in hippocampal neurons mediate N_2O's antidepressant properties. Primary murine hippocampal neurons and neuron-astrocyte co-cultures were exposed to either air (control) or 70% N_2O for 1 h. After 24 h, current evoked by low (1 μM) and saturating (1 mM) GABA concentrations were recorded using whole-cell patch clamp methods. Additionally, cultured hippocampal neurons underwent immunofluorescent staining 24 h post-treatment to measure α5 subunit cell-surface expression (relative to MAP2). Investigators were blinded to treatment groups, and data are expressed as mean ± standard deviation. Current amplitude was similar in control- and N_2O-treated primary neurons treated with 1 μM GABA (239.2 pA ± 112.3 pA vs. 279.4 pA ± 235.5 pA, n = 17 cells per group) and 1 mM GABA (4.75 nA ± 2.61 nA vs. 5.39 nA ± 3.24 nA, n = 12 cells per group). Similarly, immunostaining of primary hippocampal cultures revealed no differences in α5 subunit cell-surface expression 24 h after N_2O treatment (105.4% ± 37.2% fluorescence intensity of control, n = 147 regions of interest per group). Pilot data from co-culture experiments reveal current amplitudes evoked by 1 μM GABA in control- and N_2O-treated cells of 110.2 pA ± 40.6 pA vs. 89.0 pA ± 47.9 pA, n = 8-9 cells per group, respectively. Thus, a brief treatment with 70% N_2O does not alter $GABA_A$ receptor-mediated currents or α5$GABA_A$R cell-surface expression in primary hippocampal neurons, 24 h after treatment. Ongoing work will explore the role of astrocyte-neuron crosstalk and alternative drug concentrations and timepoints as well as N_2O's effect on mouse behaviour, to determine whether $GABA_A$ receptors contribute to N_2O's antidepressant properties. As N_2O's antidepressant effect appears to be mediated by mechanisms other than those of traditional monoaminergic antidepressants, this work aims to understand a novel mechanism to treat depression and thereby support future drug development.

Disclosures: C.T.A. Brenna: A. Employment/Salary (full or part-time):; CTAB receives salary support from the Ontario Ministry of Health and Long-Term Care (MOHLTC) Clinician Investigator Program Salary Support Grant (CIP-MOH), and a Vanier Canada Graduate Scholarship., This research was supported by the Canadian Anesthesia Research Foundation (CTAB).. **D. Wang:** None. **M. Yu:** None. **L. Kaustov:** None. **B.A. Orser:** A. Employment/Salary (full or part-time):; BAO is supported by a CIHR Foundation Grant.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); BAO collaborates on clinical studies that are supported by in-kind software donations from Cogstate Ltd. (New Haven, CT, USA).. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BAO is a named inventor on a Canadian patent (2,852,978) and two U.S. patents (9,517,265 and 10,981,954).. Other; BAO serves on the Board of Trustees of the International Anesthesia Research Society (San Francisco, CA, USA) and is co-director of the Perioperative Brain Health Centre (Toronto, Ontario, Canada).

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.17/N1

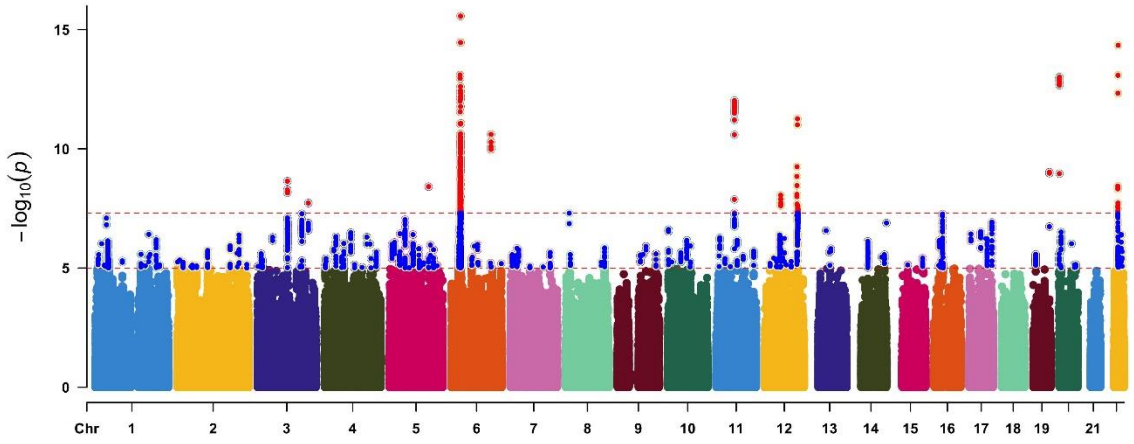
Topic: G.05. Mood Disorders

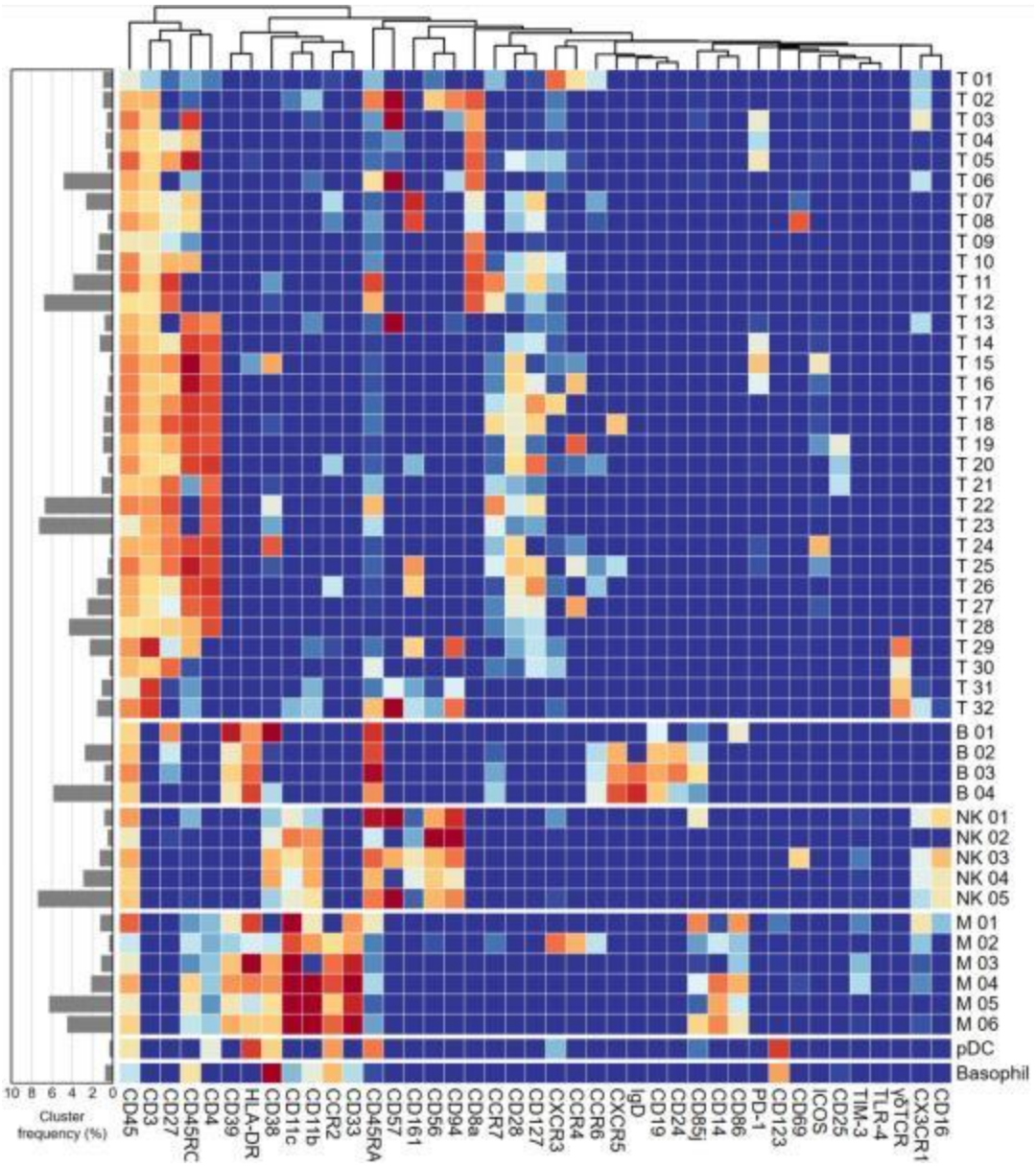
Support: NSFC82330042

Title: A GWAS study based on the Han Chinese population identified immune-inflammatory related loci in individuals with bipolar disorder.

Authors: *X. FENG;
Peking Univ. Sixth Hosp., Beijing, China

Abstract: Bipolar disorder is a severe psychiatric disorder with a heritability of 60%-90%. Previous large cohort GWAS studies have primarily focused on samples from European and American populations, with fewer studies targeting Asian populations. Earlier GWAS research using independent samples from the Han Chinese population identified bipolar disorder susceptibility loci specific to the Chinese population. This study conducted a GWAS analysis using another independent cohort of 1006 Han Chinese individuals with bipolar disorder and 2857 healthy controls, identifying 10 loci with genome-wide significance potentially associated with the risk of developing bipolar disorder, including rs60331558, rs74567609, rs28392962, rs117124314, rs800613, and rs12244112. Annotation of these loci revealed associated risk genes such as IL9RP4, PTER, SLC39A11, ANKRD22, ZNF385D, and KLF61. These SNPs and related genes are implicated to varying degrees in the processes of immunity and inflammation. Furthermore, an analysis of immune-inflammatory cell subgroups and markers in the peripheral blood of these patients revealed correlations with specific immune cell subgroups and multiple markers in bipolar disorder patients, showing differences in immune-inflammatory factors compared to both depressive patients and healthy controls.





Disclosures: X. Feng: None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.18/N2

Topic: G.05. Mood Disorders

Title: Preclinical mechanisms of lithium resistance: a rapid review

Authors: J.-M. LACROIX[1], M.-C. DURPES[2], ***S. GRIGNON**[3];
[1]Psychiatry, [2]Psychiatry & Pharmacology/physiology, Sherbrooke Univ., Sherbrooke, QC, Canada; [3]Psychiatry & Pharmacology/physiology, Univ. De Sherbrooke, Sherbrooke, QC, Canada

Abstract: Lithium (Li) is a first line treatment of bipolar affective disorder, and also presents antisuicide and neuroprotective properties. It is generally recognized, however, that partial or full resistance to the drug affects 50-70 % of patients. Given the pleiotropic effects of lithium, the mechanisms underlying response and resistance have been notoriously difficult to pinpoint. While the responder (LiR)/non responder (LiNR) dichotomy has become more regularly used in clinical studies, mechanistic studies seldom address Li resistance. The present review aims at the recension of preclinical, mechanistic studies explicitly reporting resistance to Li, with the purpose of generating research hypotheses amenable to preclinical models. Methods. Keywords (biologically relevant domains) were derived from a recent, neurobiologically oriented review (Bortolozzi et al Pharmacol Rev 76:323-357, May 2024). Pubmed query: lithium AND resistan* AND (keywords) AND (bipolar OR mood OR depression OR mania). Independent assessment by the two authors of query results (title, abstract or main text) for relevance to the research question, ie mechanisms addressing Li resistance or non-response. PRISMA criteria for documenting paper selection were used. Key findings. GSK3β, WNT, β-catenin pathway. Superoxide/H2O2 imbalance influences Li effects on GSK3β levels. An iNOS/src/FAK axis is necessary for Li induced macrophage mobility. MED10 and MSI2 are necessary to Li induced SH-SY5Y differentiation. Neurotrophins. BDNF SNPs or exon IV structural variants do not influence Li response. Apoptosis. Gene expression of pro- and anti-apoptotic pathways may differentiate LiR and LiNR, with upregulation of bcl-2 family in LiR. cAMP/CREB. Increasing cAMP levels enhances lithium response. Inflammation. TNFalpha levels are associated with lithium response (higher levels in LiNR). GWAS, exome sequencing and protein expression. Association and gene expression point to biologically plausible pathways (eg PI3K/AKT, LEF1, immune response, intercellular communication, Glu/GABA and Ach neurotransmission, cell adhesion, stress response, apoptosis). Of note, polygenic risk scores for schizophrenia and major depression are associated with worsening Li response. Discussion. Preclinical mechanisms of Li resistance appear to be relatively understudied or underreported. Available data across methods and models point to a potential, significant role for the different pathways indexed here, and notably inflammation and redox status, in Li response.

Disclosures: J. Lacroix: None. **M. Durpes:** None. **S. Grignon:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

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Topic: G.05. Mood Disorders

Support: PID2020-118582RB-I00 (MCIN/AEI/10.13039/501100011033) to MJG-F
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"FPI_022_2022" predoctoral scholarship (CAIB) to JJ-P
Travel Award SENC to JJ-P

Title: Examining ketamine's impact on adolescent female rats: antidepressant-like effects and safety profile

Authors: J. JORNET-PLAZA, *J. GARCIA-FUSTER;
IUNICS, Univ. of the Balearic Islands and IdISBa, Palma, Spain

Abstract: Currently, ketamine is approved for treatment-resistant depression in adults, but its efficacy and safety profile in adolescents has not been completely validated. Our prior studies focused on evaluating the antidepressant-like efficacy of a dose of 5 mg/kg in adolescent rats of both sexes, observing a lack of response in adolescent females. In this context, the present study aimed at evaluating different doses of ketamine in adolescent female rats, as well as its long-term safety profile. Adolescent female Sprague-Dawley rats were treated (i.p.) with ketamine (n=34) (1, 5 or 10 mg/kg) or vehicle (n=18) for 7 consecutive days (postnatal day, P33-39). While acute (30 min post first injection, P33) effects were measured under the stress of the forced-swim test (FST), repeated antidepressant-like responses were evaluated both in the FST (P40) and novelty suppressed feeding test (NSF, P43). Later on, rats were left undisturbed until adulthood when they were scored for long-term safety through the Barnes maze (i.e., cognitive performance) and the conditioned-place preference (i.e., reinforcing properties of a 10 mg/kg dose of ketamine). The results were analyzed using one-way ANOVAs (treatment as the independent variable), with Dunnett's for post-hoc analysis. Ketamine induced an acute antidepressant-like effect, but only with the 10 mg/kg dose (i.e., decreased immobility, ***p<0.001, paired with increased climbing, **p=0.005 vs. vehicle-treated rats). Moreover, ketamine showed an antidepressant-like effect for all doses tested after the repeated paradigm when compared to vehicle-treated rats: decreased immobility and increased climbing in the FST for doses 1 (*p=0.04) and 10 mg/kg (p=0.02); decreased latency to center (*p=0.049) and increased feeding time in the NSF for dose 5 mg/kg (***p=0.001). The adolescent treatment did not induce long-term changes in adulthood, both in cognitive performance and in the expected reinforcing effects induced by ketamine. The observed antidepressant-like response of ketamine in adolescent female rats were dose-dependent, since acute significance was only observed with the highest dose tested (10 mg/kg), while all three doses (1, 5 and 10 mg/kg) showed efficacy following a repeated treatment paradigm. Interestingly, drug treatment during adolescence did not induce long-term changes in adulthood in cognition or reward-mediated responses, suggesting a good safety profile. Future studies should further characterize the beneficial vs. potentially harmful effects following ketamine administration in adolescence.

Disclosures: J. Jornet-Plaza: None. **J. Garcia-Fuster:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.20/N4

Topic: G.05. Mood Disorders

Title: Antidepressant Potential Versus Neurotoxicity of Ketamine

Authors: *K. BERA[1,2,3], M. HOSSAIN[4,3], P. FENTON[4,3], Z. BLUMENFELD[5], A. ANDREEV[5], B. N. COHEN[5], D. PROBER[5], H. A. LESTER[5], L. L. LOOGER[6,7];
[1]UC San Diego Sch. of Med., San Diego, CA; [2]California Institute of Technology, Pasadena, CA; [3]Howard Hughes Medical Institute, University of California, San Diego, CA; [4]Univ. of California, San Diego, CA; [5]Caltech, Pasadena, CA; [6]Howard Hughes Med. Inst., Univ. of California, San Diego, CA; [7]University of California, San Diego, CA

Abstract: Ketamine (Ket) presents something of a paradox for pharmacology, as it exhibits rapid and robust antidepressant activity (thus it is a RAAD, rapidly acting antidepressant drug) but has potent neurotoxic potential. However, it is not yet clear precisely which molecular mechanisms produce these beneficial and deleterious effects; thus, dosing is mostly a trial-and-error affair. Here we carefully dig into the therapeutic and toxic effects of Ket and its metabolites, shedding light on the mechanisms, cell types, and timescales at issue. The first goal of our research program was to develop genetically encoded biosensors to follow the import, trafficking, and metabolism of Ket and its metabolites. We developed a family of "intensity-based RAAD-Sensing Fluorescent Reporter" [iRAADSnFR] indicators. In solution, iS-KetSnFR responds to S-Ket with an EC_{50} ~ 150 nM with maximal fluorescence increase ($\Delta F_{max}/F_0$) ~ 3.2; iS-HNKSnFR responds to (2S, 6S)-HNK with an EC_{50} ~ 110 nM with $\Delta F_{max}/F_0$ ~ 3.6; iR-KetSnFR senses R-Ket with EC_{50} ~ 130 nM and $\Delta F_{max}/F_0$ ~ 4.0; iR-HNKSnFR responds to (2R, 6R)-HNK with EC_{50} ~ 500 nM and $\Delta F_{max}/F_0$ ~ 4.0; iD-HNKSnFR responds to dehydronorketamine (DHNK) with EC_{50} ~ 160 nM and $\Delta F_{max}/F_0$ ~ 4.0. These sensors will be useful for the study of Ket, its metabolites, and related drugs in a variety of experimental settings. We have previously shown that many drugs unexpectedly act, at least in part, at intracellular targets in various organelles. We wished to assess the potential of Ket and its metabolites to traffic to, and act on, such intracellular targets. Thus we incorporated diverse organellar targeting peptides to the iRAADSnFR family and expressed them in cells. Fluorescence microscopy shows that, at the nM concentrations associated with antidepressant activity, RAADs traffic into diverse organelles within seconds, equilibrating with extracellular levels. We next sought to express the iRAADSnFRs *in vivo* in mouse, fish, and worm - the former with adeno-associated virus (AAV) transduction, the latter two with transgenesis. We have expressed the sensors in diverse locations in the central and peripheral nervous systems of the animals and subjected the animals to diverse drug regimens. We have also performed complementary experiments using proteomics, immunohistochemistry, biochemistry, and behavior tracking to profile neurotoxicity of Ket, its metabolites, and related drugs. Together, this research program will help us unravel the complicated mechanisms of action of Ket in diverse settings, and to design and synthesize next-generation ketamine analogs with preserved efficacy but minimized side effects.

Disclosures: K. Bera: None. **M. Hossain:** None. **P. Fenton:** None. **Z. Blumenfeld:** None. **A. Andreev:** None. **B.N. Cohen:** None. **D. Prober:** None. **H.A. Lester:** None. **L.L. Looger:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.21/

Topic: G.05. Mood Disorders

Title: Intravenous ketamine on explicit and implicit suicidal cognition in individuals with treatment-resistant depression and bipolar depression

Authors: *I. DOREA BANDEIRA;
Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA

Abstract: Background: Prior clinical trials have demonstrated the rapid and sustained efficacy of ketamine in improving suicidal ideation among individuals experiencing depression with suicidal thoughts. Notably, the effects were evident as early as 24 hours post-administration and endured for up to one week. Research has emphasized the positive impact of ketamine on both explicit and implicit suicidal cognition. **Methods:** Employing an open-label design, subjects underwent a single intravenous ketamine dose at 0.5mg/kg over 40 minutes. Inclusion criteria comprised a major depressive disorder or bipolar disorder type II in a major depressive episode, lasting at least eight weeks, with a history of at least one antidepressant failure in the current episode, and suicidal behavior indicated by a Beck Scale for Suicidal Ideation (BSSI) score ≥ 6. Explicit and implicit suicidal cognition assessments were conducted at baseline and 48 hours post-infusion using the BSSI and Implicit Association Test (IAT), respectively. The IAT included two variants measuring associations with "Death" and "Me" (IAT-Death) and "Escape" and "Me" (IAT-Escape). Linear mixed models were applied for statistical analysis. **Results:** Of the 36 infused subjects, 72.2% were female. Significant reductions were observed in the mean baseline BSSI total score (15.14 to 7.56, $p<0.0001$). However, no significant changes were noted in implicit suicidality measured by IAT-Death ($p=0.97$) and IAT-Escape ($p=0.53$). **Conclusions:** Preliminary findings indicate a rapid and explicit anti-suicidal effect of a single ketamine infusion, contrasting with previous data on implicit suicidal cognition. Further research is warranted to comprehensively understand ketamine's impact on distinct facets of suicidal cognition.

Disclosures: I. Dorea Bandeira: None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.22/N5

Topic: G.05. Mood Disorders

Support: JPB Foundation; the Picower Institute for Learning and Memory; George J. Elbaum (MIT ’59, SM ’63, PhD ’67), Mimi Jensen, Diane B. Greene (MIT, SM ’78), Mendel Rosenblum, Bill Swanson, annual donors to the Anesthesia Initiative Fund

Title: Influence of electroconvulsive therapy (ECT) on spectral characteristics of anesthetic-induced unconsciousness in humans

Authors: M. S. BREAULT[1], *G. KANG[2], S. ORGUC[3], B. TSENG[2], R. J. HORVATH[4], E. N. BROWN[5];
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Abstract: Electroconvulsive therapy (ECT) is a treatment for various mental disorders in humans, including treatment-resistant depression (TRD), bipolar disorder, schizophrenia, and psychosis. During the therapy, an electrical current is applied to the patient’s scalp to induce a seizure. Patients are always put under general anesthesia before and after the electrical current is applied. This creates a safe and controllable environment for the procedure as well as minimizes patient discomfort and suppresses post-treatment seizures. Anesthetics produce changes in a patient's behavioral state by disrupting brain activity. Each anesthetic has a unique mechanism, which culminates into distinct electroencephalogram (EEG) activity. The spectral features of the EEG for each anesthetic are well-known and reflect the depth of the anesthetic state of the patient. However, the mechanism by which anesthetics act on the brain may be disrupted by ECT. Furthermore, the anesthetic state pre- and post-ECT may contribute to varied clinical outcomes. In this exploratory data analysis, we investigate the impact of ECT on anesthesia state based on EEG we collected from patients with TRD undergoing ECT. First, we analyzed the anesthetic state pre-ECT and found that patients were not in the same state when ECT was applied. Second, we analyzed the anesthetic state post-ECT and found that postictal suppression initially obscured any effect anesthesia had for the majority of patients. The spectral features of anesthesia were observable later before emergence. This observation suggests there are delayed effects caused by ECT before presumably normal brain function is restored, marked by the appearance of anesthetic spectral features. Patients were not in the same anesthetic state post-ECT. We also observed that some patients emerged faster than other patients, with EEG reflecting that. Since treatments were not uniformly applied to subjects in the same anesthetic state, this could explain why some patients emerge faster than others. Our work should emphasize the importance of EEG pre- and post-ECT as well as the impact anesthetics could potentially have on patient outcomes. Our results may influence how future ECT may be applied to optimize patient outcomes to ensure patients receive consistent treatments.

Disclosures: M.S. Breault: None. **G. Kang:** None. **S. Orguc:** None. **B. Tseng:** None. **R.J. Horvath:** None. **E.N. Brown:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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Topic: G.05. Mood Disorders

Support: JPB Foundation; the Picower Institute for Learning and Memory; George J. Elbaum (MIT ’59, SM ’63, PhD ’67), Mimi Jensen, Diane B. Greene (MIT, SM ’78), Mendel Rosenblum, Bill Swanson, annual donors to the Anesthesia Initiative Fund

Title: Exploring the Influence of Demographics and Treatment Parameters on Postictal Suppression Dynamics in Electroconvulsive Therapy

Authors: *S. ORGUC[1], M. S. BREAULT[2], G. KANG[3], B. TSENG[3], M. AREGAWI[3], R. J. HORVATH[4], E. N. BROWN[5];
[1]MIT, Boston, MA; [2]The Picower Inst. for Learning and Memory, MIT, Brookline, MA; [3]MIT, Cambridge, MA; [4]Dept. of Anesthesia, Critical Care, and Pain Med., Massachusetts Gen. Hosp. / Harvard Med. Sch., Lexington, MA; [5]Brain and Cognitive Sci., MIT/Mass Gen. Hospital/Harvard Med. Sch., Cambridge, MA

Abstract: Electroconvulsive therapy (ECT) is one of the most widely used therapies for various mental disorders such as treatment-resistant depression, psychosis, schizophrenia, and so on since the 1930s. During a typical ECT treatment, the patient is administered a brief electrical shock either bilaterally or unilaterally to the temporal region. The shock has two phases: 1) the convulsive phase where the seizure is induced, and 2) the postictal phase where the EEG shows electrical suppression. The relationship between EEG changes and the response to ECT has been debated since the 1940s. According to several studies, researchers found a significant association between postictal suppression and the therapeutic efficacy of ECT. Furthermore, prior art suggests that particular features of the ictal/postictal phases may relate to subject demographics and treatment parameters. In this exploratory data analysis, we investigated the postictal phase of the ECT to see whether patient information and other ECT treatment parameters can predict its dynamic. First, we analyzed the postictal suppression phase for different patients in the time and frequency domain. Second, we used features such as demographic patient information (sex, height, weight, age), the type and amount of anesthetic used before the shock, and other treatment parameters (session number, electrode configuration, ECT pulse parameters, the duration of the seizure) to train a regression model that predicts the postictal suppression dynamics for different patients. Our observations suggest that while the ECT parameters and the choice of the anesthetic drug provide insights into the postictal suppression dynamics, we did not find a significant correlation with the demographic information. Our next step would be to investigate the relationship between the dynamics of the postictal suppression phase and the clinical outcomes of the treatment (including the therapeutic benefits and cognitive side effects) using the features presented in this study, then establish their correlations with specific patient features and information.

Disclosures: S. Orguc: None. **M.S. Breault:** None. **G. Kang:** None. **B. Tseng:** None. **M. Aregawi:** None. **R.J. Horvath:** None. **E.N. Brown:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.24/N7

Topic: G.04. Emotion

Support: Hope for Depression Research Foundation
NIMH R01 MH068542
K08MH122893
T32MH015144

Title: Dentate gyrus granule neuron plasticity evoked by electroconvulsive shock: functional and transcriptomic effects

Authors: *A. SANTIAGO[1], P. NGUYEN[2], V. LUNA[3], H. CHUNG[4], R. HEN[5], W.-L. CHANG[6];
[1]Columbia Univ., New York, NY; [2]Psychiatry, Columbia Univ., New York, NY; [3]Temple Univ., Philadelphia, PA; [4]Columbia Univ. and New York State Psychiatric Inst., New York, NY; [5]Columbia Univ., New York State Psychiatric Inst., New York, NY; [6]Columbia Univ. and NYSPI, New York, NY

Abstract: Electroconvulsive shock (ECS) is particularly effective for the improvement and even remission of treatment-resistant depression. Like other more common forms of antidepressant treatment such as fluoxetine, ECS has been shown to increase neurogenesis in the hippocampal dentate gyrus of rodent models. Yet the question of how ECS-induced neurogenesis supports improvement of depressive symptoms remains unknown. We first used x-ray ablation of neurogenesis in the dentate gyrus (DG) to show that ECS-induced neurogenesis is necessary to improve depressive-like behavior of mice exposed to chronic corticosterone (Cort). We then asked how activity from adult-born immature granule cells (iGCs) affects mature granule cell activity. Using slice electrophysiology, we show that optogenetic stimulation of adult-born neurons produces a greater hyperpolarization in mature granule neurons after ECS vs Sham treatment. Bath application of an mGluR2 antagonist blocks this hyperpolarization, indicating that direct iGC inhibition of mature granule neurons requires the activation of metabotropic glutamate receptor 2 (mGluR2). Consistent with this finding, we observe reduced expression of the immediate early gene cFos in the granule cell layer of ECS vs Sham subjects. We then asked how reduced mGluR2 expression may effect ECS-induced stress resilience. We knocked down mGluR2 expression specifically in ventral granule neurons and found that the antidepressant-like behavioral effects of ECS were blunted. Using single nucleus RNA sequencing, we reveal major transcriptomic shifts in granule neurons after treatment with ECS+Cort or fluoxetine+Cort vs Cort alone. We identify a population of immature cells which has greater representation in both ECS+Cort and fluoxetine+Cort treated samples vs Cort alone. We also find global differences in ECS- vs fluoxetine-induced transcriptomic shifts. Together, these findings highlight a critical role for immature granule cells and mGluR2 signaling in the antidepressant action of ECS.

Disclosures: A. Santiago: None. **P. Nguyen:** None. **V. Luna:** None. **H. Chung:** None. **R. Hen:** None. **W. Chang:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.26/N8

Topic: G.05. Mood Disorders

Support: NIH Grant R01DA043461

Title: Sex differences and ketamine in social isolation-induced cognitive impairment

Authors: *S. SALAND, I. YATES, M. KABBAJ;
Biomed. Sci., Florida State Univ., Tallahassee, FL

Abstract: Cognitive dysfunction is a prominent and enduring feature of depression. With equivocal effectiveness of traditional antidepressant treatments on cognitive impairment, these deficits often persist independently of other mood symptoms. More recent evidence suggests that a single subanesthetic dose of the rapid-acting antidepressant ketamine may improve some aspects of cognitive functioning in depressed patients. While promising, repeated treatments are often needed to sustain ketamine's antidepressant efficacy, and cognitive outcomes under such regimens are poorly understood. Importantly, though ketamine is generally effective in treating depressive symptoms in both sexes, sex as a biological variable has been largely neglected in studies of ketamine's efficacy within cognitive symptom domains. Accordingly, the present work examined cognitive and reward-related behavioral outcomes following social isolation stress in male and female rats, and the effects of acute and repeated low-dose ketamine on isolation-induced cognitive dysfunction. Following six weeks of post-weaning isolation, hedonic behavior was examined in sucrose preference and progressive ratio tests, followed by cognitive assessment in the attentional set-shifting task (ASST). Despite negligible effects of isolation on hedonic liking, robust motivational reward deficits were apparent in both isolated male and female rats. Interestingly, isolation impaired cognitive flexibility in a sex-dependent manner—whereas males displayed reversal learning deficits, attentional set-shifting performance was significantly impaired in isolated females. To assess ketamine's effects on isolation-induced cognitive dysfunction, ASST performance was next evaluated 24h after the 1st (acute) and 6th (repeated) intermittent injections of either saline or ketamine (10 mg/kg, *i.p.*). Findings suggest deficit-selective improvements in behavioral flexibility in both isolated males and females following acute ketamine treatment, with no effect in pair-housed controls. However, repeated administration may sex-dependently impair cognitive performance in social isolates and pair-housed animals. These effects were accompanied by sensitization to the locomotor-activating effects of ketamine selectively in female rats, suggesting a sex-dependent abuse liability under this treatment protocol. Taken together, these findings suggest ketamine's effects on cognitive flexibility are both sex- and stress-dependent. Additional work is needed to delineate the circuit-

based mechanisms contributing to ketamine's sex-dependent cognitive effects following chronic stress.

Disclosures: S. Saland: None. **I. Yates:** None. **M. Kabbaj:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.27/N9

Topic: G.05. Mood Disorders

Support: Stanley Family Foundation

Title: Targeting GSK3 for treating bipolar disorder and schizophrenia

Authors: *L. YANG[1,2], M. WEIWER[1], J. Q. PAN[3];
[1]Broad Inst., Cambridge, MA; [2]Stanley Center for Psychiatric Research, Broad Institute, Cambridge, MA; [3]Stanley Ctr. for Psychiatric Res., Broad Inst., Cambridge, MA

Abstract: Bipolar disorder (BPD) is a debilitating chronic psychiatric disease. Unfortunately, the standard of care (lithium and valproate) have narrow therapeutic index that requires close monitoring, and are associated with various side effects, limiting their use. One of the major substrates of lithium is glycogen synthase kinase 3 (GSK3). Even though itself is not implicated in human genetics for the disease etiology, many of the risk genes, such as ANK3 and AKT3, regulate its activity, therefore it is potentially a promising target for bipolar disorder therapeutics. GSK3 exists as two highly homologous paralogs, GSK3a and GSK3b. Despite numerous efforts over the past 3 decades, existing GSK3 inhibitors lack the required kinome-, and paralog-selectivity, or the desired pharmacokinetic properties for a safe translation to clinical application. Taking advantage of insights in structural biology, we report the discovery of GSK3 inhibitors with improved paralog-selectivity and brain permeability, ready for *in vivo* testing on animal models.
Preliminary data using a psychosis/mania animal model (amphetamine-induced hyperlocomotion, AIH) indicates our leading new compound (BRD7355, dual inhibitor) is potent, has wide efficacy range and large effect size, compared to our earlier compounds and the best-in-class commercial inhibitor PF-367.
We plan to test our leading and paralog-selective compounds in disease-relevant genetic animal models to gauge their effectiveness across various deficits.

Disclosures: L. Yang: None. **M. Weiwer:** None. **J.Q. Pan:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.28/N10

Topic: G.05. Mood Disorders

Support: NIH Grant R01MH124992

Title: Type 8 Adenylate Cyclase Regulates Bipolar Disorder-associated Behavior in Mice through Glycogen Synthase Kinase 3

Authors: *L. AN, Q. DING, H. WANG;
Physiol., Michigan State Univ., East Lansing, MI

Abstract: Altered cyclic AMP signaling has been shown in mood disorders. Here, we determined the function of type 8 adenylate cyclase (ADCY8), which is identified as a genetic risk factor in human studies with bipolar disorder (BD) patients. Mice with *Adcy8* deficiency show hyperactivity in the open field test, risk-taking behavior indicated by more time spent in the open arm in the elevated plus maze, impaired sensory-motor gating in the prepulse inhibition (PPI) test, and impaired contextual fear memory. Through transcriptome analysis, we identified GSK3β hyperactivity in the *Adcy8* knock-out (KO) brain. With pharmacological inhibition of GSK3 with lithium, we found that lithium downregulated GSK3β activity, reversed hyperactivity in open field test, decreased risk-taking behavior in the elevated plus maze, and rescued the contextual fear memory. Altogether, we conclude that *Adcy8* KO mice have bipolar disorder-associated behaviors and lithium is the potential therapeutic strategy.

Disclosures: L. An: None. **Q. Ding:** None. **H. Wang:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.29/N11

Topic: G.05. Mood Disorders

Title: Finding the right path to psychedelic studies in animals

Authors: J. SU[1], Z. WANG[2], *R. WU[2], H. GUOWEN[2], Y. ZHENG[2], Z. ZHENG[2], L. ZHEN[2], C. JINMENG[2], Y. XIN[2];
[1]WuXi AppTec, shanghai, China; [2]WuXi AppTec, Shanghai, China

Abstract: Emerging clinical evidence indicates that acute administration of psychedelic agonists of the 5-HT2A receptor (5-HT2AR), such as psilocybin, to patients with major depressive disorder (MDD) produce rapid antidepressant effects, which may persist up to several weeks after the treatment. Pre-clinical studies also suggest that psilocybin stimulates neurogenesis in the hippocampus and alleviates fear-related phenotypes in animals. All these boost the interest in

the study of mechanisms of psilocybin and also to develop analogs that possess the efficacy in treating depression and fear without the hallucinogenic side effects. However, inconsistent results reported in the recent studies hinder the explorations of mechanism underlying the effects of psychedelics. Meanwhile, whether single dose or chronic micro-dosing is more beneficial remains unknown. Here, we examined the efficacy of psilocybin of different doses in multiple animal models of MDD and post-traumatic stress disorders (PTSD), assessing the potential representative tools for future psychedelic efficacy studies in depression and fear-related disorders. We found that psilocybin did not affect the forced swimming test (FST) nor sucrose preference test (SPT) in the chronic corticosterone (CORT) model of depression. Furthermore, psilocybin did not decrease the escape failure in the learned helplessness paradigm. A single dose of psilocybin did not decrease the immobility time in the FST in naïve animals while produced significant head twitch responses. However, psilocybin administration in the fear conditioning paradigm enhanced the fear extinction learning in mice, indicating a potential anti-fear effect. All these results suggest that psilocybin is more pronounced in the fear paradigm than depression-related models, which may be due to the dosing regimen. In conclusion, the representative paradigm for the efficacy screening of psychedelic psilocybin needs to be further explored.

Disclosures: J. Su: None. **Z. Wang:** None. **R. Wu:** None. **H. guowen:** None. **Y. zheng:** None. **Z. zheng:** None. **L. Zhen:** None. **C. jinmeng:** None. **Y. Xin:** None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.30/N12

Topic: F.01. Neuroethology

Title: Bipolar Disorder: A Channelopathy Disorder

Authors: *W. J. WALSH;
Walsh Res. Inst., Naperville, IL

Abstract: More than 70 million are diagnosed with bipolar disorder, but treatment efficacy has been limited by major unknowns including the cause of the illness and proclivity for mania/depression cycling. A multi-year study of recent neuroscience advances was conducted in a search for bipolar insights. A 2018 APA poster presentation summarized early progress. Advances in epigenetics and cancer research along with severe oxidative overload in my 1,500 BD patient database suggest somatic weakness in DNA-repair and antioxidant-protection genes. A 2021 GWAS genomic study identified 64 small-effect bipolar variants that include DNA damage genes that may have no direct relevance to BD. An exclusion analysis revealed that 49 of the variants were major cancer genes and other DNA damage genes, with 15 expected to be “true” BD variants: ANK3, CACNAIC, SCN2A, HOMER1, CACNB2, ACDY2, GRIN2A, WFDC12, ZnF592, LMAN2L, MRPS33, PACS1, RP1-84015.2 (lincRNA), MDFIC2, and

KCNB1. About 60% of the “bipolar” variants are major ion-channel genes suggesting impaired ion channels are central to the genetic risk for bipolar disorder. This is not surprising since ion channels are directly involved in resting voltages and action potentials, and prior studies concluded BD may be a channelopathy. High DNA damage rates in BD were confirmed by greater risk for conditions unrelated to BD, including heart disease, breast cancer, diabetes, and many others. USA life expectancy is only 67 years compared to 79 years in the general population, suggesting severe DNA damage may be a prerequisite for BD onset. I propose that BD is caused by two coincident factors: (a) genetic weakness in the collaborative team of 400 ion-channel genes, and (b) genetic tendency for accelerated DNA damage. In this model, bipolar’s delayed onset results from additional damage to ion-channel genes after birth, with progressive severity caused by continuing DNA damage after onset. Studies of potential ion-channel impairments led to discovery of a potassium ion flooding mechanism consistent with (a) mania onset, (b) a switch to clinical depression, (c) symptom-free euthymia, and (d) continuous mania/depression cycling. A detailed analysis identified serotonin as the likely dysregulated NT responsible for switching. This study suggests several treatment opportunities for BD patients. For example, antioxidant therapy capable of disabling superoxide, ONOO, and hydroxyl radicals is promising. Hopefully, the knowledge gained from this investigation will lead to the development of improved therapies for this devastating disorder.

Disclosures: W.J. Walsh: None.

Poster

PSTR182: Major Depressive and Bipolar Disorder: Targeting and Treatment

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR182.31/N13

Topic: G.05. Mood Disorders

Support: MH133561

Title: Single nuclei and spatial cell type specific multiome alterations in major depression

Authors: J. MANN, G. ROSOKLIJA, ***M. BOLDRINI**;
Columbia Univ., New York, NY

Abstract: The hippocampus cellular circuit controls memory and emotional responses. Hippocampus neuroplasticity, neuron number and volume are reduced in major depressive disorder (MDD), the second-leading cause of disability worldwide. Diverse cell types have been implicated in MDD pathology and can be characterized by their gene expression. Dynamic changes in gene expression are mediated, in part, by cis-regulatory elements (CREs). However, the accessibility of CREs and their relation to gene expression in MDD is unknown in specific cell types in the hippocampus. Here, we simultaneously evaluate chromatin accessibility and gene expression (10X Genomics) in 349,847 hippocampal nuclei from 18 adult human donors. In a subsample of 6 donors, we characterize the spatial transcriptome (Visium, 10X Genomics) and

project single nuclei cell types onto in-tact anatomy. Among the 31 clusters and 11 cell types analyzed, excitatory neurons, granule neurons, ependymal cells, and oligodendrocyte lineage cells contributed mostly to differences between untreated MDD and neurotypical controls across modalities. We identified 237 significant chromatin regulatory regions involving 66 unique genes within 500 kb of the transcription start site (TSS). Peak-gene associations were enriched nearer to TSSs and the number of correlations decreased dramatically with distance. We performed marker validation using Xenium, qPCR, RNAscope (ACDio), shotgun proteomics, immunohistochemistry, and immunofluorescence. Gene ontology and pathway analyses highlight inflammation, collagen formation and extracellular matrix organization, epithelial development and cell-cell junction, and Alzheimer's Disease related pathways as altered in MDD. Some of these markers are targets of existing drugs many of which are not antidepressants and could be evaluated for drug repurposing.

Disclosures: J. Mann: None. **G. Rosoklija:** None. **M. Boldrini:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.01/N14

Topic: G.09. Drugs of Abuse and Addiction

Title: Whole-brain functional connectivity correlates of illicit substance use

Authors: *R. M. BARKER, R. F. BETZEL;
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Abstract: Substance use disorders (SUD) are hypothesized to have a distinct neural basis, traditionally explored through task-based activations and seed-based functional connectivity (FC) within select brain regions. However, how SUD is manifested at the whole-brain level remains unclear. We address this question using a whole-brain FC approach to investigate the neural correlates of substance use across a multi-substance spectrum. Using the Human Connectome Project's imaging dataset of 352 young adults (low in-scanner motion, no familial relations), we generated a reliable network-based FC signature correlating with self-reported substance use.We computed FC from regional fMRI BOLD time courses, yielding a [node x node] matrix for each participant. We then calculated the correlation of each edge (every node pair) with the substance use self-reports (number of times used during lifetime) corresponding to the categories: hallucinogens, cocaine, opiates, stimulants, sedatives, and an omnibus category labeled "illicit." Our analysis revealed significant correlations associated with all substance categories (the lone exception was cocaine). The reliability and specificity of these FC signatures were further validated through a split-half replication. The analysis highlighted a significant presence of various networks, including the temporo-parietal network and its role in multisensory integration and cognitive mediation concerning substance use. Additionally, significant connectivity patterns persisted in the somatomotor and default mode networks,

mirroring previously identified models such as myelin development mapping, sensory association axis topography, and the principal gradient of connectivity. Our study reveals significant, replicable FC patterns for all examined substance categories, except cocaine, indicating a differential impact on neural networks. This study demonstrates the complex interplay within the brain's functional architecture and its susceptibility to substance influence, opening pathways for understanding the intricate intersectionality of FC patterns across varied functional networks in substance use.

Disclosures: R.M. Barker: None. **R.F. Betzel:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.02/N15

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R21 DA047602
VA Merit I01CX001558

Title: Precision Functional Brain Mapping after Methamphetamine Administration

Authors: *W. F. HOFFMAN[1,2], R. J. HERMOSILLO[5], L. DENNIS[7], A. RANDOLPH[5], G. GRIMSRUD[5], H. ABUAD[9], D. SMITH[8], H. MCCREADY[3], W. NG[3], E. FECZKO[10], J. NAGUM[4], M. KOHNO[4], D. A. FAIR[6];
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Abstract: We used precision functional mapping to investigate the effect of orally administered methamphetamine (MA) or placebo (PBO) on network connectivity in healthy control subjects (CS). The research was approved by a joint Institutional Review Board (Veterans Affairs Portland Health Care System and Oregon Health & Science University).
Participants (n=7, mean age = 37.7 y, 5 female) CS completed 2 separate sessions in a double-blind cross-over study of 0.3 mg/kg oral MA or PBO. Subjects received a baseline scan and a scan initiated 1.5 h after receiving MA or PBO. Salivary pre and post MA levels were obtained. Scans were obtained with a 3.0-T Siemens Prisma Scanner and 32-channel head coil. High res T_{1w} and T_{2w} images were obtained. BOLD data for RSFC were collected using FIRMM software in real-time to ensure 30 to 45 min worth of low motion (FD<0.2mm) volumes. RSFC data for each participant was processed similarly to the ABCD-BIDS pipeline. Precision functional maps were constructed for each participant from 14 pre-determined network templates. Grayordinates

for each individual participant was assigned to the network template with the highest spatial similarity (eta²) value. Group connectivity matrices were obtained by assigning each grayordinate to a network if it occurred in that network in at least 6 individuals.
There was a consistent subjective response after MA administration (post scan levels 389 ± 209 ng/mL), with subjects reporting maximum subjective effects 1.5 h after MA. Correlation maps pre and post MA found robust, consistent decreased within-network connectivity in motor and sensory networks and the DMN (Figure 1). There were minimal changes in the PBO condition. Precision functional mapping is a feasible and powerful method for the study of acute drug administration. The technique produces individual maps that approach a steady state and allow detection of precise borders between networks with increased confidence. The method can yield stable group maps in modest subject cohorts. This method will be important for studies of individuals suffering from addictive disorders.

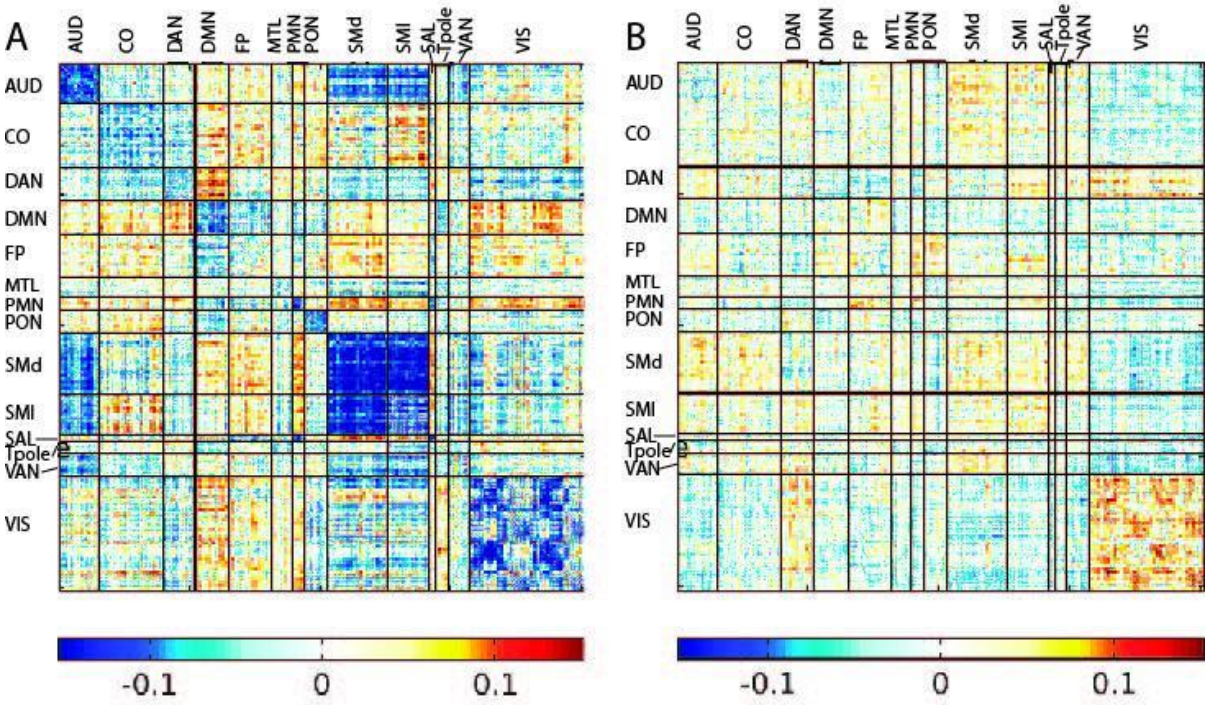

Figure 2: Post - Pre differences in RSFC (A) after MA administration and (B) after PBO administration. AUD - auditory network, CO - cingulo-opercular network, DAN - dorsal attention network, DMN -default mode network, FP - fronto-parietal network, MTL - medial temporal network, PMN - parietal medial network, PON - parieto-occipital network, SMd - dorsal somatomotor, SMI - lateral somatomotor, SAL - salience, Tpole - temporal pole, VAN - ventral attention network, VIS - visual network.

Disclosures: W.F. Hoffman: None. **R.J. Hermosillo:** None. **L. Dennis:** None. **A. Randolph:** None. **G. Grimsrud:** None. **H. Abuad:** None. **D. Smith:** None. **H. Mccready:** None. **W. Ng:** None. **E. Feczko:** None. **J. Nagum:** None. **M. Kohno:** None. **D.A. Fair:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

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Program #/Poster #: PSTR183.03/N16

Topic: G.09. Drugs of Abuse and Addiction

Support: NRF Korea Grant 2021M3E5D2A0102249311
BK21 FOUR Program Grant 5199990314123
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Title: Context dependent outcome encoding of monetary and nicotine rewards among smokers

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Abstract: Optimal decision-making often requires incorporating contextual information, which is often impaired in drug addiction. While it is crucial to utilize actual drug rewards in understanding disrupted reward processing in drug addiction, little attempts have been made to use actual drug reward as rewards. To address this gap, we used a variant of the two-armed bandit reinforcement learning (RL) task paired with computational models and a real-time vaping device to deliver nicotine reward to smokers. During each of two visits, each group (Smokers=4, Non-smokers=14) engaged in a series of RL tasks used in Bavard et al. (2021) that differed in reward probability of each stimulus and reward type. During the nicotine condition, smokers vaped nicotine instead of money as a reward, using a customized vaping device in the laboratory. We implemented three computational models using hierarchical Bayesian modeling, which differ in how each encodes choice outcomes. They were adapted from previous literatures and we annotated them as the ABSOLUTE model (Watkins, 1989), the RANGE model (Bavard et al., 2021), and the RF model, which is a variant of the frequency encoding model in Hayes & Wedell (2023). The RANGE model incorporated a context-dependent parameter and the RF model incorporated a free parameter that account for reward frequency. The results showed that both the original and the new task designs captured the systematic pattern of range adaptation. Thus, we replicated the transfer error in previous studies (e.g., Bavard et al., 2021) as we compared the ratio of optimal choices made during the transfer phase among non-smokers. When compared nicotine reward versus monetary reward among smokers, there was a marginally significant lower transfer error for nicotine rewards in the smallest EV difference condition ($t(6)=-0.6$, $p=0.086$). When we compared the model performance using the leave-one-out information criterion (LOOIC), the RF model outperformed the other two models (RF=1485, ABSOLUTE=1825, RANGE=1623), suggesting that reward frequency could have played a critical role in outcome encoding. This preliminary result may support the hypothesis that smokers adapt less to contextual information when learning about drug outcomes than monetary rewards. We show that smokers exhibited a reduced tendency to consider contextual information, specifically when anticipating nicotine, as opposed to monetary rewards. Smokers showed a distinct pattern of learning nicotine outcome comparably slower than monetary reward, and thus

making fewer transfer errors for nicotine reward, which could be explained by increased sensitivity to drug reward frequency.

Disclosures: J. Lee: None. **E. Lee:** None. **J.W. Brown:** None. **W. Ahn:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.04/N17

Topic: G.09. Drugs of Abuse and Addiction

Support: P30 DA048742
R01 MH128177

Title: The functional connectivity of the macaque striatum dynamically adapts to chronic cocaine self-administration and abstinence

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Abstract: Addiction is characterized by compulsive drug seeking and taking. Treatment options remain limited due to our incomplete grasp of the neurobiological mechanisms underlying addiction. The collection of human data spanning pre-drug exposure, initial usage, phases of addiction and abstinence, and relapse presents a considerable ethical and methodological challenge. Thus, the causes and consequences of chronic drug use are often confounded in human addiction work. Prioritizing nonhuman primate (NHP) models is key for understanding drug-induced neurobiological changes. NHPs, due to their phylogenetic proximity to humans, exhibit comparable brain organization, drug distribution, and pharmacokinetic profiles. Here, naive NHPs voluntarily triggered cocaine delivery in a self-administration (SA) paradigm. We collected resting-state fMRI data in 4 macaques: pre-drug baseline; days 5, 45, and 95 of cocaine SA; days 5, 30, and 60 of abstinence. We focused on the functional connectivity (FC) of the striatum, one of the core regions of the reward system. The intrinsic FC of the striatum exhibited two patterns of alteration: (1) a decrease in FC after early exposure, increase during mid-exposure, and decrease with prolonged exposure (i.e., caudate (CAU)-putamen (PUT) and nucleus accumbens (NAcc)-PUT); (2) a linear increase in FC during the initial two timepoints, followed by a decline in FC with prolonged exposure (i.e., NAcc-CAU). Likewise, the cortico-striatal FC alterations displayed intricate dynamics. One effect was an initial FC increase after early drug exposure, which reversed with prolonged exposure. This was the case for the FC between: (1) NAcc and insula; (2) PUT and insula/ACC/ OFC; (3) CAU and areas 12/32/VLPFC. Another effect was an increase in FC that diminished with prolonged exposure.

This was the case for the FC between (1) PUT and motor areas/DLPFC/PCC (2) CAU and area 24/attention areas; (3) NAcc and ACC/visual and attention areas. Lastly, we found linear effects of cocaine SA for the FC between (1) CAU/PUT and FEF/visual and attention areas; (2) NAcc and OFC. The FC alterations throughout abstinence exhibited similarly complex patterns. Our findings reveal the dynamic adaptation of intrinsic and cortico-striatal FC across addiction stages. We show that conceptualizing drug-induced neurobiological alterations in terms of static linear effects is incomplete. The brain undergoes gradual changes across the phases of addiction. Critically, the effects were predominantly non-linear, exhibiting complex patterns of change rather than conforming to a linear progression making the temporal dimension a critical factor to consider.

Disclosures: A.M.G. Manea: None. **G. Delgado Salazar:** None. **T. Erickson:** None. **A. Zilverstand:** None. **J. Zimmermann:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

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Program #/Poster #: PSTR183.05/N18

Topic: G.09. Drugs of Abuse and Addiction

Support: UM1 DA05224
U01 DA053629
U01 DA056003

Title: A comparative single-cell multi-omic atlas of molecular adaptations associated with opioid use in the nucleus accumbens of humans, non-human primates, and rodents

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Abstract: Substance use disorders (SUD) and addiction are associated with the dysregulation of neural circuits related to salience and habits, negative emotional states, and executive function, but the critical cell types within these brain regions are not fully described, and the best therapeutic targets within them are not known. The Single-Cell Opioid Responses in the Context of HIV (SCORCH) consortium is investigating the impacts of opioids on reward circuitry across multiple brain regions and species using single-cell and spatial genomics. Here, we report integrative analyses within the nucleus accumbens (NAc), a basal ganglia structure that is a central node of the mesolimbic dopamine reward pathway. Human post-mortem samples from opioid use disorder cases vs. controls provide insights into the variation within the human condition itself, while rodents and non-human primates are used to model specific drug exposure patterns and stages of addiction. Our integrated atlas contains 482,443 cells from the human, macaque, mouse, and rat NAc across 8 studies. We find deep conservation of spatially-resolved

neuronal subtypes, including for multiple subtypes of *DRD1*-, *DRD2*-, *DRD3*-expressing spiny projection neurons. We identified NAc cell type-specific gene expression and chromatin accessibility changes associated with drug exposure in each condition. Comparisons between humans and animal models revealed gene networks associated with specific substances and stages of addiction, as well as the dynamics of these gene networks across human donors. These analyses provide insights into the mechanisms of substance use disorders in the NAc and serve as a blueprint for collaborative studies of more than 15 brain regions.

Disclosures: B. Herb: None. **P.J. Kenny:** None. **S.A. Ament:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

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Program #/Poster #: PSTR183.06/N19

Topic: G.09. Drugs of Abuse and Addiction

Support: 1UM1DA052244-01

Title: Molecular impact of substance use disorder and HIV infection within the prefrontal cortex revealed through multi-species, atlas-level single-cell integrative analyses.

Authors: *B. R. HERB[1], J. RECEVEUR[1], O. WHITE[1], S. A. AMENT[2];
[1]Univ. of Maryland Baltimore, Baltimore, MD; [2]Dept. of Psychiatry and Inst. for Genome Sci., U Maryland Sch. of Med., Baltimore, MD

Abstract: Substance use disorders (SUD) and addiction are associated with the dysregulation of neural circuits related to salience and habits, negative emotional states, and executive function, but the cell-type specific impact within these brain regions are not fully described, and the best therapeutic targets within them are not known. The Single-Cell Opioid Responses in the Context of HIV (SCORCH) consortium has identified key regions of the brain to study the impact of opioids and HIV on reward circuitry across multiple species and with a variety of single cell transcriptomic and epigenetic approaches. Here we report on integrative analyses within the prefrontal cortex (PFC), a region which controls executive function and is highly affected during addiction, leading to impulsivity and drug-overevaluation. We have constructed cell type atlases for the PFC that encompass multiple species and SUD/HIV conditions. Rodent samples provided a single viral species paradigm which provided clear biological signatures that assisted in identifying effects in genetically diverse human samples. Our PFC atlas contains single-cell multi-omic profiles of 367,219 cells from the dorsolateral PFC of 54 human donors, and a separate rat PFC atlas encompasses 272,816 cells across 37 animals, half of which were HIV+. Our analyses describe PFC cell type-specific gene expression and chromatin accessibility changes associated with SUD, HIV, and SUD+HIV. Comparisons between humans and animal models will enable us to identify gene networks associated with specific substances and stages of addiction, then evaluate the dynamics of these gene networks in human donors. These analyses

provide insights into the mechanisms of SUD and HIV in the PFC and serve as a blueprint for collaborative studies of >15 brain regions. This work is part of the Single-Cell Opioid Responses in the Context of HIV (SCORCH) Consortium efforts.

Disclosures: B.R. Herb: None. **J. Receveur:** None. **O. White:** None. **S.A. Ament:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

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Program #/Poster #: PSTR183.07/N20

Topic: G.09. Drugs of Abuse and Addiction

Support: NINDS 1R35NS1168
Overland Foundation

Title: Brain-wide analysis of METH-induced changes in neurotransmitter phenotype

Authors: *M. PRATELLI[1,2], C. FREDMAN[1,2], D. ALVAREZ[1,2], L. WU[1,2], N. C. SPITZER[1,2]; [1]UCSD Dept. of Neurobio., La Jolla, CA; [2]Kavli Institute for Brain and Mind, La Jolla, CA

Abstract: Stimuli that cause a sustained change in neuronal activity can induce neurons to change their neurotransmitter phenotype, losing expression of their original transmitter and gaining expression of another one. These changes often involve a switch between excitatory and inhibitory transmitters and produce alterations in behavior. While more and more instances of behaviorally-relevant changes in transmitter phenotype are being discovered, the breadth of this plasticity remains unclear. We asked whether a single stimulus can induce neurons in multiple regions of the same brain to change their transmitter phenotype. As stimulus, we chose a 10-day treatment with methamphetamine (METH), which we have previously shown to induce glutamatergic neurons in the prelimbic cortex to reduce their expression of the vesicular glutamate transporter and gain GABA. Because glutamate and GABA are the most widespread excitatory and inhibitory transmitters in the brain, we developed a pipeline for whole-brain analysis of changes in these transmitter phenotypes. We generated a $VGAT^{FLP}$::$VGLUT2^{CRE}$::$TdTomato^{cON/fON}$ reporter mouse line, in which neurons that express or have expressed both the GABAergic marker VGAT and the glutamatergic marker VGLUT2 are permanently labeled with TdTomato. After treating adult mice for 10 days with either METH or saline as a control, we acquired coronal sections of the entire brain with multiphoton tomography, performed image registration into the Allen Brain Atlas, and automatically quantified the number of TdTomato+ neurons in each brain region. METH induced a gain of TdTomato in more than 1000 neurons in each of 13 brain regions. To validate the presence of changes in transmitter phenotype in TdTomato+ neurons, we performed fluorescent in situ hybridization for VGLUT2 and VGAT transcripts in three of these brain regions. We determined the METH-induced increase in the number of TdTomato+ neurons expressing only VGLUT2 or only VGAT (suggesting a switch from GABA to glutamate or vice versa), both VGLUT2 and

VGAT (indicating neurons that gained coexpression of both transmitters) or neither of the two (suggesting previous expression of both transmitters, now absent). We are now testing whether different stimuli, including voluntary running or acute exposure to foot shock, similarly cause widespread changes in transmitter phenotype. Our data-acquisition-and-analysis pipeline, although restricted to changes involving VGAT and VGLUT2, enables appreciation of the extent to which neurons can change the transmitter they express. The results provide the basis for investigation of the behavioral effects of these changes in transmitter identity.

Disclosures: M. Pratelli: None. **C. Fredman:** None. **D. Alvarez:** None. **L. Wu:** None. **N.C. Spitzer:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.08/N21

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA045664
R01 DA059602

Title: Brain-wide mapping of brain regions underlying seeking of drug reward

Authors: *L. YUAN[1], J. LEE[2], S. FAN[2], Y. YUAN[2], W. QI[2], H. SHIM[2], X. CHEN[2];
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Abstract: Drug abuse is a significant contributor to mortality in the United States, yet the intricate neural processes driving drug-seeking behavior remain poorly understood. To shed light on these mechanisms, we trained adult male mice to self-administer drugs (cocaine and fentanyl) via lever pressing in a head-fixed setup. Initially, we conducted brain-wide mapping of regions implicated in drug seeking by quantifying cFos protein levels through immunostaining brain sections post-training. Comparing cFos expression between cocaine and saline groups revealed heightened activity in nearly half of the brain regions following drug intake. Additionally, examining long-term versus short-term drug exposure unveiled differential cFos expression in select brain regions based on drug history. To delve deeper into neural dynamics, we simultaneously recorded single-unit activities using neuropixel probes and monitored relevant behavioral events during self-administration. We identified neurons exhibiting significant activity changes at seconds surrounding lever presses, spontaneous movements, and drug-associated cues, with some neurons displaying specificity to lever pressing rather than general locomotion. Furthermore, we observed cyclic activity changes correlating with minute-level intervals of drug infusion. These findings underscore the complex neural patterns associated with drug-seeking behavior and highlight potential target regions for intervention to mitigate drug seeking.

Disclosures: L. Yuan: None. **J. Lee:** None. **S. Fan:** None. **Y. Yuan:** None. **W. Qi:** None. **H. Shim:** None. **X. Chen:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.09/N22

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA

Title: Ensembles encoding drug memories in the nucleus accumbens and basolateral amygdala mediate drug-seeking after chronic stress

Authors: *A. R. LABANCA[1], R. FUTAMURA[2], A. M. MINIER-TORIBIO[3], T. MARKOVIC[4], V. KONDEV[5], A. GODINO[3], E. M. PARISE[6], L. HOLT[7], Y. YIM[3], C. J. BROWNE[2], M. SALERY[8], E. J. NESTLER[2];
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Abstract: Several overlapping environmental factors such as chronic stress and adverse life events dramatically increase the risk of developing both substance use- and stress-related psychiatric disorders in the human population. However, the neurobiological mechanisms underlying the interactions between drugs and stress are yet to be fully characterized. Growing evidence suggests that sparse populations of cells, referred to as neuronal ensembles, play a critical role in encoding drug- and stress-related memories. To better understand the interplay between these disorders, we are exploring the synergic contribution of nucleus accumbens (NAc) and basolateral amygdala (BLA) neuronal ensembles in the encoding of cocaine-related memories. We are assessing the impact of stress on the recruitment of NAc and BLA ensembles and characterizing the cells' molecular properties with the ultimate goal of elucidating neural and molecular substrates that mediate stress-precipitated drug responses. Capitalizing on *Arc*-CreER^{T2} transgenic mice, a tamoxifen-inducible system that allows for activity-dependent tagging of neuronal populations, we permanently capture cells recruited at different stages of drug exposure. Using a cocaine conditioned place preference (CPP) procedure, we tagged neurons recruited during the expression of a previously acquired place preference (test 1). Subsequently, mice were subjected to chronic social defeat stress before assessing place preference for a second time (test 2) in order to uncover the impact of stress on the recall of a drug-associated memory. We show increased place preference at test 2 in animals that were

exposed to stress between test 1 and test 2, suggesting that stress potentiates drug memory consolidation. Additionally, we found that stress exposure increased the reactivation of ensembles in both the NAc and BLA during test 2 that were previously recruited on test 1. These findings uncover a new mechanism by which stress potentiates drug responses via the modulation of drug-context associative memories through ensemble reactivation and could further contribute to our understanding of how stress and drug cross-sensitization may be encoded in the brain. We are now using RNA- and ATAC-sequencing to examine the molecular profile of the ensembles that encode place preferences and uncover how stress changes their molecular properties to prime them for reactivation. We are also extending these analyses to drug self-administration procedures to identify neuronal ensembles in these brain regions that encode drug seeking, taking, and relapse.

Disclosures: A.R. LaBanca: None. **R. Futamura:** None. **A.M. Minier-Toribio:** None. **T. Markovic:** None. **V. Kondev:** None. **A. Godino:** None. **E.M. Parise:** None. **L. Holt:** None. **Y. Yim:** None. **C.J. Browne:** None. **M. Salery:** None. **E.J. Nestler:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01DA014133
NIH P01DA047233
NIH P30DA018343

Title: Sex- and Withdrawal-Dependent Proteomic Changes in Nucleus Accumbens and Prefrontal Cortex: Insights into Synaptic Adaptations in Substance Use Disorders

Authors: *Y. YIM[1], R. FUTAMURA[2], A. GODINO[3], T. T. LAM[4], A. LABANCA[5], T. MARKOVIC[6], C. AZIZIAN[7], L. HOLT[8], V. KONDEV[1], A. M. MINIER-TORIBIO[3], E. J. NESTLER[2];
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Abstract: Substance use disorders (SUD) remain a major public health issue in the United States, posing challenges for effective treatment due to their complex neurobiological underpinnings. Dysregulated signaling within reward-related brain regions, such as the nucleus accumbens (NAc) and prefrontal cortex (PFC), is central to drug-seeking behavior and relapse.

While the transcriptional responses to drugs of abuse are relatively well-studied, our knowledge of the proteomic changes at synapses remains limited. Gaining insights into these changes could lead to more effective targets for SUD treatment. In this study, we build on previous research into cocaine-induced proteomic adaptations by examining the effects of sex and withdrawal (WD) on the synaptic proteome in the NAc and PFC. We used adult male and female C57BL/6J mice (12 weeks old) and administered daily intraperitoneal (I.P.) injections of cocaine (20 mg/kg) or saline (vehicle) for 7 days, followed by either 24 hours or 30 days of forced abstinence (withdrawal). After the withdrawal period, the NAc and PFC were extracted, and synaptosomes were isolated for analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS), followed by label-free quantification at the Yale/NIDA neuroproteomics core facility. Our analysis identified several synapse-enriched proteins, including Ephexin1, that were either upregulated or downregulated, depending on brain region, sex, and duration of withdrawal. Although most of these cocaine-regulated proteins are neuron-derived, a significant subset is enriched in astrocytes or microglia, indicating the potential role for these non-neuronal proteins in synaptic function. One key finding is that, after 30 days of withdrawal, female mice exhibited approximately 2.5 times more significant proteomic changes in the NAc than male mice. Conversely, in the PFC after 24 hours of withdrawal, male mice showed about 2.5 times more significant proteomic changes, predominantly downregulation, compared to female mice. We are currently exploring the role of Ephexin1 in relation to drug-seeking behavior, considering its brain region-, sex-, and WD-time-dependent expression. This research aims to shed light on the molecular reorganization of synapses within the reward circuitry, which may ultimately influence an individual's susceptibility to relapse. By deepening our understanding of these mechanisms, we expect to inform the development of targeted therapies for SUD.

Disclosures: Y. Yim: None. **R. Futamura:** None. **A. Godino:** None. **T.T. Lam:** None. **A. LaBanca:** None. **T. Markovic:** None. **C. Azizian:** None. **L. Holt:** None. **V. Kondev:** None. **A.M. Minier-Toribio:** None. **E.J. Nestler:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.11/N24

Topic: G.09. Drugs of Abuse and Addiction

Title: Epitranscriptomic Mechanisms in Cocaine Use Disorder

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Abstract: Repeated substance use results in long-lasting molecular changes in the brain that promote drug-seeking behaviors. While post-translational modifications (PTMs) on histone and non-histone proteins have been shown to contribute to these maladaptive neuroadaptations, RNA

modifications (i.e., epitranscriptomic alterations) remain underexplored. With over 170 distinct types, RNA modifications play important roles in regulating biological mechanisms in physiological and pathophysiological states. Using antibody-based methods, some epitranscriptomic alterations (e.g., adenosine methylation) have been recently implicated in animal models of addiction, but a comprehensive view of the RNA modifications altered by drug use has yet to be investigated. Here, we employed high-resolution mass spectrometry (MS) to characterize RNA modification altered in the nucleus accumbens (NAc) of male and female Sprague Dawley rats. Rats received acute or repeated cocaine (15 mg/kg, i.p.) or saline injections (once a day for 10 days), and the NAc and other brain regions were collected 2 h, 24 h, or 7 day after the last injection. Additionally, another cohort of male and female rats were trained to self-administer cocaine (0.5 mg/kg/infusion, i.v.) or saline for 2 hour daily sessions for 10 days, and the NAc was collected 24 h after the last session. MS analysis identified up to 40 different types of RNA modifications in NAc samples, and multiple modifications were significantly altered compared to saline including guanosine methylation, queuosine, ac4C, pseudouridine, inosine, and cytosine methylation ($p < 0.05$). In ongoing in vitro and in vivo experiments, pharmacological and siRNA-based approaches are being used to characterize the writers and erasers of specific RNA modifications altered by cocaine. Together, these results will broaden our understanding of the epitranscriptomic processes underlying cocaine use disorder.

Disclosures: S. Seyednejad: None. **D. Fabris:** None. **G.C. Sartor:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.12/N25

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA Grant U01 DA051993
University of Minnesota Doctoral Dissertation Fellowship

Title: Epigenetic regulation in the medial prefrontal cortex underlying vulnerability to opioid addiction throughout its trajectory in male and female rats

Authors: *S. X. LIU[1,2], P. MUELKEN[4], Z. L. MAXIM[1], A. RAMAKRISHNAN[5], M. S. ESTILL[5], M. G. LESAGE[1,3,4], J. R. SMETHELLS[3,4], L. SHEN[5], P. V. TRAN[2], A. C. HARRIS[1,3,4], J. C. GEWIRTZ[1,6];
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Abstract: Opioid addiction is characterized by altered patterns of gene expression and their coordinated regulation. Little is known, however, about when critical shifts in gene network expression, including those associated with disease vulnerability, occur along the trajectory from

initial drug exposure to addiction. Here, we investigated genome-wide transcription (using RNA-seq) and chromatin accessibility (using ATAC-seq) in the medial prefrontal cortex of male and female rats in three paradigms modeling the initial response to passive, repeated morphine exposure (Withdrawal-Induced Anhedonia), persistent use (Demand), and relapse (Reinstatement). Weighted Gene Co-Expression Network Analysis revealed decreased connectivity in a myelination/oligodendrocyte gene network module in morphine-exposed WIA rats and in an inflammation module in morphine-self-administering Demand rats. Follow-up Ingenuity Pathway Analysis (IPA) indicated sex-specific alterations in activity in canonical pathways and upstream regulators consistent with these functions. We next conducted a Variance Partitioning Analysis to identify transcriptional signatures associated with addiction resilience (Res) or vulnerability (Vul) based on a median split of their behavioral scores across the three paradigms. We found that addiction vulnerability in male rats across the three paradigms was associated disproportionately with differential expression of non-coding RNAs. IPA analysis of differential chromatin accessibility (ATAC-seq) in Res vs. Vul rats in both sexes revealed changes in gene networks associated with neural signaling, inflammation, and neurodevelopment, suggesting that alterations in these pathways may contribute to long-term differential vulnerability to opioid addiction. HOMER motif analysis of ATAC-seq data revealed changes in accessibility to a small set of transcription factor (TF) binding sites, some that were shared by the 3 paradigms and others that were unique to each. In conclusion, we have identified changes in gene networks, upstream regulators, biological pathways, and TF binding motifs that are either phase-specific or span all three phases along the addiction trajectory. We have also identified a set of changes that covary with individual differences in addiction vulnerability and severity.

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Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

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Program #/Poster #: PSTR183.13/N26

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute on Drug Abuse (NIDA) Grant RO1-DA054271
National Institute on Drug Abuse (NIDA) Grant RO1-DA051650
National Institute on Alcohol Abuse and Alcoholism (NIAAA) Grant RO1-AA030796
Veterans Affairs (VA) Grant IO1-BX006179

Title: Investigating habitual behavior in head-fixed mice: paraventricular thalamic projections to parvalbumin-interneurons in the dorsal striatum

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Abstract: Habitual behavior is a hallmark of several psychiatric disorders, including substance use disorders (SUDs) wherein compulsive drug-seeking and consumption override rational decision-making processes. While it has been shown that the dorsolateral (DLS) and dorsomedial striatum (DMS) interact to produce habitual vs. goal-directed behaviors, respectively, how activity within these regions could be overridden to prevent habit-like behaviors remains to be determined. Notably, while the paraventricular thalamus (PVT) is known to inhibit goal-directed reward-motivated behaviors via its projections to parvalbumin interneurons (PV-INs) in the ventral striatum, how PVT might interact with the DLS and/or DMS to influence habit-like behaviors is unclear. Using adult PV-Cre mice, we conducted electrophysiological and anatomical studies to investigate the topographical organization and cell-type specificity of PVT innervation within the DS. Optogenetic manipulation in combination with whole-cell, patch-clamp electrophysiology revealed stronger PVT innervation to $DLS^{PV\text{-}INs}$ compared to $DMS^{PV\text{-}INs}$. Viral vector tracing confirmed a higher percentage of $DLS^{PV\text{-}INs}$ receive PVT input vs. $DMS^{PV\text{-}INs}$. We also created a head-fixed self-administration model of habitual behavior, utilizing variable interval schedules with reward devaluation and omission procedures. We aim to further elucidate this circuit's role in habitual behavior by optogenetic manipulation and single-cell two-photon calcium imaging of PVT --> DLS circuitry during behavior.

Disclosures: L.M. Manusky: None. **L.M. Green:** None. **M.D. Scofield:** None. **J.M. Otis:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.14/N27

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA045764
NIH Grant U54MD007592-28

Title: The role of the insula to medial prefrontal pathway in distress tolerance, cocaine self-administration, impulsivity, and anxiety-like behaviors.

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Abstract: Distress tolerance (DT) is the behavioral ability to persist in challenging, goal-directed activities while experiencing psychological stress. Low DT (inability to persist) and high impulsivity have been associated with heightened drug-seeking and relapse. Furthermore, anxiety can be considered a symptom of distress intolerance and is associated with heightened

drug use. The insular cortex (INS) and medial prefrontal cortex (mPFC) are brain regions that have been linked to drug-seeking. Connectivity between these regions forms the key cortical nodes of the salience network, a network that has been proposed to process the most relevant stimuli (i.e., drug craving) and coordinate a behavioral response. However, no preclinical studies have examined the activity of this pathway in DT and linked it to drug-seeking, impulsivity, and anxiety-like behaviors. We hypothesize that INS-mPFC connectivity during the DT task will correlate with drug-seeking, impulsivity, and anxiety-like behaviors, and a history of cocaine use will disrupt this connectivity. To investigate this, viral infusions of fluorescent calcium sensors (Cre-dependent GCaMP6s) in the INS and a retrograde adeno-associated virus (rAAV) encoding Cre recombinase (Cre) in the mPFC were performed. In the INS, a miniscope GRIN-lens was implanted for monitoring network neural activity in freely behaving female (n=4) and male (n=8) Long Evans rats during a DT task. Subjects then underwent elevated plus maze (EPM) testing and cocaine or water/saline self-administration (SA). Animals then began an abstinence period after which DT, EPM, and neural activity/connectivity were reassessed. We used chi-square analyses to assess differences in neural activity and experimental groups. Overall, our data suggest that this neural pathway tracks events in the DT task and experiences changes in neuronal profiles before and after cocaine SA when compared to controls. Additionally, we have observed sex-dependent increases and decreases in DT after SA. Our future work will expand upon these findings by further assessing drug-seeking, impulsivity, anxiety, and INS-mPFC connectivity in DT.

Disclosures: M.A. Smoak: None. **M.A. Smoak:** None. **N.D. De Avila:** None. **A. Romero:** None. **T.M. Moschak:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.15/N28

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01 DA053328

Title: Inhibition of NMDA-type glutamate receptors within the prelimbic cortex reduces incubated cocaine-craving in rats

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Abstract: The incubation of craving is a behavioral phenomenon in which cue-elicited craving increases during a period of drug abstinence. Previously, our laboratory has demonstrated that incubated cocaine-craving is associated with an increase in extracellular glutamate within the

medial prefrontal cortex (mPFC) and that glutamate release particularly within the prelimbic (PL) subregion is necessary for incubated cocaine-craving. The precise glutamate receptors mediating the incubation-driving effects of glutamate release within the PL are not known. The present study used neuropharmacological techniques to test the hypothesis that glutamate-dependent activation of NMDA-type receptors within the PL might drive incubated cocaine-craving. For this, adult male Sprague-Dawley rats underwent long-access self-administration training (6 h/day x 10 days) for IV cocaine reinforcement signaled by a light-tone stimulus complex. At 30 day withdrawal (WD30) following cocaine self-administration training, rats underwent a test for cue-elicited craving, prior to which they were infused intra-PL with either vehicle or two doses of the competitive non-selective NMDA antagonist, D-AP5 (2.5 µg or 7.5 µg/0.5 µl per side). A control group was infused intra-PL with vehicle on withdrawal day 1 (WD1). To assess for carryover effects, rats underwent another cue test the following day with no microinjection. While the lower D-AP5 dose was insufficient to block cue-elicited cocaine-craving, the higher dose significantly reduced lever pressing, relative to WD30 vehicle controls and this effect persisted the next day. These data demonstrate that intra-PL inhibition of NMDA receptors blocks incubated cocaine-craving and argues for a role for the activation of NMDA receptors within the PL as a driving factor in incubated cocaine-craving.

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Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.16/N29

Topic: G.09. Drugs of Abuse and Addiction

Support: R01DA053328

Title: Methamphetamine and sucrose craving incubate regardless of systemic mTOR inhibition

Authors: *F. J. CANO, K. SMITH, S. ADLER, A. JOTWANI, E. LASSERS, H. NELSON, K. STUEMKE, A. T. REED APARICIO, L. L. HUERTA SANCHEZ, D. GONZALEZ, C. DENNING, K. K. SZUMLINSKI;
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Abstract: The similar temporal profile of incubated food- vs. drug-seeking has led to the hypothesis that these behavioral phenomena may involve common, time-dependent, biochemical adaptations within neural circuits governing motivated behavior. Previously, we reported that Everolimus, an FDA-approved mTOR inhibitor, dose-dependently blocks incubated cocaine-seeking during late withdrawal and this anti-incubation effect was associated with a reversal of withdrawal-induced protein changes in the prelimbic subregion of the prefrontal cortex. Herein,

we extend this work by examining the impact of Everolimus on male Sprague-Dawley rats exhibiting incubated craving for methamphetamine or sucrose. Rats were trained to self-administer methamphetamine (0.1mg/kg/infusion) or 45 mg banana-flavored sucrose pellets, paired with a light + tone compound stimulus, during daily 6-h sessions over 10 consecutive days. One or 30 days following the last operant-conditioning session, rats were gavage-infused Everolimus (1.0 mg/kg) followed by a 2-h test to measure cue-elicited responding. Methamphetamine and sucrose-experienced rats expressed incubated craving for their respective reinforcers. In contrast to our prior data for incubated cocaine-craving, Everolimus failed to block expression of incubated craving for methamphetamine or for sucrose. Also distinct from cocaine, results from our immunoblotting data for sucrose-incubated rats detected a time-dependent increase in the expression of mGlu5, p-Akt and p-mTOR within the infralimbic, but not prelimbic, cortex. Our data to date suggest that both the biochemical mechanisms and brain regions that gate the incubation of craving may be reinforcer-specific. Further, our results indicate that, in contrast to cocaine, mTOR activity is not required for the incubation of either methamphetamine or sucrose craving, arguing against heightened mTOR activity as a biochemical driver of incubation of craving per se. Taken together, our collection of data support the potential clinical utility of Everolimus for mitigating incubated cocaine, but not methamphetamine, craving during protracted withdrawal and provide evidence to support no overt effect of Everolimus on the conditioned reinforcing properties of non-drug reinforcers of relevance to off-target motivational effects.

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Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

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Topic: G.09. Drugs of Abuse and Addiction

Support: MJT Grant: NIH P30 DA048742
University of Minnesota Medical Discovery Team on Addiction
University of Minnesota Structural Circuits Core

Title: Whole-brain functional networks elicited by drug-paired cues in rodent addiction models

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Abstract: Drug-associated cues can drive relapse in substance use disorder (SUD). Cue-induced memories initiate craving, although cue reactivity is highly variable among those with SUD. In

reactive individuals, fMRI studies reveal distinct functional circuits linked to drug vs. non-drug cues, regardless of the primary drug of use. In parallel rodent studies, drug vs. non-drug cues activate distinct yet overlapping functionally connected neuronal populations (meta-ensembles). That said, the elements of these meta-ensembles (i.e., which neuronal sub-populations in which regions) that control cue-responsivity and drug craving are not well delineated. Our studies aim to address this in a rat model by comparing meta-ensembles responsive to cues paired with a psychostimulant (methamphetamine) vs. an opioid (oxycodone). The elements in common are more likely to drive addiction-related behaviors between both drug types. Given the inherent variability in behavior in our rat model, we can correlate meta-ensemble elements with these individual differences.We used a conditioned place preference (CPP) paradigm where two odors were paired with saline or drug (methamphetamine; n=4 or oxycodone; n=4) over an eight-day period. Rats received a drug-odor (CS+) or saline-odor (CS-) pairing on alternate days. Conditioning was assessed in an additional preference session by time spent with the CS+ or CS- odor. CPP produced a preference for the CS+ odor in a majority (five of eight) of the animals with useful individual variability, ranging from 25-60% of the session with the CS+ odor. After twelve days of abstinence, rats were exposed to either the drug- or saline-paired odor an hour before perfusion and brain extraction. To identify drug-cue meta-ensembles, brains were sectioned into hemispheres, cleared, and stained for NeuN and cFOS to quantify the total neuronal population and those activated by the odors, respectively. We expect to find overlapping yet distinct meta-ensembles between saline-, oxycodone-, and methamphetamine-paired odors across cortical and subcortical structures. We also expect to find meta-ensemble correlates based on the magnitude of individual preference. Identifying distinct meta-ensembles may provide a basis for new targeted and individualized SUD treatments.

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Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.18/N31

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA053328

Title: Evidence that the independent effects of, and interactions between, social isolation and binge-drinking on cognitive function are sex- and age-dependent

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Abstract: Social isolation and loneliness are major factors impacting both physical and mental health to include increased risk for alcohol and drug use and cognitive impairment. While both substance use and social isolation are well-characterized in both humans and laboratory animals to impair cognitive function, there exists few, if any studies, that have directly examined how prior histories of social isolation and alcohol consumption interact to affect cognition. Further, we are unaware of any study that has examined this question across the normal aging process. Here, male and female C57BL/6J mice, aged 3, 6, 9 or 12 months, were either maintained under group-housing conditions or were socially isolated and remained as such throughout the entire study. Three weeks following the start of isolation, mice underwent a 10-day modified Drinking-in-the-Dark (DID) binge-drinking procedure (20 and 40% v/v), followed by testing in the Morris water maze and radial arm maze. Females consumed more alcohol than males and both sexes exhibited an age-related decline in alcohol intake; however, alcohol intake was not affected by housing condition. Despite this latter observation, we detected independent effects of prior alcohol history, as well as interactions between prior alcohol history, age and/or housing condition for several variables in both maze paradigms. Consistent with an earlier report by our group, the younger mice appeared to be more sensitive overall to alcohol-related cognitive impairment than the older mice, although some alcohol effects or interaction were detected in 9 month-old mice. Generally, the Morris water maze behavior of male mice was more affected by social isolation and alcohol intake than their behavior in the radial arm maze, while females exhibited a large number of subject factor interactions in both paradigms to include the employ of a non-spatial strategy to navigate the radial arm maze. Taken together, these results support the cognitive-impairing effect of both social isolation and binge-drinking history in adult animals and provide new evidence that these subject factors can sometimes interact in a sex- and age-dependent manner to impact spatial cognition and working memory.

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Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA046403-04
UF1 NS107659-01

Title: Sex differences in the effects of social housing on stimulated dopamine release after methamphetamine exposure

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Abstract: The misuse of highly addictive stimulants such as methamphetamine (METH) has been a problem on the rise within the United States. Previous studies using cocaine self-administration have shown that single housed females had higher motivation for METH compared to socially housed females. However, males' social housing conditions had no effect on motivation for cocaine. Dopamine (DA) is a neurotransmitter in the reward system. Increased DA release is associated with rewarding properties. To investigate how social housing affects METH intake rats, the effects of electrically stimulated DA after METH exposure were investigated in single and socially housed male and female rats. DA release in the NAc core and shell of socially housed and individually housed rats was measured using fast scan cyclic voltammetry (FSCV). A chronic 16-channel carbon fiber FSCV electrode was lowered into the nucleus accumbens (NAc) and a stimulating electrode was aimed at the ventral tegmental area (VTA). Electrical stimulation was delivered to the VTA to induce the release of DA in the NAc. During the test session, prior to METH, DA release was measured and then a set of 3 electrical stimulations was given (30Hz 15p, 60Hz 30p & 60Hz 60p). Each test session consisted of an animal being given 3 i.p. injections of 0.5 mg/kg METH for a total dose of 1.5 mg/kg. After each METH injection, the same procedure of electrical stimulations was repeated. Localization of the electrode fibers was determined post-mortem. **NAc core:** Before METH, socially housed females had greater electrical stimulation-induced DA release compared with individually housed females. However, there was no effect of social housing in males. Electrically stimulated DA release, in single housed females, increased after each METH injection. By contrast, in socially housed females DA release decreased compared with pre-METH DA release. There were no significant effects of social housing or METH in males. **NAc Shell**: Before treatment, there was no effect of social housing in females or males. After METH, single housed females DA release increased after each METH injection, while in socially housed females DA release did not change significantly compared with pre-METH. There were no significant effects of housing or METH in males. The data suggests that single housed females show greater electrically stimulated DA release after METH exposure than single housed females, while social housing does not seem to affect males. Social behavior of paired rats was also analyzed after exposure to METH. Results showed that in most pairs, the social hierarchy remained the same for females, but it was less consistent in males.

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Poster

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Location: MCP Hall A

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Program #/Poster #: PSTR183.20/N33

Topic: G.09. Drugs of Abuse and Addiction

Support: R01MH125408

Title: Effects of repeated cocaine exposure on pair bonding in prairie voles

Authors: *A. AKBARABADI[1], F. DUCLOT[2], Y. LIU[3], M. CRAWFORD[1], Z.-X. WANG[4], M. KABBAJ[2];
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Abstract: While enduring social attachment with partners acts as a protective buffer against many negative consequences of life stress, the social environment can also modulate many aspects of substance use. Despite extensive empirical evidence that social relationships and social attachment play a key role in drug addiction onset, extinction, and reinstatement in humans, the underlying neurochemical mechanisms remain unclear. The prairie vole (*Microtus ochrogaster*) is a socially monogamous rodent species that forms enduring pair bonds and thus provides an excellent opportunity for studying the interactions between social environment and substance use. In this study, we investigate how a prior repeated exposure to cocaine impacts prairie voles' ability to form and maintain pair bonds. Adult sexually naive male prairie voles first underwent 10 days of daily cocaine or saline treatment to test for behavioral sensitization to the hyperlocomotor effects of cocaine. Following 10 days of withdrawal, the subjects were paired with an estrogen-primed female and the presence of a pair bond was measured after 24 hrs and 2 weeks of cohabitation using a partner preference test (PPT). Lastly, an acute cocaine challenge was performed 24 hrs following the last PPT to determine if behavioral sensitization had developed. Cocaine-treated voles traveled greater distances than saline-treated controls during the first 10 days of treatment, indicating hyperlocomotor effects of cocaine in adult male prairie voles. Interestingly, partner preference was observed in saline- but not cocaine-treated voles after 24 hrs of cohabitation, indicating that repeated cocaine treatment impairs short-term pair bonding. Following 2 weeks of cohabitation, however, both saline- and cocaine-treated voles exhibited partner preference, which suggests that repeated cocaine treatment did not prevent but rather delayed the formation of a pair bond. Altogether, these observations illustrate how long term social cohabitation can reverse the negative effects of cocaine exposure seen after short-term cohabitation in prairie voles, and strengthen the value of the socially monogamous prairie vole as a model to investigate the interaction between social environment and substance use.

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Poster

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Location: MCP Hall A

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Program #/Poster #: PSTR183.21/N34

Topic: G.09. Drugs of Abuse and Addiction

Title: A role for oxytocin signaling in social facilitation of cocaine reward but not social buffering of cocaine aversion

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Abstract: A growing body of research shows that social interactions influence motivation for drugs of abuse, drug reinforcement, and relapse. Despite this, investigations into how social interactions shape drug reward and aversion are few. Human and animal studies demonstrate that cocaine (COC) administration produces initial reward which then gives way to dysphoria. Indeed, rats develop a conditioned place preference (CPP) to the immediate effects of COC (0-5 min after administration) and a conditioned place aversion (CPA) to the delayed effects of COC (15 min after). Thus, it is likely that the rewarding and aversive effects of COC contribute to drug taking via positive and negative reinforcement mechanisms, respectively. To assess how social interaction affects COC conditioned reward and aversion we used a place conditioning procedure in which rats learn to associate a unique environment with either the immediate or delayed effects of one of three doses of COC (0.1mg/kg, 0.25mg/kg, and 1.0mg/kg, IV). Further, during each conditioning session, rats were either alone in the environment (as is typical in these procedures) or they were paired with a same-sex cage mate that never received drug. All animals underwent a post-conditioning test. We replicated previous studies showing that 1.0mg/kg COC produces robust CPP and CPA in male rats. In males conditioned alone, 0.25mg/kg COC was unable to produce a CPP, however, in rats conditioned with a conspecific this same dose produced a robust CPP, demonstrating social facilitation of cocaine reward. Further, while 1.0mg/kg COC produced a CPA in males conditioned alone, rats conditioned with a conspecific failed to develop a CPA, evidencing social buffering of cocaine aversion. Social facilitation of cocaine reward was not evident in female rats; however the presence of a conspecific did buffer cocaine aversion in females. Interestingly, conspecifics did not develop a CPP or CPA, suggesting that cocaine reward and aversion are not socially transferred. Finally, pretreatment with an oxytocin receptor antagonist significantly reduced social facilitation of cocaine reward in male rats while not affecting social buffering of cocaine aversion.

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Poster

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Program #/Poster #: PSTR183.22/N35

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R00 DA045758

Title: Oxytocin attenuates the acute and chronic endocrine disrupting effects of cocaine in female rats

Authors: *C. M. COCKRELL[1,2], A. L. COX[1], A. S. KOHTZ[1];
[1]Univ. of Mississippi Med. Ctr., Jackson, MS; [2]William Carey University, Hattiesburg, MS

Abstract: Cocaine use disorder (CUD) is a significant mental health concern, with extensive gender disparities. Clinical evidence shows a higher susceptibility in women to CUD, yet comprehensive mechanistic studies are sparse. There is evidence that vulnerability in females is linked to the endocrine-disrupting properties of cocaine, especially its impact on the estrus and menstrual cycles. Prior studies suggest that oxytocin (OXT) may alleviate these effects, although the longevity of these effects is yet to be determined. Our principal objective was to investigate the interactions between OXT and ovarian hormones during both acute and chronic exposure to cocaine, in order to evaluate the efficiency of OXT as a potential therapeutic. Herein, we used intact cycling female Sprague-Dawley rats. In acute studies, rats were administered oxytocin (0.3mg/kg, IP, 30m) or saline and cocaine (10mg/kg, IP, 15m) or saline prior to tail vein blood draw. In chronic studies, we administered cocaine or saline chronically (6 weeks of exposure) and co-administered OXT or saline every 10 days to examine the impacts on the estrus cycle. Serum samples were analyzed using an enzyme immunosorbent assay (ELISA) to quantify the levels of circulating progesterone, estradiol, oxytocin, and corticosterone. We found a significant interaction among OXT, cycle phase, and cocaine, influencing the levels of circulating progesterone in intact rats. Acutely, cocaine heightened the effects on progesterone, estradiol, and corticosterone, which were mitigated when OXT was present. The levels of circulating OXT were a significant predictor of progesterone (R^2=0.40), underscoring a robust correlation between OXT and progesterone. Conversely, our chronic observations revealed a significant interaction affecting progesterone levels due to the interplay between OXT, cycle phase, and cocaine. Chronic exposure to cocaine reduced progesterone and corticosterone levels while elevating estradiol levels. However, with the administration of exogenous OXT, levels of progesterone and estradiol were restored, and the estrus cycle became more stable as it was prior to cocaine exposure. OXT mitigated the hormonal disruptions induced by cocaine, especially affecting progesterone, estradiol, and corticosterone levels, and altered the duration of cycle phases. OXT acts as a defense against the hormonal disturbances caused by cocaine, consequently diminishing its impact on the instability of the estrus cycle. Thus, OXT is a potential treatment for the endocrine-disrupting effects of cocaine.

Disclosures: C.M. Cockrell: None. **A.L. Cox:** None. **A.S. Kohtz:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.23/N36

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH/NICHD Grant R01 HD098525
Mortimer Sackler Foundation

Title: Investigating DNA methylation of the oxytocin receptor gene in birth parents with opioid dependency undergoing attachment and biobehavioral catch-up intervention

Authors: *I. SMITH, T. CAMPBELL, T. L. ROTH;
Univ. of Delaware, Newark, DE

Abstract: The prevalence of opioid use during pregnancy in the US has been growing in recent years, with an estimated 131% increase from 2010 to 2017. Infants with prenatal opioid exposure can experience deficits in biological and behavioral regulation and could benefit from sensitive parental care, which has been shown to improve infant and parental functioning. Targeted interventions designed to enhance parental caregiving behavior such as the Attachment and Biobehavioral Catch-up (ABC) intervention have been successful in improving infant attachment, physiological regulation, and executive function, as well as parental sensitivity and neural activity. Despite a large and growing body of research supporting ABC's efficacy at fostering positive parental behaviors and enhancing infant attachment, the underlying biological mechanisms of how it leads to lasting behavioral and physiological effects are less understood. Epigenetic regulators of different genes such as DNA methylation are sensitive to early life experiences and can affect neurobiological and behavioral outcomes. Specifically, methylation of the oxytocin receptor gene (*OXTR*) is associated with early maternal caregiving behavior and infant attachment. The current study aims to assess alterations to *OXTR* methylation patterns among pregnant individuals who received opioid agonist therapy immediately preceding and/or following delivery. Participants were randomly assigned to undergo either a modified version of the ABC intervention (mABC) or a modified control intervention. Saliva samples were collected at pre-intervention, 6-, and 12-month timepoints. Maternal behaviors such as sensitivity, positive regard, and intrusiveness were assessed during the 12-month collection timepoint using scales adapted from the NICHD Observational Record of the Caregiving Environment. DNA was isolated from saliva samples and methylation status of *OXTR* was assessed by sequencing bisulfite-treated DNA using primers targeting *OXTR* exon 3 (*OXTR3*) spanning 21 CpG sites. Preliminary analyses revealed no significant main effects of intervention condition or collection timepoint. However, stepwise multiple regression analyses revealed that average methylation of *OXTR3* at 6 months ($\beta = -.858$, $p < .001$) and 12 months ($\beta = .581$, $p = .011$) postpartum inversely predicted maternal sensitivity [$F(2, 30) = 8.015$, $p < .002$, $R^2 = .348$]. The results of this ongoing study support the relationship between oxytocin gene regulation and caregiver behavior, and more work is needed to further elucidate the underlying mechanisms of behavioral interventions on caregiving experiences.

Disclosures: I. Smith: None. **T. Campbell:** None. **T.L. Roth:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.24/N37

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01 DA041808
NIH P30 DA048742
NIH T32 DA0072345
MnDrive Neuromodulation Fellowship

Title: Fundamental Sex Differences in Cocaine-Induced Plasticity of D1R- and D2R-MSNs in the Mouse Nucleus Accumbens Shell

Authors: *A. D. CHAPP[1], C. A. NWAKAMA[2], P. JAGTAP[1], C.-M. PHAN[1], M. THOMAS[1], P. G. MERMELSTEIN[1];
[1]Neurosci., Univ. of Minnesota, Minneapolis, MN; [2]Med. Scientist Training Program, Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY

Abstract: Background: Cocaine-induced plasticity in the nucleus accumbens shell of males occurs primarily in D1 dopamine receptor expressing medium spiny neurons (D1R-MSNs), with little if any impact on D2 dopamine receptor medium spiny neurons (D2R-MSNs). In females, the effect of cocaine on accumbens shell D1R and D2R-MSN neurophysiology has yet to be reported, nor has estrous cycle effects been accounted for. **Methods:** We used a 5-day locomotor sensitization paradigm followed by a 10-14 day drug free abstinence period. We then obtained *ex vivo* whole cell recordings from fluorescently labeled D1R-MSNs and D2R-MSNs in the nucleus accumbens shell of male and female mice during estrus and diestrus. We examined accumbens shell neuronal excitability as well as mEPSCs. **Results:** In females, we observed alterations in D1R-MSN excitability across the estrous cycle similar in magnitude to the actions of cocaine in males. Furthermore, cocaine shifted estrous cycle-dependent plasticity from intrinsic excitability changes in D1R-MSNs to D2R-MSNs. In males, cocaine treatment produced the anticipated drop in D1R-MSN excitability with no effect on D2R-MSN excitability. Cocaine increased mEPSC frequencies and amplitudes in D2R-MSNs from females in estrus and mEPSC amplitudes of D2R-MSNs from females in diestrus. In males, cocaine increased both D1R and D2R-MSN mEPSC amplitudes with no effect on mEPSC frequencies. **Conclusion:** Overall, while there are similar cocaine-induced disparities regarding the relative excitability of D1R-MSN versus D2R-MSN between the sexes, in males this is mediated through reduced D1R-MSN excitability, whereas in females it is due to heightened D2R-MSN excitability.

Disclosures: A.D. Chapp: None. **C.A. Nwakama:** None. **P. Jagtap:** None. **C. Phan:** None. **M. Thomas:** None. **P.G. Mermelstein:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.25/N38

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA Grant F31DA057817
NIDA Grant R01DA044925

Title: Sex-specific effects of dorsomedial stratal cannabinoid receptor-1 signaling on Pavlovian Outcome Devaluation

Authors: *C. A. STAPF[1], S. E. KEEFER[2], J. M. MCINERNEY[3], J. F. CHEER[4], D. J. CALU[4]; [1]Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [2]Psychology, Gettsyburg Col., Gettysburg, PA; [3]Neurobio., Univ. of Maryland Sch. of Med. Program in Neurosci., Baltimore, MD; [4]Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Pavlovian lever autoshaping (PLA) reveals individual-, sex-, and experience-dependent differences. Our lab previously established that sign-tracking rats are insensitive to outcome devaluation but become devaluation sensitive after extended training in PLA. However, this finding was established using only male rats. Female rats are more likely to sign-track and show increased lever pressing compared to male rats, suggesting they may be less sensitive to devaluation even after extended training. The dorsomedial striatum (DMS) regulates behavioral flexibility by promoting flexible responding in instrumental outcome devaluation. The cannabinoid type-1 receptor (CB1R) is expressed throughout the dorsal striatum but the involvement of DMS CB1R in Pavlovian devaluation has not yet been explored. To understand the contribution of DMS CB1R signaling to individual or sex-specific responses in outcome devaluation, we performed intracranial infusions of the CB1R inverse agonist, rimonabant, in the DMS before reinforced PLA sessions and outcome devaluation test sessions. We used a within-subject satiety-induced outcome devaluation procedure in male and female rats after extended training in PLA. During outcome devaluation tests, male sign-tracking rats were sensitive to devaluation while female sign-tracking rats were not. Inverse agonism of CB1Rs in the DMS reversed devaluation sensitivity in male sign-tracking rats. We saw no effect of DMS rimonabant injections on Pavlovian conditioned approach during reinforced PLA sessions. These data suggests that CB1Rs are working on inhibitory inputs in DMS of male rats to promote flexible responding. We performed whole-cell slice electrophysiology recordings to record inhibitory post-synaptic currents (IPSCs) from medium spiny neurons of the DMS. We found no differences in baseline amplitude, frequency, or interevent interval between male and female rats. We then bath applied the CB1R agonist, WIN 55,212 onto the DMS slices. We found that WIN reduced the frequency and increased the interevent interval in both male and female rats and that there were no significant sex differences in these recordings. These data suggest that male and female rats do not differ in their response to activation of CB1R on inhibitory synapses and further experiments must be done in the DMS to better understand sex differences in endocannabinoid physiology.

Disclosures: C.A. Stapf: None. **S.E. Keefer:** None. **J.M. McInerney:** None. **J.F. Cheer:** None. **D.J. Calu:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.26/N39

Topic: G.09. Drugs of Abuse and Addiction

Support: R01DA052317
T32MH065215-17

Title: Characterizing the Effects of Cocaine Delivery Rate on Neural Dynamics within the Nucleus Accumbens

Authors: *M. LEONARD[1], A. KONOMI PILKATI[2], K. THIBEAULT[1], E. S. CALIPARI[1]; [1]Vanderbilt Univ., Nashville, TN; [2]Copenhagen Univ., Kobenhavn NV, Denmark

Abstract: The rate of cocaine delivery dramatically influences its acute reinforcing effects, and is a key determinant for the risk of developing cocaine use disorder. In preclinical models, animals preferentially respond to receive fast drug infusions over slower administration. Rapid cocaine infusions also selectively potentiate neural plasticity markers within the striatum, despite producing comparable peak drug concentrations and elicited dopamine levels. It, therefore, remains unclear how the temporal dynamics of cocaine's actions within the striatum mediate differential neurobehavioral outcomes. The current experiments employ *in vivo* microendoscopy (*i.e.* miniscope) and photometric imaging techniques in freely moving mice in order to monitor multiple indicators of neural activity within the nucleus accumbens (NAc) across modes of cocaine administration. To address how both the route (IP vs IV; 3.0mg/kg) and rate (1, 10, 100s; IV) of drug administration influence patterns of neural activity within the NAc, mice (n=8) expressing the genetically-encoded calcium sensor GCaMP8 were implanted with a gradient-index (GRIN) lens for microendoscopy. We find that cocaine has reproducible effects on cellular activity within the NAc - increasing the activity of a small population of neurons, while robustly decreasing activity in the majority of remaining cells. While this general pattern was observed across all conditions, the time-course and number of cells that were sensitive to cocaine's effects were dynamically modulated by injection speed. Over repeated exposure, a subset of cocaine responsive cells was more likely to be active at the onset of drug infusion, suggesting a potential conditioned component that may further influence the pharmacodynamic properties of cocaine in NAc activity. Collectively, these studies characterize how cocaine pharmacokinetics differentially influence neural dynamics within target striatal circuitry - which may contribute to the acute reinforcing properties of cocaine and, perhaps, its long-term behavioral consequences.

Disclosures: M. Leonard: None. **A. Konomi Pilkati:** None. **K. Thibeault:** None. **E.S. Calipari:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.27/N40

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant F31 DA058157
NIH Grant R01 DA052460
P50 DA006634
P50 AA026117
Peter F. McManus Charitable Trust

Title: Diurnal rhythms in striatal acetylcholine and dopamine dynamics are modulated by differential cocaine access

Authors: *M. IACINO[1], A. BONSIB[1], L. HENDRIX[1], M. FERRIS[2];
[1]Translational Neurosci., Wake Forest Univ. Grad. Sch. of Arts & Sci., Winston-Salem, NC;
[2]Translational Neurosci., Wake Forest Univ. Sch. of Med., Winston Salem, NC

Abstract: Despite decades of research into its neurobiological mechanisms, cocaine use disorder (CUD) remains a major worldwide health problem. One variable that is often overlooked in CUD research is cocaine-induced disruption of diurnal (night/day) rhythms. The mesolimbic dopamine (DA) system is an important mediator of motivated and reward-associated behaviors that are maladapted in CUD. Acetylcholine (ACh) from striatal cholinergic interneurons (CINs) modulates mesolimbic dopamine (DA) in the nucleus accumbens (NAc) core via nicotinic acetylcholine receptors (nAChRs) on DA varicosities. Interestingly, mesolimbic DA reciprocally modulates NAc ACh via D2-like receptors (D2Rs) on CINs. Though the effect of cocaine on DA signaling has been extensively studied at single time points, cocaine-induced dysregulation of rhythms in NAc DA-ACh interactions and their mechanisms have not been investigated. Here, we used *ex vivo* fast scan cyclic voltammetry in an adult male *Sprague Dawley* rat model of cocaine self-administration under various access schedules [(Short continuous access (ShA), long continuous access (LgA), or intermittent access (IntA)] to test the hypothesis that diurnal rhythms in CIN modulation of DA release will vary based on the pattern of cocaine availability. Despite consuming less cocaine than LgA, we show that IntA significantly increased DA release across the light/dark cycle as compared to other groups. While ShA and cocaine-naïve rats maintained a rhythm of greater evoked DA release mid-light versus mid-dark cycle, IntA and LgA induced arrhythmicity of DA release. Notably, IntA promoted diurnal rhythmicity in cholinergic modulation of DA release with greater ACh control mid-light versus mid-dark cycle relative to cocaine-naïve rats. Furthermore, CIN D2R control over DA release was dependent on the light cycle and pattern of cocaine intake. ShA and cocaine-naïve rats show greater CIN D2R control over NAc DA release independent of the light/dark cycle, while IntA induced a diurnal rhythm of greater CIN D2R sensitivity to dopamine mid-dark versus mid-light cycle. Understanding the influence of diurnal rhythms and cocaine intake pattern on NAc neurochemistry will provide a rationale for targeting receptor systems, like D2Rs on CINs, as a mechanism for restoring rhythms that were present prior to cocaine history.

Disclosures: M. Iacino: None. **A. Bonsib:** None. **L. Hendrix:** None. **M. Ferris:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.28/O1

Topic: G.09. Drugs of Abuse and Addiction

Support: The Jean Phillips Shibley Endowment
Penn State College of Health and Human Development
Penn State Social Science Research Institute

Title: Adolescent nicotine exposure alters adult dendritic complexity and spine density of prefrontal cortex subregions based on genetic background

Authors: *A. TISCHER, C. NOVOA, T. J. GOULD;
Biobehavioral Hlth., The Pennsylvania State Univ., University Park, PA

Abstract: Adolescence is a critical neurodevelopmental period in which nicotine exposure may alter the normal trajectory of prefrontal cortex maturation and contribute to increased susceptibility to addiction later in life. It is less well known how genetics and sex influence long-term effects associated with adolescent nicotine exposure. Here, we studied long-term adaptations in neuronal morphology of the prefrontal cortex in adult mice exposed to nicotine during adolescence. Male and female C57BL/6J and DBA/2J mice were chronically exposed to nicotine (24 mg/kg/day free base) via subcutaneous osmotic minipumps from postnatal day 37 to 49 (PND 37-49). Brains were collected in adulthood (PND 100) and impregnated with Golgi-Cox staining for subsequent morphological analysis. Pyramidal neurons from the prelimbic and cingulate subregions of the prefrontal cortex were selectively reconstructed for quantification of dendritic complexity using Sholl Analysis. Dendritic complexity was differentially altered based on distance from the soma in only the prelimbic cortex of C57BL/6J mice exposed to nicotine during adolescence, indicating nicotine induced strain-dependent structural remodeling in a subregion-specific manner. No effect of sex was observed in prelimbic or cingulate dendritic complexity. Additionally, baseline differences in dendritic profiles of prelimbic and cingulate cortex neurons were observed in the control mice of both sexes and strains. Structural differentiation among these cell populations may reflect distinct connectivity profiles and involvement in neural circuits that are differentially altered by adolescent nicotine exposure. Spine density of prelimbic cortex neurons was then quantified as spines/10µm of secondary dendrites, factoring in distance from the soma. Adolescent nicotine exposure increased prelimbic cortex spine density of C57BL/6J mice regardless of sex. We have previously shown that adolescent nicotine exposure results in altered anxiety-like behaviors and reduced sensitivity to nicotine during adulthood in C57BL/6J and DBA/2J mice. These strain-specific structural changes may contribute to differences in adult anxiety and sensitivity to drugs related to adolescent nicotine exposure. Further exploration into the functional implications of adolescent nicotine-induced changes in prefrontal maturation among individuals of distinct genetic backgrounds may provide valuable insights into the role of genetics on the heterogeneity of addiction vulnerability.

Disclosures: A. Tischer: None. **C. Novoa:** None. **T.J. Gould:** None.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

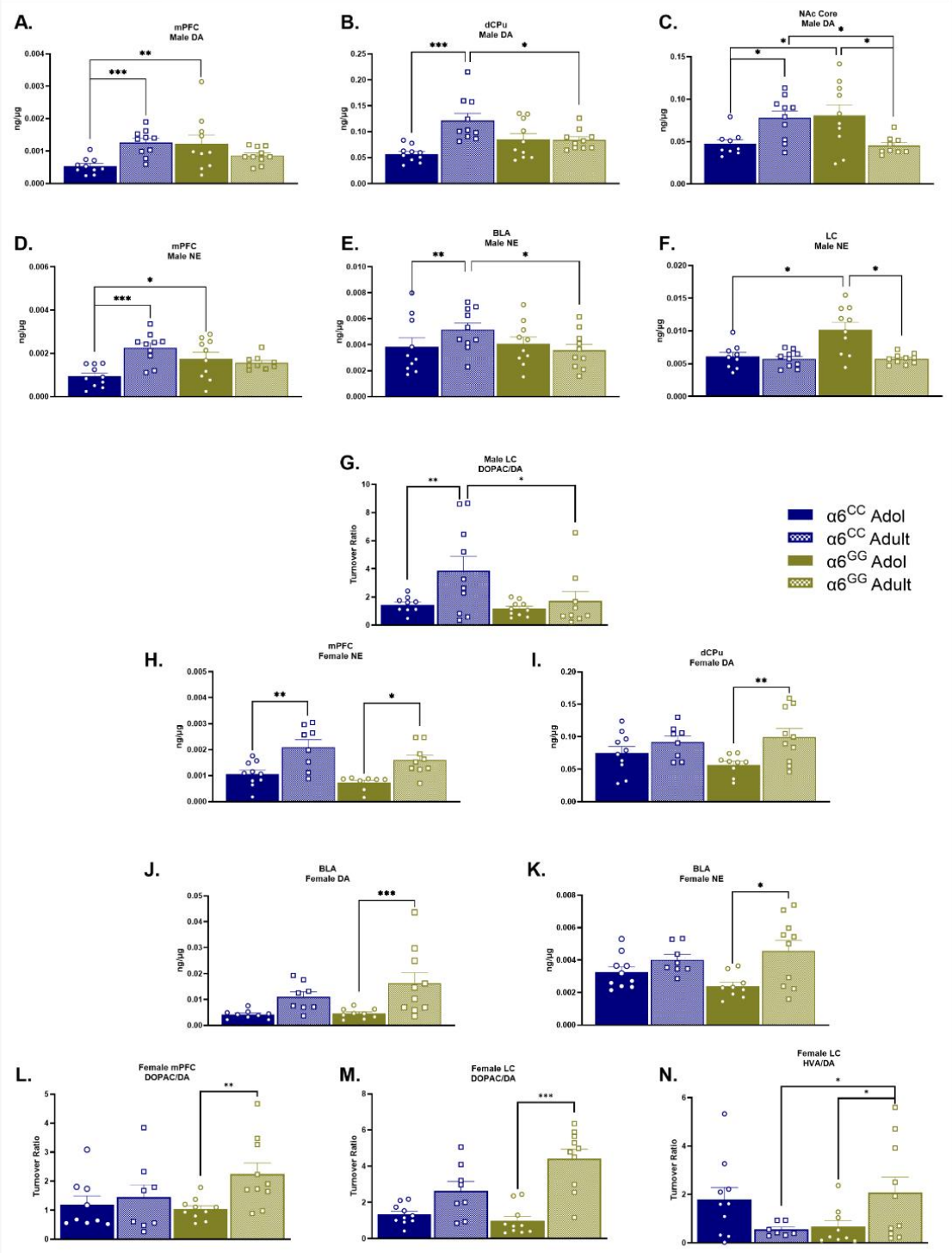
Program #/Poster #: PSTR183.29/O2

Topic: F.03. Stress and the Brain

Title: Investigating the vulnerability of the CHRNA6 3'- UTR adolescent rodents to nicotine exposure

Authors: *R. ROBERTS[1], S. LOTFIPOUR[2];
[1]Exptl. Pathology, Univ. of California Irvine, Irvine, CA; [2]Emergency Medicine, Pharmacology, & Pathology, Univ. of California, Irvine, Irvine, CA

Abstract: In 2023 6.21 million middle and high schoolers reported having used a tobacco product in the US. Although cigarette use has declined among adolescents, it has been replaced by a rise in vaping. Adolescents are vulnerable targets of the tobacco industry and experience increased susceptibility to long-lasting changes on the brain and behavior induced by nicotine, the primary agent in tobacco products. Clinical studies highlighted a key variation in the *CHRNA6* gene that results in a 'C' to 'G' allele change in the 3' untranslated region (UTR). This variation affects the alpha (α) 6 subunit of the nicotinic acetylcholine receptor (nAChR) and correlates with higher instances of nicotine use and dependence. Nicotine directly activates α6-containing nAChRs located in dopamine (DA) neurons in the ventral tegmental area (VTA). To assess the role of the *CHRNA6* genotype variation, our lab engineered a Sprague Dawley rat line containing this genetic variant. Studies in this rat line demonstrate baseline sex- and genotype-dependent brain neurotransmitter profiles and nicotine-induced anxiolytic and locomotor behavior. To explore the underlying mechanisms of the behavior observed in the *CHRNA6* 3'-UTR adolescent rats, I will use High-Performance Liquid Chromatography-Electrochemical Detection (HPLC-ECD) to quantify brain neurotransmitter level alterations after a subchronic dose of nicotine, modeling a 1-2 cigarette per day first exposure. My hypothesis is that nicotine exposure alters the levels of Dopamine, Norepinephrine, and their metabolites in reward-related regions of the brain in a sex and genotype-dependent manner. These studies are investigated in both male and female *CHRNA6* 3' UTR rats during early adolescence. The preliminary results align with previous literature demonstrating sex and genotype-dependent effects of nicotine pretreatment. Further studies will provide novel mechanistic insight into understanding nicotine addiction and creating biomarker profiles to support early risk prevention and intervention.



Disclosures: R. Roberts: None. **S. Lotfipour:** A. Employment/Salary (full or part-time):; University of California Irvine. 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.; Tobacco-Related Diseases Research Program.

Poster

PSTR183: Neural Circuits in Motivation and Mood; Genetic and Environmental Factors.

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR183.30/O3

Topic: G.09. Drugs of Abuse and Addiction

Title: Prelimbic cortex inputs to the laterodorsal tegmental nucleus critically contribute to the development of cocaine locomotor sensitization in mice.

Authors: M. KORUNA, K. M. SCHREITER, ***S. STEIDL**;
Psychology, Loyola Univ. Chicago, Chicago, IL

Abstract: Sensitization - an enhancement in the ability of drugs of abuse to activate dopamine (DA) neurotransmission, increase locomotion and support self-administration following previous drug exposure - is thought to reflect underlying neural changes important for drug addiction. Drug-induced potentiation of ventral tegmental area (VTA) glutamate (GLU) signaling and changes in GLU receptors have long been known to critically contribute to the induction of sensitization. We have previously identified the laterodorsal tegmental nucleus (LDTg) as the critical source of VTA GLU for the development of locomotor and nucleus accumbens DA sensitization (Puranik et al., 2022). The LDTg may thus give rise to the VTA GLU synapses at which the GLU plasticity, known to contribute to the enhancement of addictive behaviors, occurs. How cocaine exposure activates LDTg-VTA GLU afferents is not known, but lesions of the prelimbic region (PrL) of the medial prefrontal cortex prevent cocaine locomotor sensitization. Here we show that viral transfection of PrL cells in mice results in dense labeling of eYFP-expressing terminals in the LDTg. We then used optogenetic techniques to test the role of PrL GLU projections to the LDTg in the development of cocaine locomotor sensitization. Halorhodopsin (NpHR) or a control viral vector (eYFP) was expressed in PrL GLU neurons of wildtype mice. Separate groups of NpHR and eYFP mice were implanted with bilateral optic probes aimed at the LDTg. Mice were then injected with either cocaine (COC, 15 mg/kg; i.p.) or saline (SAL; 10 ml/kg; i.p.) for five consecutive days and locomotion was measured for 1 hour on each day. During each of these sessions light stimulation (532 nM, 5-8 mW, 5 sec on 5 sec off) was provided into the LDTg to inhibit PrL afferents. One week later all mice received a COC challenge injection (15 mg/kg; i.p.) in the absence of light stimulation. Locomotion was increased following COC pretreatment relative to SAL pretreatment in both female and male eYFP control mice. By contrast, inhibition of PrL GLU afferents to the LDTg during the induction phase blocks the subsequent expression of locomotor sensitization in both female and male NpHR mice. We hypothesize that enhanced DA signaling during cocaine exposure increases excitability of PrL output to the LDTg, and that this contributes to a maladaptive positive feedback that further enhances mesolimbic DA output, known to contribute to the enhancement of addictive behaviors.

Disclosures: M. Koruna: None. **K.M. Schreiter:** None. **S. Steidl:** None.

Poster

PSTR184: Mechanisms of Attention

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.01/O4

Topic: H.01. Attention

Support: SIUMED RSG 2024

Title: Early life stress results in female specific cognitive impairments and reduced LC neuron excitability

Authors: *S. BRANNAN[1], B. D. RICHARDSON[2];
[1]SIU-School of Med., Springfield, IL; [2]Pharmacol., SIU - Sch. of Med., Springfield, IL

Abstract: Adverse Childhood Experiences (ACEs) have been associated with many neurodevelopmental and affective disorders, with more exposures increasing negative risks. Females in general have an increased prevalence of stress-related psychopathologies beginning after adolescence, indicative of female-specific adolescent stress sensitivity. To understand underlying neuronal components mediating the relationship between sex, stress, and cognitive/affective behaviors, we focus on understanding changes in/involvement of the norepinephrine (NE)-releasing locus coeruleus (LC) for several reasons. The LC has a key role in attention and memory along with regulating affective behaviors such as anxiety. Corticotropin-releasing hormone/factor (CRF), the neuropeptide responsible for central stress signaling, directly alters LC neuron firing differently in female rodents due to variations in CRF receptor signaling properties. Although NE signaling is important in regulating attention and NE dynamics vary with sex and stress, the sex-specific effects of early life stress on persistent attentional capacity has not been explored. We hypothesized altering the LC-NE pathway through CRF-mediated effects during stress in early life will induce sex-specific persistent LC physiology changes paralleled by changes in cognitive and affective behaviors. The effects of an early life variable stress (ELVS) paradigm inducing stress at both early life and adolescence were assessed using behavioral and electrophysiological measures for attention & impulsivity (5 serial choice reaction time task), hyperactivity (open field), anxiety (elevated zero maze), and LC neuron excitability (in vitro patch clamp electrophysiology) in C57bl6/J mice (B6J). Relative to control mice, B6J female ELVS mice display hyperactivity, disrupted short-term memory, and impaired attention that was absent in B6J male ELVS mice. Female ELVS B6J mice exhibited decreased LC neuron excitability in comparison to female controls that was also not observed in B6J ELVS males. An increase in ELVS female LC neuron's evoked action potential delay time suggests an underlying change in transient voltage-activated potassium channels that are further evaluated pharmacologically to unveil a potential mechanism of ELVS-induced sex-specific change in LC neuron firing dynamics. Using this novel animal model, we have identified sexually dimorphic changes in behavior paralleled by changes in LC neuron activity. These

findings contribute to understanding sex differences in adverse outcomes of early life stress, identifying the LC as a potential target of ACE-related disorder sensitivity and treatment.

Disclosures: S. Brannan: None. **B.D. Richardson:** None.

Poster

PSTR184: Mechanisms of Attention

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.02/O5

Topic: H.01. Attention

Support: JP15dm0207001
JP23wm0625001

Title: Non-primary thalamocortical circuit controls temporal expectation sharpening

Authors: Y. ATSUMI[1], I. OOMOTO[1], T. KARAKI[1], Y. SAITO[2], Y. OISI[1], K. KOBAYASHI[3], S. KATO[4], K. KOBAYASHI[5], K. OTA[6], ***M. MURAYAMA**[7];
[1]Ctr. for Brain Sci., RIKEN, Wako, Japan; [2]Ctr. for Brain Sci., RIKEN Ctr. for Brain Sci., Wako, Japan; [3]Natl. Inst. For Physiological Sci., Okazaki, Japan; [4]Fukushima Med. Univ., Fukushima-City, Japan; [5]Fukushima Med. Univ., Fukushima 960-1295, Japan; [6]The Univ. of Tokyo, Tokyo, Japan; [7]RIKEN Ctr. For Brain Sci., Wako, Japan

Abstract: Expectation driven by predictable stimuli modulates perceptual experiences. Although this modulation is thought to involve sensory-evoked cortical responses, the specific neural circuits involved are still not fully understood. In this study, we explored the neural circuits involved in the expectation modulation of tactile responses in the forepaw primary somatosensory cortex (fpS1) of mice. Extracellular recordings from fpS1 revealed that temporally predictable stimuli with fixed intervals to the forepaw sharpened the representation of tactile evoked responses by suppressing a dominant population of layer 5 (L5) pyramidal neurons with low stimulus selectivity (non-responders) while facilitating neurons with high stimulus selectivity (responders). The facilitative effect on responders was related to an increase in their burst firing responses. Two-photon imaging from the distal dendrites of L5 neurons also confirmed that temporally predictable stimuli sharpen the dendritic representation of the tactile stimulus. Based on these results, we hypothesized that the expectation-related sharpening effect would be implemented by dominant suppression derived from local inhibitory neurons and facilitation of burst firing responses by dendritic activation. We identified that the Ventral Medial thalamic nucleus, a non-primary thalamic region projecting axons to superficial layers of S1, is involved in the expectation modulation. The predictable stimulus preferentially facilitated the thalamus but not by unpredictable stimuli with variable intervals. Thalamic axons mono-synaptically activated distal dendrites of L5 neurons and facilitated a firing activity of responders. On the other hand, the thalamic axons also recruited a cortical di-synaptic inhibitory circuit that dominantly inhibited the somatic activity of L5 neurons. Optogenetic deactivation of

the thalamic input to fpS1 attenuated the expectation related suppressing effect on firing responses of L5 neurons. These results indicated that temporal expectation would drive non-primary thalamocortical input, which sharpens the representation of cortical evoked responses by mono-synaptically activating distal dendrites and di-synaptically inhibiting somata.

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Poster

PSTR184: Mechanisms of Attention

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.03/O6

Topic: H.01. Attention

Support: Boehringer Ingelheim

Title: The role of learned irrelevance and perseveration when shifting attention in rats with and without mPFC inhibition

Authors: T. S. KNOTT[1], J. F. DU HOFFMANN[2], *D. S. TAIT[3], V. BROWN[4];
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Abstract: There is a well-known cost of learning when intact subjects are required to redirect their attention from previously relevant cues to other cues that were previously irrelevant, termed extradimensional shifting. Rats with lesions of medial prefrontal cortex (mPFC), have a greater cost of attentional shifting. This study asked whether the greater cost in impaired rats is due to an exacerbation of the same mechanism that underlies the learning cost of intact rats, or whether there is an additional factor accounting for the impairment. Using overtraining to induce attentional set, we tested how readily rats, with an intact or inactivated mPFC, shifted attention *away* from previously relevant cues and *to* previously irrelevant cues. Twelve female Lister hooded rats with bilateral mPFC inhibitory DREADDs (AAV5-hSyn-hM4D(Gi)-mCherry - Bryan Roth, Addgene) all completed three modified bowl digging tasks four times: with and without overtraining (30 additional post-criterion trials) and with and without Compound 21 (DREADDs receptor ligand; 3 mg/kg), in a pseudorandom order. Each task began with a compound (multidimensional) two-choice discrimination (CD), followed by a reversal of contingencies (REV), and then a final attentional shift stage. The final shift stage was either (a) a non-reversal shift (NRS), where the irrelevant and relevant dimensions switched; (b) a learned irrelevance shift (LIS), challenging rats to shift attention *to* a previously irrelevant exemplar and ignore exemplars in a novel dimension; or (c) a perseveration shift (PS), challenging rats to shift

attention *from* a previously relevant exemplar and respond to an exemplar in a novel dimension. Overtraining facilitated attentional set formation, slowing learning when the relevant and irrelevant dimensions were switched (NRS). The origin of the shift cost in intact rats was slower attentional shifting *to* cues that had been irrelevant (LIS) with no difficulty in shifting attention *away* from previously relevant exemplars (PS). By contrast, when the mPFC was inactivated, the rats showed more rapid shifting *to* previously irrelevant exemplars (LIS), but an increased cost of shifting attention *away* from previously relevant exemplars (PS). These findings are consistent with the suggestion that impaired set-shifting following mPFC inhibition is attributable to a failure to downregulate attention to cues.

Disclosures: T.S. Knott: 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.; BOEHRINGER INGELHEIM. **J.F. Du Hoffmann:** A. Employment/Salary (full or part-time):; BOEHRINGER INGELHEIM. **D.S. Tait:** None. **V. Brown:** None.

Poster

PSTR184: Mechanisms of Attention

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.04/O7

Topic: H.01. Attention

Support: This research study was funded by Philip Morris International.

Title: Low-dose nicotine-induced improvement of attentional performance in rats is not blocked by nicotinic antagonist co-treatment

Authors: M. STOUT[1], ***E. LEVIN**[2];
[1]Psychiatry and Behavioral Sci., Duke Univ. Med. Ctr., Durham, NC; [2]Duke Univ. Med. Ctr., Chapel Hill, NC

Abstract: Nicotine has been found in a variety of studies to improve cognitive function, including attention. It is unclear if this is due to the receptor-stimulating or -desensitizing effects of nicotine. This study was conducted to determine whether nicotine effects would be reversed with co-administration of nicotinic antagonists, which would occur if the effect was mediated by receptor stimulation. Young adult (~8-week-old) female Sprague Dawley rats (N=41) were trained on an operant visual signal detection task to assess attention in an FR1 schedule to respond to the presence of absence of a light signal. They were administered low doses of nicotine (0.01, 0.02, or 0.04 mg/kg), with the control group given vehicle (saline) subcutaneous (sc) injections (N=10-11/group) 20 minutes before the test sessions. In the next phase of the study, which followed just after nicotine-alone testing, nicotine or vehicle was given alone or in combination with 4 or 8 mg/kg (sc) of the α4β2 antagonist dihydro-β-erythroidine or the α7 antagonist methyllycaconitine to determine whether any effects induced by nicotine would be

blocked by co-administration of a nicotinic antagonist. The percent correct response data were analyzed by the analysis of variance with a significance threshold of $p<0.05$. Animals treated with 0.01 and 0.04 mg/kg nicotine doses had modest, but significantly higher percent correct rates on the attentional test compared to control (both $p<0.05$), and with marginal ($p<0.10$) improvement with the 0.02 mg/kg nicotine dose that did not reach statistical significance. The improvement in percent correct performance on the attention test continued to be evident with co-administration of nicotinic antagonists during the second phase of the study. Rats given 0.01 mg/kg nicotine continued to show a significant ($p<0.05$) improvement in percent correct relative to the saline vehicle, and this persisted with both doses of each nicotinic antagonist. Marginal improvements continued to be seen with the 0.02 and 0.04 mg/kg nicotine doses when administered with the nicotinic antagonists. The results provide support for the hypothesis that low doses (0.01mg/kg) of nicotine improve attentional performance in an animal model likely through a receptor-desensitizing effect rather than a receptor-stimulatory effect. While this study improves understanding of biological mechanisms, it is important to recognize the inherent limitations when extrapolating the findings in a broader context. Thus, the results should be interpreted with caution and considered as preliminary evidence that requires further validation.

Disclosures: M. Stout: None. **E. Levin:** None.

Poster

PSTR184: Mechanisms of Attention

Location: MCP Hall A

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Topic: H.01. Attention

Support: Natural Sciences and Engineering Research Council of Canada Graduate Scholarship
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Title: The effects of multimodal distractors on sustained attention of sign-tracker and goal-trackers.

Authors: *F. HUPPÉ-GOURGUES;
Univ. De Moncton, Moncton, NB, Canada

Abstract: During Pavlovian conditioning, Sign-Tracker (ST), Goal-Tracker (GT) and Intermediate (IG) phenotypes emerge. They are characterized by the degree to which they tend to attribute incentive salience to cues associated with rewards. Research has shown that these phenotypes also differ in other aspects. For example, in humans, STs tend to favor bottom-up while GTs tend to favor top-down attention. Some researchers have found the same pattern in rats. However, the evidence is limited. Therefore, it is hypothesized that if the addition of a distractor increases the difficulty of the task, then the performance will decrease when distractors

are added compared to the absence of distractors. It is also hypothesized that if STs favor bottom-up and GTs favor top-down attention, then light and auditory distractors will particularly affect the performance of STs in a sustained attention task (SAT). This study evaluates the signal detection performance of rats during nine different SAT with distractors. The sample was Long-Evans rats. Findings show a main effect of distractors, but no clear effect of phenotypes in detection performance. These results support that adding distractors increases the difficulty of the task but negates that the performance of STs is more affected, suggesting that distinction between phenotypes in terms of attention capacity is less important than previously presented. This study nuances the current findings and highlights the importance of future studies to clarify the use of bottom-up attention phenotypes.

Disclosures: F. Huppé-Gourgues: None.

Poster

PSTR184: Mechanisms of Attention

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.06/O9

Topic: H.01. Attention

Title: A low-cost automated setup for assessing sustained attention in rats

Authors: *B. NAMDARZADEH;
UNIVERSITY OF TEHRAN, Tehran, Iran, Islamic Republic of

Abstract: Sustained attention, crucial for understanding disorders like ADHD, is often costly to study. Our research introduces a low-cost, automated system for assessing this in rats, enhancing the accessibility and consistency of cognitive research. This system is designed for detailed studies of attention mechanisms, paving the way for novel therapeutic interventions.
Our study utilized seven male hooded rats, adhering to ethical guidelines. The experiment involved a bespoke, low-cost automated behavioral apparatus designed to assess cognitive behavior in response to auditory and visual stimuli. The setup is controlled by an Arduino board interfaced with MATLAB, ensuring precise execution and data capture of behavioral tasks. The tasks required rats to initiate trials by nose-poking into a center port, maintaining this position until a delayed go cue signaled them to select a side port for a reward. The difficulty of the task was progressively increased by extending the waiting period up to six seconds, systematically training the rats to enhance their sustained attention. The entire system was meticulously assembled to ensure uniformity and reliability, with continuous monitoring of task execution and environmental conditions, including temperature and humidity control, to maintain experimental integrity.
In our preliminary analysis, rats demonstrated a significant improvement in their ability to maintain a center nose-poke as the delay period was extended. Specifically, the duration for which rats could hold the nose-poke increased significantly over trials, from an initial average of 0.01 second to 3.05 ± 1.65 seconds by the end of the training period. Statistical analysis using a

repeated measures ANOVA indicated that these changes were significant, highlighting an enhancement in sustained attention capabilities. Additionally, variability in performance was observed across subjects, with some rats achieving maximum delay maintenance faster than others. This variability emphasizes individual differences in attentional capacity among the rats. These results demonstrate the effectiveness of our setup in measuring sustained attention improvements in rats, offering insights into ADHD and potential for broader neuropsychiatric research. The system's affordability and scalability make it a valuable tool for exploring attention mechanisms and developing targeted interventions.

Disclosures: B. Namdarzadeh: None.

Poster

PSTR184: Mechanisms of Attention

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Topic: H.01. Attention

Support: Lundbeck Foundation Grant R276-2018-792

Title: Determining the role of norepinephrine in attention by chemogenetic manipulation and fiber photometry measurement of transmitter release dynamics

Authors: *L. P. POSSELT, J. COLL MARQUÈS, S. H. JØRGENSEN, S. OVROM, F. HERBORG, A. TOFT SØRENSEN, U. GETHER;
Dept. of Neurosci., Univ. of Copenhagen, Copenhagen N, Denmark

Abstract: Norepinephrine (NE) transmission in the frontal cortex plays a critical role in promoting goal-directed, attentive, and adaptive behavior. Dysregulation in NE signaling has been linked to many different neurological and psychiatric conditions, including attention deficit hyperactivity disorder (ADHD). Although prior research has consistently shown that NE shortage significantly decreases attention, it is still unclear how high NE levels influence attentive behavior. A better understanding of the neural processes underlying NE signaling in attention may result in improvement in diagnosing and treating attentional impairments. To investigate NE release patterns in the rodent medial prefrontal cortex (mPFC) and its correlation to attentive performance *in vivo*, we express a noradrenergic biosensor (GRABNE1m) in the mPFC and in combination an excitatory Designer Receptor Exclusively Activated by Designer Drug (DREADD) in the Locus Coeruleus (LC). Upon DREADD activation in the LC by systemic injection of a specific DREADD ligand, we observed a significant as well as a dose-dependent rise in basal NE fluorescence in the prefrontal cortex. Crucially, a clear link was found between a rise in fluorescence in mPFC, hence an increase in NE release, and a significant decline in attentive performance, whilst gross motor functions were not altered. Attentive performance was measured in the rodent Continuous Performance Test, additionally gross motor functions were observed in an open field test. Concluding, our findings provide strong evidence

that excessive NE levels in the rodent medial frontal cortex impair attentional performance. Our study provides a foundation for understanding the relationship between NE dynamics and attentive behavior, and for clarifying temporal aspects of NE signaling in more discrete behavioral outputs. Future studies are furthermore focusing on the functional significance of NE dynamics in other brain regions involved in attentive processes.

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Poster

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Topic: H.01. Attention

Support: Brain Research Foundation: BRFSG-2023-13
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Title: Cortical circuit dynamics contributing to spatially directed attentional control in complex auditory environments

Authors: M. IRANI[1], Z. QU[2], K. K. SEN[3], S. SADAGHIANI[4], *H. J. GRITTON[5];
[1]Univ. of Illinois, Champaign, IL; [2]Univ. of Illinois Urbana-Champaign, Champaign, IL; [3]Biomed. Engin., Boston Univ., Boston, MA; [4]Psychology, Univ. of Illinois, Urbana-Champaign, Urbana, IL; [5]Univ. of Illinois, Urbana, IL

Abstract: The ability to segregate specific sound features from statistically competing sound sources is an essential component for animals and humans to extract meaningful information in a complex sound environment. Sound source localization depends on comparison between intensity and timing of sound information as it arrives to the subject. Furthermore, attentional mechanisms at the level of the auditory cortex are thought to play an integral role in processing sound source location. However, how the auditory cortex represents spatial sound information at the cortical level and how attention modifies this representation remains poorly understood. In this study, our goal was to characterize how sound location is represented across cortical layers and how attention might alter sensory processing representation in the primary auditory cortex. To address this question, we collected local field potential and single-unit recordings across cortical layers from fourteen mice in a multi-speaker environment. During the task, mice were presented with auditory stimuli from speakers arranged along the azimuth at four locations. The experiment was divided into a passive block, where locations were not relevant, and an active block, where one of the locations were associated with reward. Our results reveal an enhancement in frequencies in the alpha/beta range (9-16 Hz) for non-relevant locations, and an enhancement delta and gamma range (2.5-4 Hz) for relevant locations during the active block. This suggests a top-down modulation of alpha-beta is critical for suppressing activity in the

primary auditory cortex associated to non-relevant locations. Moreover, our results show flexible modulation of these rhythms when introducing new relevant locations. We further discovered these changes were associated with changes in physiological measures of attentional load including location specific changes in pupil diameter. Single unit analyses revealed that the active condition alters the preferential tuning of the neural population in the active block. Finally, our preliminary findings indicate that changes in these frequency bands emerge differentially across cortical layers, and we are currently exploring how these changes in rhythms contribute to the discriminability of sound location by modulating the activity of single neurons in the auditory cortex.

Disclosures: M. Irani: None. **Z. Qu:** None. **K.K. Sen:** None. **S. Sadaghiani:** None. **H.J. Gritton:** None.

Poster

PSTR184: Mechanisms of Attention

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Support: CIHR Project Grant
NSERC Dicovery Grant

Title: Dorsal Raphe Serotonin Neurons Encode Visual Attention Signals in Mice

Authors: *J. LEHNERT[1], K. CHA[2], A. KHADRA[2], E. P. COOK[1], A. KRISHNASWAMY[1]; [1]McGill Univ., Montreal, QC, Canada; [2]Dept. of Physiol., McGill Univ., Montreal, QC, Canada

Abstract: Visual perception arises when internal states of the brain focus our visual system on the most behaviorally salient stimuli. The neuromodulator serotonin (5HT) is among the most important carriers of such state information, but little is known about how its release in visual areas impacts the processing of visual-guided behavior. Here we show that 5HT release by the dorsal raphe (DR) regulates visual attention and detection in the awake and behaving mice. We trained mice to perform a cued visually-guided detection task in which mice search for a 3-bar grating pattern atop dynamic checkerboard noise, and simultaneously collected fiber photometry recordings of DR neural activity. We found that DR activity decreases when animals attend to a screen to detect the grating stimulus. By employing a genetically encoded sensor of 5HT release, we found that this decrease in DR activity corresponds to a drop in 5HT release in mouse visual cortex. By using optogenetic actuators to elevate or suppress DR neural activity while mice perform our tasks, we demonstrated that suppression of DR activity enhances attention and stimulus detection, whereas elevation of DR activity attenuated attention and stimulus detection. These results demonstrate that DR-derived 5HT conveys a novel attentional modulation to visual cortex and influences detection of visual stimuli. These results provide a new framework to understand 5HT neuromodulation in visually-guided behavior and provide an entry point to

study diseases (eg. autism spectrum disorder) in which 5HT signaling and visual perception are disrupted.

Disclosures: J. Lehnert: None. **K. Cha:** None. **A. Khadra:** None. **E.P. Cook:** None. **A. Krishnaswamy:** None.

Poster

PSTR184: Mechanisms of Attention

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Program #/Poster #: PSTR184.10/O13

Topic: H.01. Attention

Title: Investigating Anesthetic Mechanisms, Insights from Midazolam, Ketamine, and their Combination on Cortical Neural Pathways

Authors: *F. HAIDER;
Technion, Haifa, Israel

Abstract: Understanding the intricate mechanisms underlying general anesthetic-induced loss of consciousness (LOC) continues to pose a significant challenge in biomedical research. Recent investigations have revealed the distinct impacts of anesthetics on thalamo-cortical (TC) and cortico-cortical (CC) pathways. Despite advancements in identifying the molecular targets of anesthetics, their specific effects on cortical neural networks remain unresolved. This study focuses on the examination of Midazolam and Ketamine, two widely used sedative-anesthetic agents. Midazolam, a GABA agonist, is known for its amnestic properties and safety. Ketamine, an NMDA receptor antagonist, induces dissociative anesthesia. This research aims to elucidate the effects of Ketamine, Midazolam, and their combination on post-synaptic responses in TC and CC pathways. Hypothesizing a preferential effect on CC responses, we directly measured anesthetic effects on ascending and descending pathways terminating in the primary auditory cortex (A1). Specifically, we gauge excitatory post-synaptic potentials (EPSP) in layer 5 cortical pyramidal neurons following stimulation of the TC and CC pathways. Our findings reveal significant differences in their impacts: Ketamine notably reduces CC responses, while Midazolam enhances both TC and CC responses. The statistical analysis shows Ketamine significantly reduces EPSP response to TC and CC stimuli. Particularly, at 200μM ketamine, the EPSP response decreased from a control value of 6.22±2.55mV to 5.55±2.84mV, and further decreased to 4.14±2.47mV at 400μM ketamine, with statistically significant differences observed in both cases ($p < 0.05$). Conversely, Midazolam's effect on the CC response was smaller than the effect on TC response (CC: 5.82±3.31mV in control, 8.30±2.87 mV with 250ng/ml midazolam and 8.05±3.80mV with 500ng/ml midazolam, TC: 3.09±2.63mV in control, 5.05±3.33mV with 250ng/ml midazolam and 5.53±4.79mV with 500ng/ml midazolam), the EPSP changes were all significant for both doses ($p<0.001$). Additionally, the combination of Midazolam and Ketamine has a complex effect on EPSP responses. At low doses, the TC response significantly increases (4.40±2.04 mV), while at high doses, it returns to baseline

(3.52±2.14mV). Conversely, the CC response remains stable at low doses (3.68±2.10 mV) but decreases at high doses (2.17±1.52mV). The analysis reveals that CC pathways are more sensitive to anesthetic effects compared to TC pathways, despite differing impacts of the drugs. This underscores the crucial role of cortico-cortical feedback in loss of consciousness mechanisms, modulated differently by each drug.

Disclosures: F. Haider: None.

Poster

PSTR184: Mechanisms of Attention

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Title: Noradrenergic modulation of population activity in the primary somatosensory cortex, striatum, and amygdala in a tactile selective detection task

Authors: *C. KELLEY, C. SLATER, M. SORRENTINO, D. NOONE, Q. WANG; Biomed. Engin., Columbia Univ., New York, NY

Abstract: Selectively responding to behaviorally relevant stimuli and ignoring distractions is essential for survival in dynamic environments. Primary somatosensory cortex (S1) processes relevant and irrelevant stimuli, striatum mediates sensorimotor transformations, and amygdala processes the emotional valence of rewards. Noradrenergic modulation is known to enhance representations of relevant stimuli and regulate arousal, but its role in selective detection remains unclear. To investigate this role, we crossed DBH-Cre and Gq-DREADD mouse lines to selectively express excitatory DREADD receptors in noradrenergic neurons. Mice were trained to selectively respond to whisker deflections on a target side by licking a water spout within a window of opportunity. Successful responses were rewarded with water. Deflections on the other side were not rewarded, requiring mice to withhold licks to avoid a time out. Chronic Neuropixels 1.0 implants recorded spiking activity in S1, striatum, and amygdala contralateral to the target stimulus. On hit trials, stimuli were followed by sustained spiking across cortical layers. Spiking activity also increased following target stimulus and reward in striatum and amygdala. Single units were classified by their spike waveforms: positive spiking (PS), triphasic spiking (TS), compound spiking (CS), fast spiking (FS), and regular spiking (RS). FS and RS cells in S1 exhibited strong stimulus encoding. Striatal RS cells which were driven by target stimuli (significantly higher firing rate 200 ms after stimulus) exhibited the strongest stimulus

encoding. We observed significantly higher spontaneous spiking variablilty in RS cells before correct trials than before incorrect trials across regions.
To understand the effects of noradrenergic stimulation, mice were injected with deschloroclozapine (DCZ) or saline in randomly selected sessions. We showed that noradrenergic stimulation promoted sustained population activity during the response window across regions and resulted in greater population activity immediately following reward in striatum and amygdala on hit trials. DCZ also altered the fraction of cell types which were driven or suppressed by target stimuli. The fractions of PS and TS cells driven by target stimuli increased and the fraction of FS cells driven by target stimuli decreased in S1 with DCZ. In striatum the fraction of FS and TS cells driven by target stimuli increased, while the fraction of all stimulus driven cell types increased in amygdala. Together, our preliminary results suggest that noradrenergic stimulation has a profound effect on neural activity in those task-relevant brain structures.

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Poster

PSTR184: Mechanisms of Attention

Location: MCP Hall A

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Program #/Poster #: PSTR184.12/O15

Topic: H.01. Attention

Title: Arousal-linked levels of norepinephrine and acetylcholine in the cortex change across the lifespan in a mouse model of Alzheimer's Disease

Authors: *E. NEYHART[1], N. ZHOU[2], J. CHIN[3], J. REIMER[2];
[1]Baylor Col. of Med., Houston, TX; [2]Neurosci., Baylor Col. of Med., Houston, TX; [3]Dept. of Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Spontaneous transitions between high and low arousal, indexed by behavioral variables like pupil size and locomotion, have been directly linked to cortical levels of acetylcholine (ACh) and norepinephrine (NE). Deterioration of these neuromodulatory systems and accompanying deficits in attention have also been implicated in neurodegenerative diseases such as Alzheimer's Disease (AD). However, we don't yet have a clear picture of how release and clearance of NE and ACh in the cortex change over the lifespan, and pinpointing the precise timepoint when deviations occur in a disease model could help us understand the mechanism behind attention and memory deficits. The current study used in vivo 2-photon imaging to record spontaneous activity of ACh and NE using GACh3.0 and GrabNE2h in the cortex of J20 and age-matched non-transgenic mice at various ages. We found that the magnitude of ACh & NE release during a transition to a high-arousal state indexed by run onset differed between the two genotypes and at different ages, as well as differences in the clearance time of these two

neuromodulators at run offset. Similarly, during quiescent states marked by lack of movement, the magnitude of neuromodulator release at the moment of pupil dilation differed between the two genotypes. We further recorded activity of the locus coeruleus (LC) and NE activity simultaneously by imaging the red-shifted rNE sensor along with cortical-projecting LC axons expressing GCaMP, and found differences in the timing of the relationship between LC activity and NE activity.

Disclosures: E. Neyhart: None. **N. Zhou:** None. **J. Chin:** None. **J. Reimer:** None.

Poster

PSTR184: Mechanisms of Attention

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Program #/Poster #: PSTR184.13/O16

Topic: H.01. Attention

Title: Insula-frontal cortical interactions during a touchscreen-based attention task (rCPT) in mice

Authors: *A. E. HARR[1], S. SURESH[2], H. L. HALLOCK[3];
[1]Lafayette Col., Easton, PA; [2]Lafayette Col., Easton, PA, ; [3]Neurosci., Lafayette Col., Easton, PA

Abstract: Previous work has demonstrated that the locus coeruleus and frontal cortex functionally interact during a rodent analog of the touchscreen-based continuous performance test (rCPT) of attention (Hallock et al., 2024). However, it is unlikely that this circuit is the only circuit that contributes to successful rCPT performance. Many other brain areas send efferent projections to the locus coeruleus and frontal cortex, suggesting that these two brain regions function as part of a broader network of areas that supports behavior during this task. In order to identify such regions, we used a dual viral-targeting strategy to express either the red fluorescent protein tdTomato or green fluorescent protein (GFP) in neurons with axonal output to both the locus coeruleus and frontal cortex. To do this, we injected either a retrograde virus expressing tdTomato (AAVrg-CAG-tdTomato) or a retrograde virus expressing GFP (AAVrg-hSyn-EGFP) into either the locus coeruleus or frontal cortex of adult mice, and looked in whole brain slices for areas of overlapping fluorescence. One such area that we identified was the anterior insula. The anterior insula has been heavily implicated in attentional processing, but its role in the rCPT remains to be elucidated. To identify neural correlates of behavior in the anterior insula network during rCPT performance, we implanted stereotrodes into both the anterior insula and frontal cortex of male and female mice. We recorded local field potentials (LFPs) from both regions simultaneously during rCPT habituation, early training, overtraining, and a probe session in which attentional demand was increased. Our findings will shed light on the neural mechanisms of attention in a rodent task that has high translational validity for human tests of attention.

Disclosures: A.E. Harr: None. **S. Suresh:** None. **H.L. Hallock:** None.

Poster

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Topic: H.01. Attention

Support: NIH Grant R21MH130066

Title: Effect of chemogenetic manipulation of locus coeruleus (LC)-projecting prelimbic cortex (PrL) neurons on sustained attention

Authors: *J. J. REHG, J. A. MIRANDA-BARRIENTOS, K. MARTINOWICH;
Lieber Inst. for Brain Develop., Baltimore, MD

Abstract: Sustained attention, the ability to focus on a stimulus or task over an extended period, is a fundamental cognitive process that is impaired in individuals diagnosed with several neuropsychiatric disorders, including attention-deficit/hyperactivity disorder, schizophrenia, and depression. Of the many tasks developed to assess sustained attention, the continuous performance task (CPT) is the most frequently used in both clinical and research contexts. Recently, a translational rodent version of the human CPT (rCPT) was developed as a touchscreen based operant task that requires discrimination between target and non-target visual stimuli over an extended testing session (Kim et al., 2015). Various modifications to the rCPT, such as extended sessions or degraded visual stimuli, have been made to increase the cognitive load of the task and determine their effect on sustained attention. We recently demonstrated that stimulus degradation impairs performance on the rCPT by increasing the number of incorrect responses (DeBrosse et al., 2023). While attentional processes recruit a broad network within the brain, functional and electrophysiological studies have highlighted the role of the prelimbic cortex (PrL), and the locus coeruleus (LC) as a key nodes for attentional control (Wu et al., 2017, Sara, 2016). The PrL sends projections to the LC, and we recently reported that PrL oscillations lead the LC during incorrect responses in mice performing the rCPT (Hallock et al, 2023). However, the limited spatial resolution and source ambiguity of LFPs make it difficult to infer the cellular mechanisms underlying this signal. To test the causal role of PrL-LC circuit in sustained attention, we chemogenetically manipulated the activity of PrL-LC projection neurons in mice performing the rCPT by selectively expressing either excitatory (hM3Dq) or inhibitory (hM4Di) DREADDs in this population. We found that chemogenetic manipulation of PrL-LC projectors altered the number of false alarms during a degraded stimulus probe, demonstrating a role for this population in regulating accuracy in the task. To dissect the cellular and temporal mechanism by which chemogenetic manipulation of PrL-LC projectors control false alarms in rCPT, we are currently performing in vivo Ca2+ imaging recordings of PrL-LC projectors during rCPT.

Disclosures: J.J. Rehg: None. **J.A. Miranda-Barrientos:** None. **K. Martinowich:** None.

Poster

PSTR184: Mechanisms of Attention

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NIH R00 EY025768

Title: Characterizing LPFC Response Properties that Contribute to Attentional Dynamics

Authors: *J. C. GONZALEZ-AMORETTI, A. C. SNYDER;
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Abstract: Selective attention is a perceptual mechanism that allows organisms to prioritize objects in an environment filled with continuous streams of stimuli. Selective attention is dependent on higher-level structures exerting top-down modulation of bottom-up signals processed by lower-level structures. Structures within and around the lateral prefrontal cortex (LPFC) play a role in generating top-down influence of responses in lower- and intermediate-level visual processing regions, such as MT, V4 and IT, among others. Visual information diverges early in different pathways dedicated to processing spatial and feature signals. However, these pathways eventually converge on the LPFC, suggesting an integration of these spatial and feature signals. This projects aims to understand the mechanisms of signal integration within the LPFC which then enable top-down modulation mechanisms of lower-level visual processing regions. We hypothesize an interplay between regions in LPFC, where the integration and transformation of visual signals are implemented by mixed-selective population dynamics. The first aim of this project is to characterize the response properties of sub-populations in the ventral prearcuate region (VPA) and the frontal eye fields (FEF) during a visual search task designed to disengage feature-based attention (FBA) and spatial attention (SA). We hypothesize degrees of mixed-selective responses in both regions where preference may vary along a spatiofeatural index (SFI) based on the recorded region, task demands or different periods within a trial. We used singular value decomposition (SVD) to characterize response properties of neurons at an individual- and at a population-level. This allows us to compare individual responses of neurons to the broader population-level response, as well as the contribution of each recorded neuron to the collective response. By pairing the SVD and our SFI scoring system we can find how different sub-population responses in LPFC vary based on preferences to feature (*what* object is shown), space (*where* an object is shown) or both. We expect to find higher degrees of mixed-selectivity, suggestive of a role in integrating feature- and spatial-signals. These results will help us understand how specialized sub-populations contribute to population dynamics that serve to encode internal representations of task-relevant search templates. Following this aim, we will further analyze the temporal dynamics of these different sub-

populations during single-trials to understand the foundation in which visual signals are transformed into spatial-coordinates to enable oculomotor behavioral responses.

Disclosures: J.C. Gonzalez-Amoretti: None. **A.C. Snyder:** None.

Poster

PSTR184: Mechanisms of Attention

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.16/O19

Topic: H.01. Attention

Support: NIH Grant R01 NS120987
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Title: Sex differences in striatal dopamine dynamics after acute amphetamine exposure in mice.

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Abstract: There are no pharmacological treatment options for many types of substance use disorders (SUD) including amphetamine use disorder. Investigating individual differences in SUD has the potential lead to more effective options. For example, large sex differences exist throughout SUD, which females transition quicker from initial use to dependance and have higher instances of relapse. However, sex differences in the neural mechanisms underlying altered cognition throughout SUD are unknown. To understand the sex differences in SUD-related cognitive impairments, we trained mice on an interval timing task, which requires working memory and attention. We quantified striatal dopamine dynamics with dLight, a florescent dopamine indicator, in the dorsal striatum during the interval timing task. We observed robust modulation in dLight dynamics during interval timing in the striatum. Strikingly, temporal and reward signals were attenuated by amphetamine (1.5 mg/kg). Finally, males and females can have distinct dopamine dynamics, and we found that dLight signals related to trial-start differed between the sexes. Understanding dorsal striatal dopamine dynamics during amphetamine exposure will inform SUD treatments and could provide insight into sex-specific mechanisms.

Disclosures: H. Stutt: None. **M.A. Weber:** None. **A. Bova:** None. **N.S. Narayanan:** None.

Poster

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Support: NIH Brain Initiative Grant U19NS107616
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Title: Differential effects of forebrain and brainstem cholinergic systems on attentional function

Authors: B. GAMALLO LANA[1], C. F. LABORC[1], P. LEONE[1], R. P. MACHOLD[1], *A. MAR[2];
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Abstract: Acetylcholine (ACh) plays an important neuromodulatory role in regulating arousal and cognition. Brain ACh derives from two main sources: the basal forebrain and the mesopontine tegmentum in the rostral brainstem. While growing evidence supports the importance of these brain areas in cognitive performance, little is known about the specific contributions of ACh signaling from these two regions to visual discrimination and attention. To examine this, we assessed mouse models in which choline acetyltransferase (ChAT) - an enzyme essential for ACh synthesis - was conditionally knocked out (cKO) using region-specific genetic approaches, thereby selectively eliminating ACh production in the forebrain (f-ChATcKO, brainstem (b-ChATcKO), or both (fb-ChATcKO) regions. We tested adult male and female ChAT-cKO model mice on a touch screen rodent continuous performance test (rCPT), during which a sequence of visual patterned images are presented and the mouse must discriminate and respond selectively to target over nontarget images to maximize rewards. We found that, relative to wildtype littermate controls, f-ChATcKO mice had a significantly increased false alarm rate and reduced discriminative sensitivity (d'), whereas b-ChATcKO mice had a decreased hit rate with an increased response criterion (c). Double fb-ChATcKO mice exhibited profound performance deficits consistent with a combined effect of the f-ChATcKO and b-ChATcKO behavioral impairments. No deficits were observed in a 2-alternative forced choice visual discrimination task, suggesting that the rCPT performance phenotypes observed were related to the attentional demands of selecting among rapid, sequentially-presented visual patterns. These data support a model in which forebrain and brainstem cholinergic systems exert differential and additive contributions to visual attentional function.

Disclosures: B. Gamallo Lana: None. **C.F. Laborc:** None. **P. Leone:** None. **R.P. Machold:** None. **A. Mar:** None.

Poster

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Title: Cholinergic modulation of frontal sensory cortical projections is associated with post-error attention adjustment

Authors: *T. NISHIOKA[1], A. SERRATELLI[2], S. ALLEN[3], K. J. NORMAN[4], P. MACCARIO[5], Y. LI[6], H. MORISHITA[7];
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Abstract: The frontal cortex, especially the anterior cingulate cortex area (ACA), is essential for exerting cognitive control after errors, but the mechanisms that modulate ACA neurons to improved performance after errors are poorly understood. Among neurons in ACA, our recent study found that top-down frontal sensory cortical projections to the visual cortex (ACAvis) causally contribute to post-error attentional enhancement (Norman et al Neuron 2021). However, little is known about the inputs that activate the ACAvis neurons and how they contribute to post-error attentional adjustment. Here, we focused on glutamate, dopamine, acetylcholine (ACh), and norepinephrine, known as excitatory inputs onto ACAvis neurons, to elucidate which input is crucial for post-error attentional enhancement.To investigate how excitatory inputs onto ACAvis neurons contribute to attentional adjustment, we employed an intersectional viral approach to selectively express the various biosensors in the ACAvis neurons and performed fiber photometry imaging from mice performing the 5-choice serial reaction time task (5-CSRTT). Fiberphotometry imaging in ACAvis neurons showed that unlike glutamate, dopamine, and norepinephrine, ACh input is preferentially elevated toward the end of the anticipatory period immediately before an animal makes correct choices after error trials. Consistently, calcium imaging showed ACAvis neurons are also activated at the same timing during post-error correct trials.Our findings underscore the pivotal role of cholinergic inputs onto ACA neurons in cognitive control by enhancing attentional performance following errors. This elucidates a novel mechanism underlying post-error attentional adjustment, shedding light on potential targets for cognitive enhancement strategies.

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Poster

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SFARI pilot award #977966

Title: Altered history-dependent frontal-visual cortical projection activity underlies visual attention deficits in a mouse model of Fragile X Syndrome.

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Abstract: Attention deficits in Fragile X syndrome and in the relative mouse model (Fmr1KO) have long been demonstrated, but the underlying cognitive circuit disruptions remain unclear. Previously, frontal-visual projection activity before stimulus presentation was found to be essential for attention function, specifically following error trials. Moreover, we recently demonstrated in mice that frontal-visual projection neurons undergo a developmental shift in local input connectivity during adolescence that is disrupted in Fmr1KO mice, leading to a persistent hyperlocal connectivity state in adulthood. To understand how this increased local connectivity promotes attention deficits in Fmr1KO mice, we utilized fiber photometry recording of calcium and glutamate signaling in frontal-visual projections in adult Fmr1KO mice during 5 Choice Serial Reaction Time Task (5CSRTT) to monitor circuit activity. Previously, our lab found that frontal-visual projection calcium activity during the intertrial interval (ITI), before the stimulus is presented, in WT (Wildtype) mice was highest in correct trials following errors (E-C) during the late (2.5-5 seconds) ITI. However, in Fmr1KO mice, we found that calcium signaling during the ITI was significantly driven by post correct performance history (C+1). Similarly, glutamate input onto frontal-visual projection neurons during the ITI in both WT and Fmr1KO mice were highest following correct trials. These findings demonstrate that frontal-visual projection activity is altered in adult Fmr1KO in the context of failed maturation of local connectivity. Moreover, the output calcium activity of projections in Fmr1KO is shifted closer to the glutamatergic input dynamic which may be a consequence of retained hyperlocal connectivity.

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Poster

PSTR184: Mechanisms of Attention

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Topic: H.01. Attention

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Title: Validation of RAIL behavioral training system for freely behaving mice on complex visually guided decision tasks

Authors: *D. MURALIKRISHNAN[1], N. B. KOTHARI[1], B. A. D. HOLT[1], S. P. MYSORE[2]; [1]Johns Hopkins Univ., Baltimore, MD; [2]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Research in our laboratory has recently developed touchscreen-based tasks for the study of visuospatial selective attention and decision-making in freely behaving mice using commercially available operant boxes. Investigating the neural circuit bases of these behaviors necessitates the use of a range of cell-type and projection-specific interrogations, in turn requiring a large number of well-trained mice. A significant obstacle to high-throughput training of large cohorts of mice, however, is the very high cost of commercial behavioral training systems. In response, by making use of off-the-shelf hardware and open source software, we have developed the Rodent Automated and Integrated Learning (RAIL), which offers a promising alternative for large-scale studies. In this project, we present a detailed comparison of behavioral outcomes between the established commercial system that we have used previously, and the emerging RAIL system. Our focus is particularly on the efficacy in conducting step-by-step, multistage behavioral shaping for complex visually guided tasks (such as forced-choice orientation discrimination). By examining the performance and utility of both systems within this specific training paradigm, our results showcase the feasibility and effectiveness of training rodents on complex visually-guided tasks using the low-cost and scalable RAIL system.

Disclosures: D. Muralikrishnan: None. **N.B. Kothari:** None. **B.A.D. Holt:** None. **S.P. Mysore:** None.

Poster

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Support: 1R21MH128601
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Title: High-throughput system for parallelized training of freely behaving mice on touchscreen based tasks

Authors: *B. A. D. HOLT, S. P. MYSORE;
Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Training large cohorts of freely behaving mice on visually-guided tasks can greatly facilitate investigation of the underlying cell-type and projection-specific neural bases during naturalistic behavior. For efficient high-throughput training (of >20 mice at a time), hardware and software infrastructure capable of running complex processes in parallel is essential. Yet, such commercial systems for visually-guided tasks in unrestrained mice are prohibitively expensive and contain proprietary components. Previously, we have shown proof-of-principle for how Internet of Things (IoT) h/w and open-source s/w may be integrated into a modular and cost-effective solution for automated operant training. However, the movement from a single prototype to an operational network of a large number of training boxes is more than the multiplication of its parts. Given the diverse networking and security environments in which users will be running experiments, as well as the nature of continuously changing commercial ecosystems for h/w and s/w, it is important to create a solution that is both reliable at scale and capable of evolution. The first major challenge is the expansion of the s/w infrastructure. Here, we present a refined and optimized community version of our Rodent Automated and Integrated Learning (RAIL) software system, redesigned for cross-platform compatibility, ease of use, and practical scalability. This system is capable of synchronous communication and control for parallelized training operations at scale. To improve accessibility and ease of use of our RAIL software, we have developed both a desktop application capable of offline access to device h/w and a web-based application capable of real-time access. Through consultation with industry professionals, we have audited, stress-tested, and optimized this s/w at scale to meet current industry standards and comply with best practices. The second major challenge is on the h/w side. In our newest version of RAIL hardware, we have improved upon our recommended calibration techniques and transitioned from machine shopped parts to 3D-printable designs for the reduction of scaling costs and to enable adoption by users without access, or expertise to operate, workshop machinery. Additionally, RAIL hardware and software are designed to be modular and mutable, allowing for ease of synchronized interaction with modern tools of neuroscientific interrogation during behavioral tasks, such as 1p calcium imaging or optogenetics. Overall, our RAIL system aims to reduce both financial and technical barriers for entry into neuroscientific research on visually guided behavior in unrestrained mice.

Disclosures: B.A.D. Holt: None. **S.P. Mysore:** None.

Poster

PSTR184: Mechanisms of Attention

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Title: Accuracy of winner-take-all in saliency detection

Authors: *O. HENDLER[1], R. SEGEV[2], M. SHAMIR[3];
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Abstract: Visual search involves examining an environment to locate specific target objects amidst numerous irrelevant distractors. A commonly accepted theory is that saliency detection is governed by a winner-take-all (WTA) competition between contextually modulated cells that form a saliency map. However, existing research suggests that the WTA mechanism's capability to aggregate information from large populations is limited, raising questions about its effectiveness in visual search tasks. In our investigation, we performed a modeling study to assess the accuracy of WTA in detecting the salient object in a pop out task. We analyzed two algorithms: a single-best-cell WTA, which bases its decision on the most active neuron, and a generalized population-based WTA, where the decision is made by a collective of active neurons. In both cases, our findings demonstrate that the performance of the WTA models does not match the high accuracy observed in behavioral experiments. Specifically: WTA was significantly impacted by neuronal heterogeneity. While robust to heterogeneity, population WTA was affected by experimentally reported neuronal noise correlations. Given these insights, we suggest that the traditional understanding of the winner-take-all (WTA) mechanism needs revising. Our ongoing research is focused on determining whether leveraging heterogeneity to counteract noise correlations can improve the accuracy of the WTA model in a biologically realistic manner. Previous studies have indicated that the detrimental effects of noise correlations on population codes might be mitigated by capitalizing on heterogeneity-strategically utilizing the most informative neurons while minimizing input from less informative ones. This strategy, however, requires fine-tuning that may not be biologically plausible. Our findings necessitate a reevaluation of the mechanisms driving pop out visual search and underscores the need for additional experimental studies to further investigate the underlying readout mechanisms of saliency detection.

Disclosures: O. Hendler: None. **R. Segev:** None. **M. Shamir:** None.

Poster

PSTR184: Mechanisms of Attention

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Title: Functional stability and recurrent STDP in rhythmogenesis

Authors: *G. SOCOLOVSKY[1], M. SHAMIR[2];
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Abstract: Stable neural activity plays a pivotal role in various cognitive, behavioral, and physiological functions, including information processing, memory consolidation, behavioral regulation, and attentional mechanisms. The orchestration of such stability is governed by the intricate interplay of synaptic connections within neural populations. Extensive theoretical and empirical investigations have underscored the influential role of synaptic weights in shaping both the collective dynamics and the fluctuations of neural activity. A salient inquiry arising from this understanding pertains to the mechanism responsible for fine-tuning synaptic weights to values conducive to neural stability. In our conjecture, we posit asymmetrical Spike-Timing-Dependent Plasticity (STDP) as a plausible candidate underlying this tuning process. STDP claims that the modification in synaptic efficacies depends on the time interval between pre and post synaptic spikes. In our theoretical study, we employed a firing rate model to describe the dynamics of a recurrent neural network comprising excitatory and inhibitory neuronal populations. The synaptic plasticity dynamics were governed by the temporal overlap between neural activity correlations and the STDP learning rule. Our investigation revealed that STDP originates a robust emergence of critical rhythmogenesis—a phenomenon characterized by intermittent rhythmic neural activity—while concurrently fostering neural stability. Furthermore, STDP facilitates synaptic fluctuations that preserve this stable behavior.

Disclosures: G. Socolovsky: None. **M. Shamir:** None.

Poster

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Title: A causal role for superior colliculus in spatial attention modulation in pulvino-cortical pathway

Authors: *Y. WU[1], R. BOSHRA[1], M. HARRIS[1], M. RODRIGUEZ[1,2], M. BERG[3], S. KASTNER[1];
[1]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [2]Princeton Neurosci. Inst., Princeton, NJ; [3]Univ. of Washington, Seattle, WA

Abstract: The superior colliculus (SC), a main area for saccade generation, is thought to be involved in target selection and spatial attention control. Recent studies show that a newly identified node of the attention network, the dorsal posterior inferotemporal area (PITd), is

strongly modulated by SC during spatial attention. However, the temporal dynamics and the subcortical pathway of such a high-level modulation signal from the midbrain to PITd are still unknown. We hypothesized that the projections of SC to pulvinar may be instrumental in the attentional modulation of extrastriate cortex. To investigate this idea, we reversibly inactivated SC with muscimol (a GABA agonist), and simultaneously recorded spiking activities and local field potentials (LFPs) from SC, pulvinar and PITd with dense linear electrode arrays, while two macaques performed an object- and spatial-attention task (i.e. Egly-Driver task). In each trial, two horizontal or vertical bar-shaped objects were presented on each side around a central fixation point. A spatial cue was presented around the edge of one bar end to indicate the location of the near-threshold visual target (contrast change) with 80% cue validity. After a random period (300-1500 ms cue-to-target interval), a valid (same location) or invalid (the other end of the same object or the end of the opposite object with the same distance) target was presented at threshold. Monkeys released a lever upon detection to receive a juice reward. Monkeys' hit rates were around 75% and exhibited 11.5% attention facilitation. Muscimol was injected into the middle and deep layers of SC, which resulted in increased saccade latencies to the affected visual field (as measured in a delayed saccade task), and sometimes mild nystagmus. After SC inactivation, the monkeys' overall hit rate significantly decreased by 6%, and the attentional facilitation effect was reduced. The attention modulation of firing rates at the attended location (relative to an equidistant uncued location) also decreased in both pulvinar and PITd after SC inactivation. This change was primarily due to a response facilitation in the unattended condition as compared to firing rates in the same condition before muscimol injection. Our preliminary findings suggest that SC provides suppressive modulation to distractors through the pulvino-cortical pathway and underlines both SC and the transthalamic pathways as important parts of the attention network.

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Poster

PSTR184: Mechanisms of Attention

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Topic: H.01. Attention

Title: Modulation of attention rhythms following systemic administration of methylphenidate

Authors: *M. J. HARRIS[1], R. BOSHRA[2], B. MOREA[1], S. KASTNER[3];
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Abstract: While the cellular mechanisms for pharmacological treatments of attention disorders like methylphenidate (Ritalin) and dextroamphetamine (Adderall) have been thoroughly researched, it remains less clear how these compounds affect specific characteristics of

attentional modulation while it is being deployed. As a means to better understand the dimensions along which psychostimulants modulate attention-related behaviors, methylphenidate was administered systemically to two rhesus macaques, previously trained to perform a spatial attention task - the Egly-Driver task. In particular, it was unknown whether the rhythmic property of spatial attention allocation observed during the Egly-Driver task's variable cue-to-target (CT) interval may be one such dimension affected by methylphenidate. Preliminary results indicate that methylphenidate modulates attentional sampling of potential target locations. Rhythmic fluctuations in behavioral performance were calculated using a shifting 50-ms time bin across the CT window (350-1600 ms), in steps of 10-ms. Trial-wise permutation testing (1,000 iterations) of the behavioral data was performed to establish values of statistical significance for the frequencies of behavioral rhythms. Following methylphenidate, attentional theta rhythms (4-5 Hz) were reduced below significance at the cued target location. In contrast, enhanced amplitudes of higher frequency behavioral oscillations (9-10 Hz) at equidistant non-cued target locations emerged. Interestingly, the changes in frequency of the behavioral rhythm were independent of changes to hit rate, which remained relatively constant between conditions. This shift between cued and uncued location frequencies following methylphenidate may highlight one potential property of psychostimulants to alter the way in which underlying neural dynamics support flexible attentional sampling of the environment at the behavioral level.

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Poster

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C.V. Starr

Title: Population spiking and field potentials in superior colliculus and frontal eye fields exhibit theta coordination during covert spatial attention

Authors: *R. BOSHRA[1], M. HARRIS[2], B. MOREA[3], K. BANAIE BOROUJENI[4], K. DOUGHERTY[4], M. RODRIGUEZ[3], M. BERG[5], S. KASTNER[4];
[1]Princeton Neurosci. Inst., Princeton, NJ; [2]Neurosci., Princeton Neurosci. Inst., Plainsboro, NJ; [3]Princeton Univ., Princeton, NJ; [4]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [5]Vet. Services, Washington Natl. Primate Res. Ctr., Seattle, WA

Abstract: Covert attention enables prioritized processing of stimuli to meet task demands. Recent findings suggest that spatial attention is dynamic in time, exhibiting theta (4-8) rhythms

observed in behavior and in underlying neural signals. The explicit manner by which attention control signals are coordinated by these rhythms across cortical and subcortical nodes is not well understood. Here, we investigate local population activity and coupling between the frontal eye fields (FEF), a primary cortical attention node in prefrontal cortex, and the superior colliculus (SC), a midbrain region predominantly associated with oculomotor control and implicated in attention control. We investigate whether SC exhibits rhythmic signatures of attention previously reported in cortex and thalamus. Specifically, we hypothesize that SC and FEF will be strongly modulated by attentional theta and that SC modulation will be especially dominant during attentional shifts. To probe our questions, we trained two rhesus macaques to perform a modified Egly-Driver task. We recorded neural spiking and local field potentials (LFPs) from FEF and SC synchronously using dense 128-channel probes. We inspected local theta oscillations and cross-frequency coupling in both areas and the relationships between neural theta and behavioral performance (hit rate) to replicate previous findings in FEF and extend them to SC. Then, we utilized population decoding techniques to test our hypothesis that theta cycles alter the encoded moment-to-moment location of attention in population level spiking activity. Lastly, we inspected the cross-areal communication between FEF and SC using spike-phase coupling. Preliminary results showed that both areas exhibited a relationship between neural theta phase (4-8 Hz) and target detection (hit rate), indicating that SC's role in attention may also be coordinated by theta rhythms. Decoding analysis of spike rates prior to target (-500-0 ms) exhibited a rhythmic increase in decoded cue information that had a period of ~200 ms (5Hz) in FEF, but not SC. This finding suggests that theta modulation in SC does not stem from rhythmic changes in local firing rates. Spike-field coherence between the two areas showed an FEF-based theta increase during cued compared to non-cued trials. Our results suggest that coordination by theta activity of the cortico-thalamic attention network extends to the SC.

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Poster

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European Young Investigator Award

Title: Rhythmic perceptual sampling in awake macaque area V4

Authors: *S. PATEL[1], E. PSAROU[1], A. PETER[2,1], P. FRIES[1,3];
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Abstract: Overt sensory sampling often proceeds rhythmically by whisking, sniffing or saccading, and covert attentional sampling might similarly sample stimuli rhythmically. Rhythmic sampling is suggested by the fact that the ability of human subjects to detect a weak target is modulated by the 7-8 Hz phase of frontal EEG (Busch and VanRullen, 2010), or the 4-6 Hz phase of occipital EEG (Harris et al., 2018). Here, we aimed at investigating these EEG phenomena in more detail by using recordings of multi-unit activity (MUA) and local field potentials (LFPs) from awake macaque area V4 during performance of a detection task. Similar recordings and task have previously been used to relate pre-stimulus MUA and LFP power in areas V1, V4 and PFC to stimulus detection performance (van Vugt et al., 2018). Yet, whether detection performance fluctuates rhythmically requires to relate it specifically to the pre-stimulus phase. Estimating the phase of ongoing neuronal rhythms at the moment of stimulus presentation is challenging, because conventional phase estimation approaches require a finite-length estimation window and a taper, which unfortunately entails reduced sensitivity at the edge, i.e. just before the stimulus. To overcome this, we optimized spectral estimation at the edge by developing a new method. The method was built on previous approaches using autoregression-based extrapolation, yet compared favorably to them and other published approaches. Using this new method, we found that the pre-stimulus LFP phase at 6-10 Hz was predictive of the strength of the post-stimulus neuronal response. The post-stimulus neuronal response was in turn predictive of behavioral detection performance. These results provide a bridge from the EEG findings in human subjects to intracortical recordings in non-human primates that allow the further investigation of the underlying mechanisms. The mechanistic understanding is essential, as the direct dependence of behavioral performance on the phase of brain rhythms is most parsimoniously explained by a causal role of those rhythms.

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Disclosures: S. Patel: A. Employment/Salary (full or part-time):; Ernst Strüngmann Institute. **E. Psarou:** None. **A. Peter:** A. Employment/Salary (full or part-time):; MIT. **P. Fries:** F. Consulting Fees (e.g., advisory boards); CorTec GmbH.

Poster

PSTR184: Mechanisms of Attention

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR184.28/O31

Topic: H.01. Attention

Support: NIH Grant 1RF1NS128974-01

Title: Limitations of pupil diameter as a proxy for locus coeruleus activity

Authors: *L. W. THOMPSON[1], J. I. GOLD[2];
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Abstract: Non-luminance-mediated changes in pupil diameter can reflect neural activity patterns in multiple brain pathways, including the locus coeruleus-norepinephrine (LC-NE) system (Joshi and Gold, 2020). This relationship has been used extensively to draw conclusions about LC-NE function based on pupil measures. Many of these studies have been motivated by the known relationship between arousal and cognitive performance, which follows a Yerkes-Dodson ("inverted-U") curve, and proposed, associated LC-NE-mediated changes in neuronal gain (Ghose et al., 2024). Specifically, arousal is thought to track baseline LC activity, which at moderate levels corresponds to strong, transient task-related LC responses and better performance ("phasic mode"), but at low or high levels corresponds to weak task-driven LC responses and worse performance ("tonic mode"; Aston-Jones and Cohen, 2005). These observations have led to the suggestion that pupil diameter can serve as a proxy for LC phasic/tonic dynamics; e.g., using task-driven pupil responses to infer baseline LC activity and the associated neuronal gain (Eldar et al., 2013). However, although evoked pupil and LC responses are each inversely related to their respective baseline values, those relationships are also sensitive to other factors (e.g., biophysical properties of the iris) that may influence the link between LC activity and pupil diameter but have not been examined in detail.
Here we compared evoked and baseline measurements of LC spiking activity (N = 107 single units from 2 monkeys) and pupil diameter during passive fixation (Joshi et al., 2016). We confirmed previous findings by showing that the baseline activity of many individual LC neurons was correlated reliably with baseline pupil diameter, even after accounting for potential drift in both measures across a recording session (N = 43). Likewise, we found that baseline activity and pupil diameter were negatively correlated with their respective evoked responses to auditory beeps presented on a randomly selected subset of trials. However, after accounting for these relationships, we found that evoked pupil responses were rarely related to baseline LC activity, using either a linear (N = 7) or quadratic (N = 4) regression model. These results suggest that, at least during passive fixation, pupil-dilation responses to startling events (e.g., auditory beeps) do not provide an inverse measure of tonic LC activity. Future work is needed to determine whether similar results are obtained across a broader range of cognitive states.

Disclosures: L.W. Thompson: None. **J.I. Gold:** None.

Poster

PSTR184: Mechanisms of Attention

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Topic: H.01. Attention

Support: National Institute of Mental Health of the National Institutes of Health Award Number R01MH129641

Title: Gaze preferences predict fast learning of new attentional targets and slow disengagement from distractors during adaptive behavior

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Abstract: Gaze is preferentially directed to visual objects that are informative, rewarding, or novel. These gaze dimensions are in conflict in volatile environment when reward values of objects change, or new objects appear. For these volatile environments gaze may support learning by sampling information of newly introduced objects, or alternatively gaze might slow down learning by continuously sampling objects that are no longer rewarded.
We tested these scenarios in two nonhuman primates performing a feature-reward learning task. In blocks of 30-50 trials monkeys learned through trial-and-error which of three objects had a feature that was rewarded. Block transitions either introduced new object features, or only reassigned reward to previously shown features. In each trial monkeys freely looked at the objects before they committed choosing an object to receive reward. We quantified gaze preferences as the duration of object fixations prior to and independent of the choice of an object. Analysis was aligned to the trial within a block at which a 70% learning criterion was reached. We found that gaze preferences towards a newly rewarded object increased together with choices of that object, reaching a plateau shortly after learning completed. However, gaze to non-rewarded objects only gradually declined and continued declining after learning criterion was reached. This gaze bias varied for distractor objects that were introduced newly in the current block, shared visual features with the rewarded target object of the previous block, or shared features with distractor objects of the previous block. While gaze only gradually avoided all three types of distractors, it maintained a bias to look at newly introduced distracting objects even after the target was learned, and showed a continued bias for distractors that were previously rewarded targets. This result illustrates a long-lasting influence of relative novelty and target reward history on gaze preferences.
In summary, these findings document that gaze preferences to objects predict subsequent choices of targets and distractors during trial-and-error learning. Gaze is biased towards rewarded targets as soon as they have been learned, but gaze towards non-rewarded objects prevails. The slow disengagement from distracting objects was based on a memory bias (for objects that were previously rewarded visual objects) and on a relative novelty bias (for objects only recently introduced as new distractors). Taken together, gaze preferences predict not only the fast learning of new target features, but also the lingering influence of memory and novelty biases during adaptive behavior.

Disclosures: X. Wen: None. **T. Womelsdorf:** None.

Poster

PSTR184: Mechanisms of Attention

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Topic: H.01. Attention

Support: NIH Grant EY033726
Searle Scholars Program

Title: The neural bases of attention-related suppression in macaques

Authors: *Z. REDDING[1], J. KLEMBCZYK[2], I. C. FIEBELKORN[2];
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Abstract: Imagine searching for ingredients in a bustling market. Even in this very routine context, we are presented with a vast array of sensory information, some important and some not. Given that the brain has a limited processing capacity, it must rely on a set of filtering mechanisms to enhance the processing of behaviorally important information and suppress the processing of potentially distracting information. This collection of filtering mechanisms is broadly referred to as selective attention. Much has been learned about the neural bases of attention-related enhancement; however, despite recent advances from research using EEG/MEG with human subjects, there remains considerable debate over the neural bases of attention-related suppression. Recent evidence from humans indicates that suppression can be voluntarily deployed, independent from enhancement. Yet the neural circuits and neural mechanisms underlying this voluntary suppression remain unclear. We simultaneously recorded neural activity from the frontal eye fields (FEF), lateral intraparietal area (LIP), and extrastriate visual cortex (V4), while macaques performed a detection task. Spatially informative cues promoted anticipatory enhancement at a likely target location and/or anticipatory suppression at a likely distractor location. We compared the effects of cueing target and/or distractor locations relative to uninformative cues. Salient distractors occurred on half of the trials. Behavioral results reveal evidence of voluntary suppression. Monkeys used the distractor cue to actively suppress specific locations when the target location was unpredictable. Preliminary neurophysiological results suggest unique contributions from different brain regions, as distractor cues led to anticipatory increases in spiking activity in LIP but no change in anticipatory spiking activity in FEF. Distractor cues also led to the suppression distractor-evoked responses in both LIP and FEF. In contrast, target cues—without concurrent distractor cues—did not lead to the suppression of distractor-evoked responses (relative to distractor-evoked responses following uninformative cues). That is, we observed no evidence that target cues were associated with attention-related suppression elsewhere in the visual field. These findings (i) provide evidence of voluntary suppression, independent of attention-related enhancement and (ii) begin to reveal the neural bases of such voluntary suppression. Further analyses of the interactions between brain regions

will reveal the unique network-level interactions underlying attention-related suppression and attention-related enhancement.

Disclosures: Z. Redding: None. **J. Klembczyk:** None. **I.C. Fiebelkorn:** None.

Poster

PSTR185: Neural Mechanisms of Decision Making in Rodents

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.01/O34

Topic: H.03. Decision Making

Title: Neuronal signatures of contextual decision-making in mouse prefrontal cortex and mediodorsal thalamus

Authors: *X. WANG, D. HÄHNKE, A. RANGANATH, T. W. BERNKLAU, S. N. JACOB; Translational Neurotechnology Lab., Dept. of Neurosurg., Klinikum rechts der Isar, Tech. Univ. of Munich, Munich, Germany

Abstract: The ability to modify behavior in the face of varying task demands is a central component of cognitive flexibility. In situations with insufficient evidence, one should refrain from premeditated actions and collect additional evidence. To isolate a state of cognitive flexibility, we trained head-fixed mice on a two-alternative forced-choice task in which animals rotated a response ball to the left or right. An auditory context cue that was either predictive or not predictive for the upcoming instruction was followed by an auditory instruction cue signaling which side to rotate to. As expected, animals performed better and faster in the predictive context than in the non-predictive context. Decomposition of sub-movements following the context cue (indexing the animals' 'state of mind') revealed a preparatory bias towards the animals' preferred response side before the actual instructed movement. We hypothesized that this behavioral strategy might draw upon executive centers of the prefrontal cortex (specifically, the prelimbic cortex, PL) and the interconnected mediodorsal thalamus (MD). Therefore, we extracellularly recorded single-neuron activity in these regions (1049 total units across 4 animals; 629 in PL and 420 in MD) as the animals performed the task. During the context epoch, non-predictive and non-preferred cues induced stronger neuronal responses than the preferred cue in both regions. In non-predictive trials, PL responded to the context cue earlier than MD, while MD responded earlier in predictive trials. During the instruction epoch, instruction to the preferred side elicited stronger activity than to the non-preferred side in both regions. MD encoded the instructed side more stably than PL but did not show context-dependent changes in neuronal responses as opposed to PL. Notably, neuronal functional connectivity in PL distinguished between contexts, while in MD it distinguished between response sides. These findings suggest that mice employ optimized asymmetric behavioral strategies, supported by prefrontal-driven executive control over thalamus-driven movement planning.

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Poster

PSTR185: Neural Mechanisms of Decision Making in Rodents

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.02/O35

Topic: H.03. Decision Making

Support: NIH Grant E889140
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Title: Adaptation of counter-strategies by mice in matching pennies task

Authors: *J. INDAJANG, L. SHEN, A. C. KWAN;
Biomed. Engin., Cornell Univ., Ithaca, NY

Abstract: When playing a game against an opponent, an individual may employ a specific strategy to maximize reward. If the opponent changes their game plan, it would be advantageous for them to adapt the strategy in response to the competitive pressure. However, the neural implementation of such a flexible switch in strategy has not been well characterized. To better understand such value-based decision-making behavior in mice, we have adapted the matching pennies task, a two-player competitive game. Under the matching pennies task, we trained mice to compete for a reward against a computer opponent that will shift its strategy throughout the session. Importantly, this strategy shift is not cued to the mouse, so the mouse must adapt its behavior based only on intuition. We have demonstrated that mice can not only learn this paradigm but can also adapt their behavior when the opponent changes its strategy over time. In addition, we have silenced specific regions of the brain through chemogenetic DREADDs inactivation to assess the dependence of these regions in adaptive decision-making. Two areas of interest we perturbed are the retrosplenial cortex, a region previously hypothesized to be necessary for persistent value coding and spatial memory, and the dorsal cingulate and secondary motor sub-regions, which have been linked to matching antecedent events to current decisions. Ultimately, our work will provide new insight into how mice perceive shifts in strategies during dynamic competition, as well as illustrate which regions in the brain are important for devising counter-strategies. These results may pilot further investigation into how these networks may be modeled for adaptive decision algorithms in organisms.

Disclosures: J. Indajang: None. **L. Shen:** None. **A.C. Kwan:** None.

Poster

PSTR185: Neural Mechanisms of Decision Making in Rodents

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Simons Collaboration on the Global Brain

Title: Anterior cingulate cortex encodes spontaneously formed choice priors during perceptual decision-making

Authors: *L. T. OESCH[1], D. SANDBERG[1], J. COUTO[2], A. K. CHURCHLAND[2];
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Abstract: During decision-making, animals must select appropriate weights for incoming sensory information and internal priors (beliefs formed from previous experience). A growing body of work suggests that the anterior cingulate cortex (ACC) is required for different types of sequential and probabilistic tasks where choice priors are experimentally manipulated and are beneficial for task performance. However, far less is known about the function of ACC during instances where priors can negatively affect performance, such as when subjects spontaneously form choice priors based on incorrect beliefs about the system (for instance, that rewarded choices should be repeated). Here, we used miniature fluorescence microscopes to measure excitatory neural activity in the ACC in freely behaving mice making sensory accumulation-of-evidence decisions. Imaging was performed during expert performance on visual decisions and during learning of auditory decisions. We found that upcoming choices can be decoded from ACC activity during the inter-trial interval in novices and in experts performing trials with more ambiguous sensory evidence but not in the same experts when the sensory information was unambiguous. Decoding accuracy was higher in animals that based their decisions on previous choices and outcomes rather than sensory evidence. Because previous choice, upcoming choice, and movements can become correlated in subjects that use choice priors we used a linear encoding model to disentangle the representation of these variables in ACC neurons. We found that the representation of neither upcoming choice nor previous choice and outcome could solely be explained by movements. Together, these results suggest that ACC neurons integrate information about past choices and outcomes to form a choice prior that can be deployed when sensory evidence is ambiguous.

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Poster

PSTR185: Neural Mechanisms of Decision Making in Rodents

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Topic: H.03. Decision Making

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Title: Striatal iSPN activation and reward conflict promotes exploration in mice during a two-alternative forced choice task with probabilistic rewards

Authors: *J. BAHUGUNA[1], J. K. BADYNA[2], K. BOND[3], E. A. YTTRI[4], J. E. RUBIN[5], T. D. VERSTYNEN[6];
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Abstract: How do the decision policies during a forced choice task get modulated in response to activation of different basal ganglia pathways and environmental uncertainty remains an unsolved question. Here, we use behavioral data from mice to test the roles of indirect pathway spiny projection neurons (iSPN) and reward uncertainty in modulating decision policies in response to environmental feedback. Animals (N=9) performed a two-alternative forced choice task with probabilistic rewards with varying levels of conflict, or similarity between reward probabilities. The action outcome contingencies were changed every 10-20 trials and the striatal iSPNs were optogenetically stimulated (A2A-cre ai32 mice) while the animal performed the task. The trial-by-trial behavioral data were analyzed using a drift diffusion model (DDM) that describes the process of decision making as a noisy accumulation of evidence (with a certain drift rate) up to a decision threshold (at a certain boundary height) and provides an intuitive framework to map behavioral features into a decision policy. We explore the decision policies observed at 1) steady state, and 2) change points (reward contingency switches). In control conditions, the steady state is characterized by high drift rate and boundary height which corresponds to an exploitative decision policy with high accuracy. At change points, upon encountering errors, mice decrease the drift rate and boundary height, thereby switching to an exploratory decision policy. The sharp drops in drift rate and boundary height indicate a slowing down of evidence accumulation and relaxation of decision threshold, followed by a gradual recovery to the steady state rate and threshold. An increase in the reward conflict also pushes the overall decision trajectory into an exploratory regime (decrease in both drift rate and boundary height). iSPN-stimulation also makes the steady state and change point decision policy more exploratory (lower drift rate and higher boundary height), however, the exploration is slower relative to that observed due to high conflict and at change points. Our results suggest that while iSPN stimulation and high reward conflict both promote exploration in mice, the exploration strategies are different.

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Poster

PSTR185: Neural Mechanisms of Decision Making in Rodents

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Topic: H.03. Decision Making

Support: NIBIB R01EB029857
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NINDS R01NS115327

Title: Decision-related activity in mouse PPC during somatosensory accumulation of evidence

Authors: *D. A. WEISS[1], A. M. BORSA[2], A. KIM[3], N. H. CHANG[1], C. WAIBLINGER[1], G. B. STANLEY[1];
[1]Wallace H. Coulter Dept. of Biomed. Engin., [2]Electrical Engin., [3]Georgia Inst. of Technol., Atlanta, GA

Abstract: As we explore and interact with the world, most perceptions and decisions require integration of many pieces of evidence collected over a period of time. As the world is dynamic and changing, our brains must be able to make sense of and act upon time-varying information, such as determining if a car is hurtling towards us, and we must move to avoid it. However, even after two decades of studies on evidence accumulation, it is still unclear how neuronal populations cooperate to dynamically accumulate evidence over time towards perceptual decisions. To address this gap, we trained mice to perform a two-alternative forced choice task that requires accumulation of somatosensory evidence over time. Animals were exposed to trains of discrete, Poisson distributed random whisker stimuli to both sides of their face and tasked to discriminate which side received the higher number of stimuli over a 2 sec window, indicated through licking either left or right water ports. While animals performed the task, using silicon probes, we recorded population spiking activity in a multisensory posterior parietal cortical (PPC) region of the mouse (Rostrolateral, or RL). PPC has previously been shown to contain correlates of decision-making. As a preliminary test leading up to evidence accumulation, we evaluated whether neurons in RL contained choice specific information. In this neural population, we found neurons that are responsive to left and right choice, and from the population we were able to decode left choice, right choice, and misses (no response). Our decoders were still able to discriminate the animals' choice even when their answers disagreed with stimulus direction, i.e. incorrect responses, suggesting that the RL population is sensitive to choice over stimulus. Furthermore, by randomly permuting spike times within a trial while holding the total number of spikes constant, we found that decoding accuracy decreased significantly, but remained above chance levels. This indicates that average firing rates hold some choice-related information, but dynamic firing rates are even more informative. In ongoing work, we are modeling the underlying accumulation process with latent variable models to estimate single-trial and moment-by-moment decision variables to elucidate how the population activity relates to behavior, providing potential opportunities for real-time manipulation and optimal control of decision making.

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Poster

PSTR185: Neural Mechanisms of Decision Making in Rodents

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Topic: H.03. Decision Making

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Title: Role of orexin/hypocretin neurons in multivalent decision making

Authors: *A. L. TESMER, D. PELEG-RAIBSTEIN, D. BURDAKOV;
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Abstract: Decisions are rarely made in isolation; adaptive behaviour requires successful navigation of multivalent spaces wherein multiple options may directly compete. Brain circuitry underlying complex value-based decisions is traditionally attributed to mesolimbic dopamine and cortical circuits, whereas other subcortical nuclei, such as the hypothalamus, are much less studied. Physiological processes of motivation, arousal, and reward-seeking are long understood to be regulated by orexin/hypocretin (OH) neurons in the lateral hypothalamus. However, how OH neuron involvement in these processes translates into the context of multivalent decision-making is much less understood. Here, we use a multi-armed maze containing several options wherein freely behaving mice can choose to interact with their environment. Causal manipulations and behavioral modeling revealed that OH neurons can bias comparative decisions, but not perception, of value in our maze. Correlative photometry recordings similarly show acute phasic bursting patterns of OH neurons aligned to unique decision events. Together, our data suggests that subcortical nuclei may be far more involved in multivalent decision making than previously believed, and specifically OH neurons have an acute role in guiding comparative choices.

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Poster

PSTR185: Neural Mechanisms of Decision Making in Rodents

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Topic: H.03. Decision Making

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NIH R01 MH128746
NSF 2024776
Simons Collaboration on the Global Brain Pilot Award

Title: Neural correlates of adaptive behavioral strategies in changing environments

Authors: *Y. E. ZHANG[1], B. P. DANSKIN[2], Y. SHAO[1], M. ZHOU[1], E. UPPALAPATI[1], T. KOMIYAMA[1];
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Abstract: Value-based decisions are often guided by outcomes of similar decisions made in the past, based on the animal's subjective strategies shaped by environmental feedback. In fast-changing environments, animals rely solely on very recent experiences, whereas in slower-changing environments, they benefit from integrating a longer history of past experiences. The neural mechanisms underlying flexible history integration yielding such adaptive behavioral strategies remain largely unknown. To address this question, we designed two structurally similar dynamic foraging tasks to simulate fast-changing and slow-changing environments for mice. Mice dynamically adjusted their strategies, exhibiting reliance on very short past history in fast-changing environments and a much longer history in slow-changing environments. Next, we investigated the neural correlates of these adaptive adjustments of behavioral strategies. In our previous studies, we identified that history-encoding cortical neurons exhibit diverse neuron-specific time constants, with larger time constants encoding longer history information. In this current study, we found that when mice transitioned their behavioral strategies, these history-encoding neurons undergo a population shift of their time constants consistent with behavior. Among the six dorsal cortical regions we examined, the retrosplenial cortex (RSC) showed an overrepresentation of larger time constants when mice relied on longer history, and exhibited the most significant population shift across strategies. We propose that dynamic shifts of history integration time windows of RSC neurons may mediate adaptive changes in behavioral strategies.

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Poster

PSTR185: Neural Mechanisms of Decision Making in Rodents

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Topic: H.03. Decision Making

Support: NINDS Grant UF1NS122040-01

Title: A causal role of visuo-sensory superior colliculus in perceptual decision-making in mice

Authors: *V. THAKUR[1], E. Y. WALKER[1], M. A. BASSO[2];
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Abstract: Traditional models of decision-making propose a cortico-centric feedforward architecture for decision formation. Recent studies demonstrate the involvement of the brainstem superior colliculus (SC) in decision-making, but the results suggest varying roles in aspects of the decision process. Thus, different neuronal cell types and the microcircuits they comprise may play different roles in decision-making. Here, we investigate how one specific SC cell type - the wide field vertical (WFV) cell which projects to the lateral posterior nucleus of the thalamus - influences decisions during evidence accumulation. We hypothesize that the WFV cells of the visuo-sensory SC, are involved in decision-making, in addition to intermediate layer neurons which are well-known to play some role. We capitalized on the mouse model with its rich molecular and genetic tools to probe cell-type specific mechanisms. Trained mice performed the random dot motion direction discrimination task (RDM) and using a within-trial evidence manipulation (transient pulses of motion information), we discovered that mice perform the RDM task using an accumulation strategy like humans and monkeys. Using neuronal cell-type specific chemogenetic inhibition and hierarchical drift-diffusion modeling we found that inhibition of the activity of WFV neurons influences decision-making, creating an ipsilateral bias by altering the starting point of evidence accumulation. This finding differs from that recently found with manipulation of the intermediate layers of the SC in primates in which ipsilateral biases resulted from changes in evidence accumulation. Based on our new results we proposed that a recurrent pathway involving superficial WFV cells plays a crucial role in decision-making in addition to the intermediate layers neurons. Moreover, our new training methods in mice indicate that the mouse is a powerful and highly complementary approach to the primate model for probing how evidence-based decisions are formed in the brain and how this process and the underlying circuitry may be shared or different across species.

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Poster

PSTR185: Neural Mechanisms of Decision Making in Rodents

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Topic: H.03. Decision Making

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Title: Integration of bias and sensory information in the striatum for decision-making

Authors: *E. HWANG[1], A. OWUSU-OFORI[2,3], S. KORDE[2];
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Abstract: Many decisions we make in everyday life are guided by sensory stimuli, such as the color of traffic lights, the aroma of foods, and the sound of fire alarms. These sensory-guided decisions are also influenced by non-sensory factors including context (e.g., prior notification of fire alarm testing) and biases (e.g., familiarity or novelty biases). Here we investigate the neural circuits that mediate the interaction between non-sensory factors and sensory signals in decision-making processes. Previous studies identified that (1) the posterior parietal cortex (PPC) encodes an internal decision bias that updates based on the history of previous choices and their outcomes, and (2) this history-dependent bias information is transmitted to the striatum (STR) to influence decision-making. Intriguingly, this region of the STR also receives axonal projections from the visual cortex (VC), suggesting a potential interaction between bias information from the PPC and sensory information from the VC within the STR. In a simple additive scheme, the output from the STR would be predominantly influenced by the history-dependent bias from the PPC, in conditions of weak sensory signals (e.g., dim visual stimuli). In contrast, strong sensory signals could override this bias. This hypothesis leads to a testable prediction that inactivating the pathway from the VC to STR would weaken visual inputs to the STR, making choices less sensitive to visual stimuli and more subject to history-dependent bias.
To test this prediction, we assessed the impact of inactivating the VC-STR pathway on decision-making in mice using a standardized international brain laboratory (IBL) task. In this task, mice were presented with visual stimuli of varying contrast levels on either side of a screen and indicated the stimulus location by turning a wheel. The VC-STR pathway was optogenetically inactivated through the expression of inhibitory opsin Jaws in the VC and the implantation of fiber optic cannula in the STR. Consistent with our predictions, inactivation of the VC-STR axon terminals significantly diminished the performance of visually-guided decisions, reducing the rate of correct choices. Moreover, responses to strong visual stimuli (i.e., high contrast stimuli) were more susceptible to the influence of history-dependent bias in inactivation than control trials. In the non-opsin control group, we found no significant changes associated with light delivery to the STR. These results support our hypothesis that the STR integrates bias and sensory information transmitted from the cortex, producing complex and flexible sensory-guided decision-making.

Disclosures: E. Hwang: None. **A. Owusu-Ofori:** None. **S. Korde:** None.

Poster

PSTR185: Neural Mechanisms of Decision Making in Rodents

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR185.10/P5

Topic: H.03. Decision Making

Support: NHMRC GNT2011633
DP240100128

Title: Corticothalamic ensembles predict decision time during motivational conflict

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Abstract: Among the most difficult choices are those requiring the balance of conflicting motivational needs. A hungry foraging animal, for example, must balance the need to feed against risk of capture; the need to act speedily against acting cautiously. In a dynamic environment, where one need may be reprioritised over another on a moment-to-moment basis, decision-making requires dynamic mechanisms that integrate speeded action with choice outcomes. However, these mechanisms are not well understood. Here we describe the dynamics of identified cellular ensembles in prefrontal cortex as mice resolve competing demands.We trained mice on a linear track to approach a goal with conflicting values of reward and punishment and tracked the speed of their decisions in relation to their proximity to the goal and the outcome of their choice (e.g. approach or avoid). We applied sequential sampling models of decision making to identify mechanisms of choice under conflict. We also imaged calcium activity from individual cells in the prelimbic cortex (PL, N=1441 cells) and in output-defined PL cells (targeting midline paraventricular thalamus; PL-to-PVT, N=161 cells) during this decision-making. Agglomerative hierarchical clustering of cellular activity time-locked to the onset of approach or avoid choices in PL-to-PVT cells revealed heterogeneity among these projectors, though a majority were excitatory (39.8%; inhibitory: 29.2%; other: 31.1%). When we clustered cellular activity timed-locked specifically to avoid choices, the proportion of excitatory cells was lowest when mice were furthest from conflict (38.51%) and highest near the goal (68.9%). Conversely, for cellular activity timed-locked to approach choices, the proportion of excitatory cells was highest when mice were furthest from conflict (72.7%) and lowest when mice neared the goal (31.7%). We then used an encoding model to identify cells belonging to specific choice-related ensembles. Fewer cells in PL overall (13.39%) than in projection-defined PL-to-PVT cells (39.75%) significantly encoded at least one choice event. Critically, latent constructs of choice (decision caution) as well as decision time could be predicted by choice-related activity of these PL-to-PVT ensembles. Together these findings suggest that PL-to-PVT cells are tuned to conflict proximity and encode latent cognitive constructs of choice.

Disclosures: Z. Millan: None. **G.P. McNally:** None.

Poster

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Topic: H.03. Decision Making

Support: NIH Grant R01 MH123679
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Title: Impact of choice freedom, choice number and cognitive demand in a decision-making task involving working memory

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Abstract: Modeling decision-making and working-memory processes in rodents receives substantial interest. However, difficulties in shaping mouse strategies through reinforcement learning, as well as big discrepancies in the design of paradigms across species, are two big challenges that make comparisons between studies difficult. Here, we designed a task that relies on the ability of mice (C57Bl/6 strain) to maintain sensory information (two distinct tones,1 kHz and 11 kHz; 1.5 s) for several seconds, and successfully to associate them with the retrieval of rewards in the two opposite arms of a T-maze. We found that when animals are tested on the working-memory version of the task (3-s delay) without any previous training, and in a block design (4-6 trials blocks), animals gradually shift their strategy to maximize the number of rewards by increasing the motor expense and completing more trials, but decreasing their accuracy (20 sessions, n = 4 males). Furthermore, when animals are provided with extended cues that last until decision, accuracy increases with increasing free choice trials proportion (2-7 sessions; n = 4, 3 males). Additionally, accuracy increases when animals are provided with multiple delay choices compared to only one (1-18 sessions; n = 11-34, 6-16 males). Together, these findings support the importance of proper training design, as well as finely controlling the maintenance time and providing high intra-session variability for optimizing performance in decision-making tasks.

Disclosures: I. Ibarra-Lecue: None. **A.Z. Harris:** None. **S. Haegens:** None.

Poster

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Program #/Poster #: PSTR185.12/P7

Topic: H.03. Decision Making

Title: Influence of Travel Costs and Sex Hormones on Patch-Leaving Foraging Decisions in Rats

Authors: *Y. DAI[1], R. M. BURCH[2], A. STOICA[2], A. SEIELSTAD[2], K. CASTILLO[2], M. JIN[2], P. PORAYATH[2], J. R. HINMAN[2];
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Abstract: Foraging is a fundamental behavior for animals to satisfy their nutritional needs and ensure survival in their natural habitats. Patch-leaving (PL) foraging requires animals to decide when to depart from a resource-constrained area; a decision critical to optimizing foraging

strategies and resource management. We tested male and female rats on a PL foraging task involving two open field patches with depleting reward rates connected by a corridor where different time delays could be instituted, thus modeling the travel time between patches. Both male and female rats display patch residence times in line with the marginal value theorem (MVT) by staying longer in a patch when the time delay is longer, but all animals tend to stay longer than the optimal patch residence time. Yet female rats overstayed significantly less, consumed rewards at a greater rate, and traveled a shorter distance than male rats, thus demonstrating greater efficiency in foraging. The performance of female rats actually fluctuated across the estrous cycle, with shorter patch residence durations associated with high estrogen (proestrus and estrous) than low estrogen (metestrus and diestrus). While shorter patch overstay durations can be viewed as closer to optimal, an alternative interpretation is that animals are making impulsive choices. We tested rats on both an operant chamber-based delay discounting task and the PL foraging task to determine whether rats that make impulsive choices during delay discounting also display shorter patch residence times. Rats that showed a higher preference for the small immediate reward during delay discounting demonstrated shorter patch residence times in the PL foraging task, suggesting that shorter patch residence times reflect an impulsive choice on the part of the rat. In nature, foragers voluntarily move from one patch to another patch, rather than simply waiting out a time delay between patches and then teleporting to the next one. Next, we modified the PL foraging task to require rats to run different distances in a running wheel in between patches, thus modeling travel distance with actual distance rather than a time delay. Similar to the time delay version, rats increased their patch residence times as the distance cost between patches increased. Interestingly, the correlation between patch residence time and travel distances was weaker than the correlation between patch residence time and the actual time taken by the rats to complete the travel distances. This indicates that the time cost of travel, rather than the distance between patches, influences PL decision-making.

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Poster

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Topic: H.03. Decision Making

Support: Summer Mentored Research Fellowship at UCLA
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Title: Comparing time and effort costs in a free-choice foraging task for rats

Authors: *R. K. KENDALL, A. K. GARCIA, B. A. BEJARANO, A. M. WIKENHEISER; Psychology, UCLA Chapter, Los Angeles, CA

Abstract: To investigate how decisions are made when animals have full autonomy to explore options, we tested rats on a free-choice patch foraging task with two open-field arenas comprising foraging patches. In each session, the reward schedule assigned to the patches was selected randomly from a set of four possible schedules. The initial rate of reward ranged from low to high in evenly-spaced steps across the four reward schedules, but reward rate always decreased monotonically while rats remained in the patch. Leaving a patch reset the reward rate to its maximum level, but incurred a cost. In one experiment this cost took the form of a "travel time" delay (n = 10 rats, 5 female) during which food was not available; in a separate experiment rats (n = 10 rats, 5 female) were forced to scale two 30-cm barriers to switch between patches, imposing a physical effort cost. Unlike previous versions of this task, here rats were free to choose where to forage and how long to remain in each location throughout each 30-minute session. Thus, optimal task behavior required rats to both identify the patch with the better reward schedule, and determine the optimal amount of time to spend in the better patch on each visit. We hypothesized that rats would solve the task by exploring both patches early within each session before eventually committing the remainder of their visits to the patch with the better reward schedule. Consistent with theoretical models of foraging, rats remained longer in patches with higher initial reward rates and chose longer patch residence durations when the cost of switching patches increased, whether that cost was imposed as a delay or a physical effort requirement. Surprisingly, rats rarely returned to the same patch on successive visits. Patch revisits were rare across all sessions (median: one revisit/session) and did not increase as rats gained more experience on the task. The probability of revisiting a patch was only weakly influenced by task parameters such as reward schedule and patch-switching cost. Preliminary data indicate that revisits were equally rare whether the switching cost entailed physical effort or a delay. These data suggest that rats have a strong tendency towards serial alternation in spatial foraging scenarios. Although rats modified the amount of time they spent in patches in response to task conditions, the order in which they visited patches was close to perfect alternation. These data connect with previous work on spatial alternation on T-mazes, and suggest that alternation may be an adaptive default strategy for sequential choice situations.

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Poster

PSTR185: Neural Mechanisms of Decision Making in Rodents

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Topic: H.03. Decision Making

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Title: Task-dependent neuronal interactions across cortical areas

Authors: *E. DIAMANTI[1], O. KARNIOL-TAMBOUR[1], L. PINTO[2], M. SCHOTTDORF[1], S. THIBERGE[1], C. D. BRODY[3], J. W. PILLOW[1], D. W. TANK[1];
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Abstract: Behaviors requiring working memory (WM) recruit the whole cortex, whereas simpler behaviors do not (Pinto et al. 2019). How do interactions between neurons across the cortex change with task demands? We trained 9 transgenic mice to perform a WM task (towers) in virtual reality that combined navigation with sensory evidence accumulation. The towers alternated in blocks with a simpler (visually guided) task featuring the same virtual environment without requiring WM. While mice performed the two tasks with the same motor behavior, we simultaneously imaged excitatory neurons in 3 cortical areas, anteromedial visual (AM), retrosplenial (RSC), and premotor (M2), using a random-access two-photon microscope. We first characterized functional interactions across areas using pairwise correlations between neurons from all areas, estimated for each task. As in our widefield study (Pinto et al. 2019), pairwise correlations dropped in the towers compared to the visually guided. If pairwise correlations differ between tasks, then the subspace of neural dynamics defined by the eigenvectors of each task's correlation matrix may also differ. Specifically, we asked how well each task's subspace captured the variance of the population activity in both tasks. Although the towers subspace explained activity in both tasks, the visually guided subspace did not explain activity in the towers equally well as in the visually guided. Does this discrepancy depend on interactions across areas? We used within-area pairwise correlations to identify subspaces for each area. AM's task subspaces were almost overlapping. In contrast, in RSC and M2, the visually guided task's subspace explained less variance of the activity in the towers task, although this difference was smaller than when we estimated the subspace on the full three-area correlation matrix. To investigate the task-dependence of interareal interactions beyond the linear regime of pairwise correlations, we developed a model of a nonlinear dynamical system with multi-region structure (Karniol-Tambour et al., 2024). By decomposing dynamics in the latent space into local and cross-region, we estimated the latent communication profiles between regions, and used decoding to determine the task-related information contained in these profiles. We are now investigating how these profiles vary with task demands. Our results suggest that, during WM, population activity is not contained in the subspace of simpler behaviors, particularly in association areas M2 and RSC. These areas can thus differentiate between WM and simpler tasks, and this differentiation is further amplified through functional interareal interactions.

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Poster

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Title: Sex differences in change-of-mind neuroeconomic decision-making is modulated by LINC00473 in medial prefrontal cortex

Authors: R. DURAND-DE CUTTOLI[1], O. ISSLER[3], S. J. RUSSO[4], ***E. NESTLER**[5], B. M. SWEIS[2];
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Abstract: Changing one's mind is a complex cognitive phenomenon involving a continuous re-appraisal of the trade-off between past costs and future value. Recent work modeling this behavior across species has established associations between aspects of this choice process and their contributions to altered decision-making in psychopathology. Here, we investigated the actions in medial prefrontal cortex (mPFC) neurons of long intergenic non-coding RNA, LINC00473, known to induce stress resilience in a striking sex-dependent manner, but whose role in cognitive function is unknown. We characterized complex decision-making behavior in male and female mice longitudinally in our neuroeconomic foraging paradigm, Restaurant Row, following virus-mediated LINC00473 expression in mPFC neurons. On this task, mice foraged for their primary source of food among varying costs (delays) and subjective value (flavors) while on a limited time-budget during which decisions to accept and wait for rewards were separated into discrete stages of primary commitments and secondary re-evaluations. We discovered important differences in decision-making behavior between female and male mice. LINC00473 expression selectively influenced multiple features of re-evaluative choices, without affecting primary decisions, in female mice only. These behavioral effects included changing how mice (i) cached the value of the passage of time and (ii) weighed their history of economically disadvantageous choices. Both processes were uniquely linked to change-of-mind decisions and underlie the computational bases of distinct aspects of counterfactual thinking. These findings reveal a key bridge between a molecular driver of stress resilience and psychological mechanisms underlying sex-specific decision-making capabilities.

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Poster

PSTR185: Neural Mechanisms of Decision Making in Rodents

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Title: Task-evoked pupil responses reflect belief states in mice

Authors: *M. MAHEU[1,2], J. COPELAND[1,2], A. CERVÁN[3], J. DE LA ROCHA[3], J. S. WIEGERT[4,5], T. H. DONNER[1];
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Abstract: The arousal systems of the brainstem shape the state of cortical circuits and control the size of the pupil, providing a peripheral 'readout' of cortical state. Brainstem activity and the pupil are also rapidly modulated during difficult decisions. Work in humans has shown that key decision variables, such as decision uncertainty, are reflected in the amplitude of this decision-evoked arousal response. It is unknown whether evoked pupil responses reflect such high-level decision variables also in rodents, which are amenable to detailed mechanistic interrogation. We aimed to test whether decision uncertainty modulates evoked pupil responses in mice in two phases of the task: (i) during the formation of a protracted decision and (ii) after receiving feedback about the choice (reward or no-reward). A model that accounts for behavioral, neural, and human pupil responses (Kepecs *et al.*, 2008; Urai *et al.*, 2017) predicts an interaction between sensory evidence strength and choice outcome in the scaling of uncertainty-dependent internal signals. Critically, the model predicts a sign flip in this interaction from decision to post-feedback phases (Lak *et al.*, 2017). Head-fixed mice performed an auditory discrimination task based on a binaural stream of successive auditory samples with fluctuating intensity, and reported the side with the stronger mean intensity by licking one of two ports. Mice could only lick and collect a water reward after a post-stimulus delay period of 1s. Behavioral choices related lawfully to the sign and strength of the cumulative sensory evidence and were influenced

by evidence fluctuations, with primacy and to a lesser extent recency effects. Response times varied as a function of both evidence strength and outcome, as predicted by the model. Task events elicited two distinct pupil responses: one during decision formation and a second (larger) one after feedback. Critically, both responses were modulated by the model-predicted evidence-outcome interaction, with a sign flip between decision (positive) and feedback (negative) phases: while during the decision phase, the pupil response decreased with evidence in correct trials and increased in incorrect trials, this modulation reversed during the feedback phase. We conclude that pupil-linked arousal responses in the mouse are modulated by high-level computational variables during decisions under uncertainty.

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Poster

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Title: Large-scale geometry of cortical dynamics underlying evidence accumulation and short-term memory

Authors: *R. M. COSTA[1], P. S. SALVINO[1], J. LUO[1], S. IBARRA[2], L. PINTO[1];
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Abstract: To generate effective behavior in complex environments, the brain must learn to represent many pieces of information about the world and perform cognitive computations upon them. Recent findings suggest that cortical microcircuits arrange these representations in particular geometries in population activity space to facilitate cognitive computations. But does this principle hold at the level of large-scale cortical dynamics? To answer this, we developed a new virtual reality (VR) task for mice to study the large-scale cortical dynamics supporting distinct cognitive computations. Mice accumulate evidence to determine the predominant color of a random checkerboard pattern in the sample region, retain it in memory during a delay region, and make a choice at the end of the maze by matching the test stimulus to the predominant color of the sample. We vary sample coherence (proportion of squares of the predominant color), and sample and delay duration by changing the VR movement gain in different maze regions. Imaging mesoscale excitatory activity from across the dorsal cortex during task performance, we observed distinct large-scale activity patterns during evidence

accumulation, short-term memory, and choice. This was such that areas of the frontal cortex were preferentially engaged during longer-lasting computations, regardless of their identity, while the pattern of posterior recruitment was computation-specific. We used demixed principal component analysis (dPCA) to estimate a set of axes that best capture activity related to each task variable. Strikingly, computing the angles between these axes revealed that they are near-orthogonal. This geometry suggests that evidence accumulation and short-term memory rely upon largely non-overlapping patterns of large-scale cortical dynamics. Finally, because some of the task axes were defined by the duration of the computation, we asked how the networks mapping onto each axis related to the intrinsic timescale hierarchy across the cortex. Notably, the timescale of each area was strongly positively correlated with its contribution to the short-term memory, but not the evidence-accumulation, axis. Thus, the role of the hierarchy of timescales in organizing cortical activity appears to be computation dependent. Our findings thus disentangle evidence accumulation and short-term memory to reveal a surprising independence between the large-scale dynamics engaged by each computation.

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Poster

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Support: NIMH: R00MH120047
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Title: Distinct input-output relationships between cholinergic basal forebrain subnuclei

Authors: *J. TAN[1], R. LU[1], L. PINTO[1,2];
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Abstract: The basal forebrain (BF) provides the only source of long-range cholinergic neuromodulatory input to the cortex. Unpublished findings from our laboratory suggest the BF crucially modulates cortex-wide computations during cognitive behavior in a task-dependent manner, with heterogeneous cholinergic activity patterns across the cortex. This heterogeneity is potentially subserved by cholinergic projections originating from distinct subnuclei within the BF. However, the source of task-related information to BF subnuclei remains unclear. Here, we asked whether these subnuclei have distinct input-output relationships in mice. First, to compare input patterns, we used dual-color retrograde viral tracing and whole-brain projection analysis to compare inputs to two BF subnuclei, the horizontal diagonal band (HDB) and the substantia

innominata (SI). This revealed differential laminar and subregional input to the subnuclei from across the mouse brain. Notably, we identified distinct dorsoventral and anteroposterior gradients of projections to SI and HDB from neurons within the medial prefrontal cortex (mPFC), potentially explaining the source of task-related information to the BF. Additionally, we quantified the output patterns of HDB and SI using anterograde tracing data from the Allen Brain Mouse Connectivity Atlas. We found that HDB and SI innervate non-overlapping cortical territories along the anteroposterior and mediolateral axes and preferentially target different cortical layers. Overall, our result confirms previously reported heterogeneity within the BF and offer a potential anatomical basis for task- and area-specific orchestration of cortical activity. Future behavioral and functional experiments will elucidate the role of cholinergic heterogeneity in flexible cognitive behavior.

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Poster

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Topic: H.03. Decision Making

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Title: A cholinergic mechanism enabling task-specific computation across the cortex

Authors: *J. LUO[1], P. S. SALVINO[1], E. MYHRE[1], J. TAN[1], A. RAPP[1], J. CANTON-JOSH[1], R. LU[1], L. PINTO[1,2];
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Abstract: Global activity patterns in the dorsal cortex are reorganized when mice perform perceptual decision-making tasks involving different underlying computations with varying complexity. However, the circuit mechanisms responsible for these large-scale activity changes remain unknown. An interesting possibility is that they involve neuromodulatory input to the cortex. In particular, cholinergic neurons in the basal forebrain project widely across cortical areas while exhibiting topographical and functional heterogeneity. They are therefore well equipped to serve as an orchestrator of task-dependent large-scale cortical activity. To test this hypothesis, we imaged cholinergic dynamics across the dorsal cortex while the mice performed a cued task-switching paradigm in virtual reality, with dozens of unpredictable switches within a session. Specifically, they rapidly switched between a complex task that requires evidence accumulation over time and a simple task where the correct choice on each trial is indicated by a salient visual cue. We observed clear task differences in cholinergic activity, which appeared

heterogeneous across cortical areas and task epochs. To investigate whether cholinergic input is required for behavioral performance in a task-specific manner, we injected the pan-muscarinic cholinergic antagonist scopolamine or saline prior to behavioral sessions. Strikingly, blocking cholinergic input specifically impaired performance in the complex task while leaving performance in the simple task intact. In addition, muscarinic acetylcholine receptor blockade diminished task differences in cortical excitatory activity and reduced decorrelation across areas in the complex task. These findings suggest that cholinergic input is recruited during perceptual decision-making in a task-dependent manner to shape cognitive computation across the cortex. Further experiments targeting specific task epochs will allow us to disentangle specific components of decision-making supported by cortical cholinergic inputs.

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Poster

PSTR186: Executive Function and Cognitive Flexibility

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Title: Intracranial EEG correlates of concurrent demands on cognitive stability and flexibility

Authors: *J. ZHANG[1], A. EARLE-RICHARDSON[2], D. G. SOUTHWELL[3], T. EGNER[4], G. B. COGAN[5];
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Abstract: Two core capacities of cognitive control are *cognitive stability*, the ability to focus attention on task-relevant stimuli while ignoring distracters, and *cognitive flexibility*, the ability to shift between different rule-sets to guide behavior. Whether these capacities arise from distinct or overlapping neural mechanisms remains unclear as prior studies investigated them in isolation and used only macroscale neural data (fMRI, scalp EEG) that lack the temporal/spatial resolution to elucidate neural mechanisms. Here, we obtained mesoscale neural data (iEEG) from 13 epilepsy patients while they completed a local/global Navon letter task. The task independently varied demands on stability (congruent vs. incongruent stimuli) and flexibility (repeating vs. switching tasks between trials), allowing us to analyze neural activity associated with congruency, task switching, and their interaction. Patients demonstrated robust behavioral congruency effects and switch costs. Prior work suggests the lateral prefrontal cortex (LPFC) is a primary neural substrate for cognitive stability and flexibility. We examined high-gamma activity within individual LPFC electrodes (N=105), focusing on the electrodes that had

significant task-related activity (N=44, 42%). Most of these task-sensitive electrodes were sensitive to at least one effect of interest (N=30, 68%), mostly emerging ~500ms post-stimulus-onset. About half of these effect-sensitive electrodes displayed exclusive effects of congruency or task switching (N=16, 53%), while the others exhibited additive or interactive effects (N=14, 47%). These results suggest concurrent demands on cognitive stability and flexibility have both distinct and overlapping mesoscale neural substrates in LPFC.

Disclosures: J. Zhang: None. **A. Earle-Richardson:** None. **D.G. Southwell:** None. **T. Egner:** None. **G.B. Cogan:** None.

Poster

PSTR186: Executive Function and Cognitive Flexibility

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.02/Q4

Topic: H.04. Executive Functions

Support: BMBF
Max Planck Society

Title: Single cell correlates of multitasking in higher associative areas of the pigeon brain

Authors: *J. M. TUFF[1,2], G. MALDARELLI[1], J. PACKHEISER[1], R. PUSCH[1], N. ROOK[1], O. GUNTURKUN[1];
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Abstract: Multitasking can be described as a form of goal-directed behaviour that relies on the integration and sequential execution of several different tasks for successful completion of an overarching goal**.** It has been shown that the parallel activation of multiple tasks leads to less efficient behaviour compared to doing tasks one after another. In humans, a widespread network of frontal and basal ganglia areas have been associated with this type of behaviour. However, it is still unknown how multitasking manifests itself on cellular level. Pigeons are able to show this type of behaviour and exhibit a network of higher associative and striatal areas that are activated during multitasking with the nidopallium caudolaterale (NCL) and the nidopallium intermedium medialis pars laterale (NIMl) being highly relevant. In this study, we investigated single cell correlates of multitasking in these areas while the animals perform a STOP-CHANGE task. We found activity patterns that correspond to different task demands of this experimental paradigm. In particular, we found differential activity patterns between phases of sequential and parallel task activation, which might explain the effects on behavioural efficiency.

Disclosures: J.M. Tuff: None. **G. Maldarelli:** None. **J. Packheiser:** None. **R. Pusch:** None. **N. Rook:** None. **O. Gunturkun:** None.

Poster

PSTR186: Executive Function and Cognitive Flexibility

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.03/

Topic: H.04. Executive Functions

Support: NWO VICI

Title: Phasic alertness generates urgency and amplifies competition between evidence accumulators

Authors: *J. J. TROMP[1], F. WURM[2], F. LUCCHI[2], R. DE KLEIJN[1], S. NIEUWENHUIS[1]; [1]Cognitive Psychology, Leiden Univ., Leiden, Netherlands; [2]Health, Med. and Neuropsychology Unit, Leiden Univ., Leiden, Netherlands

Abstract: The alerting system plays a crucial role in our ability to rapidly process and act upon abrupt changes in the environment. Generally, inducing phasic alertness via auditory or visual cues enhances perceptual processing and speeds up behavioral responses. However, their effects on cognitive control present a notable divergence from the beneficial effects of alerting cues. Despite generally improving performance, alerting cues often exacerbate interference from distracting stimuli, impairing cognitive control. The mechanisms underlying this phenomenon remain elusive, with previous theoretical frameworks yielding conflicting results. In this study, we propose a novel, biologically informed account of how phasic alertness speeds up behavioral responses while impairing cognitive control.

We advance three key assumptions. First, we posit that alerting cues induce a transient increase in phasic arousal, driven by neuromodulatory nuclei such as the locus coeruleus. We validate this assumption through pupil dilation response measurements, a well-established correlate of phasic neuromodulatory activity. Second, we hypothesize that the phasic arousal response constitutes an evidence-independent neural urgency signal, expediting the decision process by driving evidence accumulators closer to a fixed decision threshold. We examine this hypothesis through EEG signatures of decision urgency in motor cortical activity, revealing a link between alerting cues, phasic arousal, and decision urgency. Third, we suggest that decision urgency increases response conflict, exacerbating interference from distracting information. We find support for this assumption through both behavioral (flanker congruency effect) and neural manifestations of response conflict (midfrontal theta-band activity).

Our empirical findings provide robust evidence for the proposed account, shedding light on the intricate interplay between phasic alertness, perceptual decision-making, and cognitive control. Additionally, a drift-diffusion model incorporating urgency and lateral inhibition replicates the behavioral effects. This biologically grounded framework offers valuable insights into the nuanced dynamics among distinct attentional systems and sheds light on computational trade-offs inherent in the cognitive system.

Disclosures: J.J. Tromp: None. **F. Wurm:** None. **F. Lucchi:** None. **R. de Kleijn:** None. **S. Nieuwenhuis:** None.

Poster

PSTR186: Executive Function and Cognitive Flexibility

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Program #/Poster #: PSTR186.04/Q5

Topic: G.04. Emotion

Support: MnDRIVE Neuromodulation Research Fellowship
5T32DA007234-36
1R01NS120851-01A1

Title: Optogenetic deep brain stimulation of medial PFC projections in mid-striatum improves cognitive flexibility

Authors: *E. SACHSE[1], J. BENNEK[2], E. M. DASTIN-VAN RIJN[3], M. BUCCINI[4], M. MENSINGER[5], F. IACOBUCCI[6], M. ESGUERRA[6], A. S. WIDGE[7];
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Abstract: Neuromodulation of cortico-striatal circuits can ameliorate deficits in flexible decision-making. Deep brain stimulation (DBS) of the ventral internal capsule/ventral striatum (VCVS) in humans and DBS-like stimulation of mid-striatum in rats, both improve cognitive flexibility. Clinical DBS is thought to work by stimulating axons within the VCVS, but the exact mechanisms remain unclear. By using optogenetic DBS and selective activation of axons vs. cell bodies we can determine which neural elements are required for DBS's behavioral effects. Four male and four female rats were transfected with AAV5-CaMKII-Chronos-GFP or AAV5-CaMKII-GFP (control) in bilateral subregions of the mPFC and implanted with optic fibers in mid-striatum. The rats were trained on an extradimensional set-shifting task requiring flexible decision-making to successfully suppress more habitual responses . Rats underwent testing periods of within-task bilateral stimulation (470nm,~10mW, 130Hz, 2ms pulse width), or no stimulation (OFF condition) for a balanced number of ON/OFF sessions. Performance (reaction time and errors) was quantified during periods of flexibility using a generalized linear mixed-effects model (GLM). Successful viral transfection of mPFC and activation of Chronos was confirmed by evoked-response potentials in a separate cohort of rats prior to this experiment. Fiber localization and viral expression were confirmed by histology. Bilateral opto-DBS reduced reaction times ($p = 0.00023$) without increasing errors ($p = 0.1221$) in rats with active Chronos compared to control-virus rats. This is not an artifact of learning, sex, individual rat, or set-shift protocol as these effects were accounted for by the GLM. Optogenetic DBS of mPFC axons improved cognitive flexibility, replicating effects of electrical DBS. This indicates mid-striatal electrical DBS effects in rats are mediated by mPFC axons that project to and pass-through mid-

striatum, as opposed to local striatal cell bodies, supporting the hypothesis that VCVS DBS in humans works through axonal stimulation..

Disclosures: E. Sachse: None. **J. Bennek:** None. **E.M. Dastin-van Rijn:** None. **M. Buccini:** None. **M. Mensinger:** None. **F. Iacobucci:** None. **M. Esguerra:** None. **A.S. Widge:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); unlicensed intellectual property related to the topic of this poster. F. Consulting Fees (e.g., advisory boards); Abbott Medical.

Poster

PSTR186: Executive Function and Cognitive Flexibility

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.05/Q6

Topic: H.03. Decision Making

Support: Neurological Foundation Grant 2325 PRG

Title: Manipulating thalamocortical pathways to alter deficits in cognitive flexibility

Authors: *J. J. HAMILTON[1], X. RAM[1], L. BERRIMAN[1], J. C. DALRYMPLE-ALFORD[1,2,3], A. S. MITCHELL[1];
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Abstract: Cognitive flexibility, the ability to switch thoughts and responses rapidly as we encounter different task demands has been attributed to the prefrontal cortex. However, the mediodorsal thalamus (MD) and the nucleus reuniens of the thalamus (RE) are also implicated. Crucially, thalamocortical dysfunction has now been identified across a range of neurological conditions with cognitive deficits. Interventions at the level of thalamocortical interactions may provide new clinical strategies to mitigate some of these cognitive difficulties. In this experiment, we performed bilateral excitotoxic lesions to either the MD or RE in 8-month-old male rats to determine if these thalamic manipulations influenced performance on an adapted version of the Wisconsin Card Sorting Task that measures cognitive flexibility in rats. This adapted version of the attentional set-shifting task the measures the ability to attend to stimuli dimensions that reliably predict reward (intra-dimensional shift; ID) for three consecutive ID subtasks, followed by the need to shift attention to another, previously ignored stimulus dimension when contingencies change (extradimensional shift). We found that lesions to these two thalamic regions produced impairments in this version of attentional set shifting and reversal learning. Specifically, MD lesioned rats required a greater number of trials to complete the first reversal of the compound discrimination and both MD and RE lesioned animals showed significant impairments on the first of three ID subtask (ID1). Once a stable response strategy has been learnt, lesioned animals were readily able to implement the attentional set strategy for the remaining ID shift tasks (ID2, ID3) involving the same sensory dimension. Across all

subtasks, both MD and RE lesioned animals made more errors during learning for each discrimination and made significantly more errors than sham lesion control animals. Intraperitoneal injections of noradrenaline given 30 minutes prior to completing the attentional set-shifting task reduced the overall numbers of errors. Our evidence indicates that both the MD and RE thalamus are involved in cognitive flexibility. This contributes to knowledge around the influence of the thalamus on cognitive function and its suitability as a therapeutic target for patients with cognitive decline.

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Poster

PSTR186: Executive Function and Cognitive Flexibility

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Program #/Poster #: PSTR186.06/R1

Topic: H.04. Executive Functions

Support: JSPS KAKENHI Grant Number 23H04680
Kagoshima University Megumikai Medical Research Promotion Fund

Title: Improved training procedure for serial reversal task of touch screen-based visual discrimination in mice

Authors: *Y. KIYAMA[1], M. KAKEYAMA[2], H. OKUNO[1];
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Abstract: The touch screen operant learning system has been widely used for analyzing various brain functions in animals. Our focus extends to evaluating cognitive flexibility through touch screen-based visual discrimination tasks in mice. Serial reversal learning paradigm is often used for evaluating an animal's cognitive flexibility. However, reversal discrimination learning has been reported to be challenging in mice, due to their inability to acquire the reversal rule (Tamada et al., Front. Neurosci., 2021). We hypothesized that such inability might originate from slow learning processes of visual discrimination in mice. Therefore, in this study, we aimed to improve the learning efficiency by optimizing task parameters. In our visual discrimination task, mice underwent training to discriminate between two distinct visual patterns in a touch-panel operant chamber (O'HARA & CO., LTD, Japan). Mice were required to touch the designated pattern (S+) to obtain a food pellet. An erroneous touch to the non-designated pattern (S-) led to no reward and a ten-second inter-trial interval. We first changed the number of touches for the stimulus to be selected as choice; two or three successive touches were required for choice selection, as opposed to the conventional single touch. To our surprise, this modification dramatically improved task performance with a single S+ and S- pair. In our conventional single touch procedure, it usually took more than ten daily sessions to exceed a

correct rate of 75%, where reaching a plateau. In contrast, in the new three-touch procedure, the correct rate reached close to 75% within 5 days and the plateau levels exceeded 80%. When we increased the S+ and S- to three pairs each, the mice learned significantly faster in the three-touch procedure than in the conventional single-touch choice selection ($p < 0.01$). After further modifications, the initial acquisition required only four daily sessions to surpass an across-animal average of 75% correct rate with five sessions with three pairs. Performance plateau levels also showed significant enhancement, nearing 90% with the three-pair stimulus set. With this optimized protocol, we next trained mice in a serial reversal learning task in which the switching of the S+ and S- stimuli was repeated ten times. The mice exhibited progressive improvement of task performance over ten reversal repeats ($p<0.001$), indicating that the mice gradually developed a reversal "learning-set", in other words, they succeeded to "learn" the rule of reversal task. Thus, our optimized protocol allows the rapid and reliable assessment of cognitive flexibility, and would facilitate development of more demanding tasks in mice.

Disclosures: Y. Kiyama: None. **M. Kakeyama:** None. **H. Okuno:** None.

Poster

PSTR186: Executive Function and Cognitive Flexibility

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Topic: H.04. Executive Functions

Support: NIH Grant AG050518

Title: Interactions between positive allosteric modulation of muscarinic receptors and orexin-1 receptor antagonism in cognitive flexibility in rats (Rattus norvegicus)

Authors: *M. LI, J. A. BURK;
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Abstract: Cognitive flexibility is the ability to adjust to different rules according to context. Previous research has shown independent effects of orexin and acetylcholine (ACh) on cognitive flexibility. Nonetheless, there are strong neural connections between orexin and ACh, where orexin neurons project to ACh neuron cell bodies, suggesting potential interactions between these neurotransmitters. In the present project, rats received drug treatments to manipulate their orexin and ACh systems at different timepoints and their cognitive flexibility was tested. Rats were first water restricted and trained to associate lever pressing with water rewards. Then, two tasks were used to test cognitive flexibility: first the cue task, where the rule is to press the lever under the illuminated cue light, and then the response task, where the rule is to always press the lever in the opposite direction of side preference. The main measure of cognitive flexibility is the number of trials until 10 consecutive correct responses are made for the response task because it measures how well rats learn when the rule changes. For drug treatment, rats were intraperitoneally injected with to VU0453595, an M1 PAM (positive allosteric modulator of the

M1-subtype muscarinic receptors) which upregulates ACh, for seven days of training and three days of cue task (ten days total). Then, rats were injected with orexin-1 receptor antagonist, SB-334867, on the first day of the response task, which is when the initial rule shift occurs. Our preliminary results suggest that M1 PAM improves task performance, whereas orexin receptor antagonist impairs performance. Results from this study can help understand neural mechanisms of cognitive flexibility and inform treatment of cognitive flexibility deficits.

Disclosures: M. Li: None. **J.A. Burk:** None.

Poster

PSTR186: Executive Function and Cognitive Flexibility

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Program #/Poster #: PSTR186.08/R3

Topic: H.04. Executive Functions

Support: CRC1193

Title: A novel neural mechanism for emotion-cognition interaction

Authors: *A. DIETRICH[1], E. PINZUTI[2], Y. CABRAL-CALDERIN[3], F. MÜLLER-DAHLHAUS[4], M. WIBRAL[5], O. TÜSCHER[6];
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Abstract: The interactions between emotional and cognitive processing critically determine human behaviour -- often with significant consequences in daily life. Previous research focused on the medial prefrontal cortex as the key brain region involved in emotion-cognition interaction. However, the underlying neuronal mechanisms and their translation to behavior remain poorly understood. We investigated the role of transient ß-oscillatory activity in the lateral prefrontal cortex, specifically the right inferior frontal gyrus (rIFG), in dynamically resolving emotion-cognition competition, clearly arguing against the traditional view of separating processing streams. Using EEG recordings in a large cohort (N=121) of human participants and advanced source-reconstruction techniques with finite-element head-modeling, we observed spatial overlap of emotion and cognition processing within a sub-area of the rIFG called the pars triangularis with remarkable overlap to known fMRI localization supporting the idea that rIFG plays a critical general role in interference inhibition, regardless of the type of information causing the interference. Interestingly, the emotion-cognition interaction in the rIFG occurred transiently rather than continuously, peaking during the transition from emotional to cognitive processing. This interaction was most pronounced in the beta-frequency band. Our analysis of information flow using Granger-causality revealed that the pars triangularis of the rIFG communicated with

other functional subdivisions of the rIFG and parieto-occipital areas (Precuneus, V2) in a frequency-specific manner. This communication pattern predicted individual emotional and cognitive interference effects. Overall, our findings highlight the functional and temporal segregation and integration of emotion, cognition, and their interaction in the rIFG. Furthermore, the activity in the rIFG modulates top-down connections to parieto-occipital visuo-attentional areas, ultimately shaping behavioral performance.

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Poster

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.09/R4

Topic: H.04. Executive Functions

Support: NSERC Discovery Grant

Title: Exercise does not alter oculomotor preparatory phase cortical hemodynamics

Authors: *M. HEATH, G. JEYARAJAN;
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Abstract: A single bout of exercise improves executive function (EF). The *neural efficiency* hypothesis' asserts that improved EF is related to decreased brain activity; however, a paucity of work has employed an event-related protocol to determine whether preparatory phase cortical hemodynamic changes account for the postexercise EF benefit. Participants (N=23) completed 20-min sessions of light-intensity active (i.e., via volitional cycle ergometry), and passive (i.e., via mechanically driven cycle ergometry) exercise and a non-exercise control session. Prior to and after each session, participants completed an event-related design wherein transcranial Doppler ultrasound measured middle cerebral artery velocity (MCAv) to assess preparatory phase cortical hemodynamics for pro- (i.e., saccade to veridical target) and antisaccades (i.e., an EF task requiring a saccade mirror-symmetrical to a target). Antisaccades were evaluated because they provide the basis to detect subtle EF changes and are mediated via frontoparietal networks that show task-dependent changes following a single bout of exercise. Antisaccades produced longer reaction times (RT) and an increased preparatory phase MCAv than prosaccades – a result linked to the increased neural activity related to an EF task. Notably, passive and active exercise produced a postexercise reduction in antisaccade – but not prosaccade – RT; however, frequentist and Bayesian statistics indicated that antisaccade preparatory phase cortical hemodynamics did not vary from pre- to postexercise, and did not correlate with the antisaccade RT benefit. Accordingly, the postexercise EF benefit did not manifest via an exercise-based change in functional hyperemia. As such, our findings support the evolving view that the

interplay between interdependent neurophysiological changes – as opposed to a unitary change in cerebral blood flow – supports a postexercise EF benefit.

Disclosures: M. Heath: None. **G. Jeyarajan:** None.

Poster

PSTR186: Executive Function and Cognitive Flexibility

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.10/R5

Topic: H.04. Executive Functions

Support: NSERC Discovery Grant

Title: Passive exercise provides a simultaneous and postexercise executive function benefit

Authors: *A. RAHIMIDARABAD[1], C. DALTON[2], C. EDGAR[2], B. TARI[2], M. HEATH[2]; [1]Schulich Sch. of Med. and Dent., Univ. of Western Ontario: Western Univ., London, ON, Canada; [2]Fac. of Hlth. Sci., Univ. of Western Ontario, London, ON, Canada

Abstract: Background: Passive exercise involves movement of the limbs via an external mechanical force and supports a postexercise executive function (EF) benefit. It is, however, unclear whether EF is improved simultaneously with passive exercise - a salient question given the rise of passive and active exercise workstations aimed at enhancing productivity and well-being in sedentary occupations. **Methods:** Participants (N = 23) completed 20-minute sessions of active (i.e., volitional muscle activation) and passive (i.e., via mechanically driven cycle ergometer) cycling and a non-exercise control condition. EF was assessed before, simultaneous with, and after each session via the antipointing task. Antipointing requires a response mirror-symmetrical to a target and is an ideal tool for the current study, given that the task is mediated via EF frontoparietal networks that show task-dependent changes following a single bout of active exercise. **Results:** Passive exercise reduced antipointing reaction time (RT) at simultaneous ($p = 0.042$) and postexercise ($p = 0.01$) assessments, whereas active exercise selectively produced a post-intervention - but not simultaneous - RT reduction. ($p = 0.011$). **Conclusions:** Passive and active exercise elicited a postexercise EF benefit; however, only passive exercise produced a simultaneous benefit and is a result suggesting that the intervention provides the physiological/psychological changes necessary to improve EF efficiency without the associated dual-task cost(s) of volitional muscle activity.

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Poster

PSTR186: Executive Function and Cognitive Flexibility

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Topic: H.04. Executive Functions

Support: NSERC Discovery Grant

Title: Passive and active exercise differentially modulate executive function and mental fatigue

Authors: *G. JEYARAJAN[1], L. BUWADI[2], D. HAILE[1], A. AYAZ[2], L. NAGAMATSU[1], M. D. HEATH[1];
[1]Sch. of Kinesiology, [2]Schulich Sch. of Med. & Dent., Western Univ., London, ON, Canada

Abstract: Background: Mental fatigue (MF) impairs executive function (EF) and is linked to a concurrent reduction in cerebral blood flow (CBF). Notably, passive exercise, which involves movement of the limbs via an external mechanical force, increases CBF and elicits a postexercise EF benefit. Accordingly, passive exercise may provide a neuroprotective benefit (i.e., increase in CBF) to ameliorate the sustained attention deficits associated with a MF-inducing task. **Methods:** Healthy young participants (13 female, 9 male) (N=22) completed three 20-min conditions consisting of: (1) a non-exercise control, (2) active (i.e., via volitional muscle activity) cycle ergometer exercise at 37 W, and (3) passive cycle ergometer exercise. EF was examined prior to and immediately following each condition via the antisaccade task (i.e., saccade mirror-symmetrical to target). Following the post-intervention EF assessment, a 20-min psychomotor vigilance task (PVT) was performed to evaluate MF. Transcranial Doppler ultrasound measured middle cerebral artery velocity (MCAv) throughout the PVT to estimate CBF. **Results**: Active and passive exercise produced a pre- to postexercise reduction in antisaccade reaction times (RT) (ps=.03), whereas control condition values did not vary (p=.07). Notably, MCAv and RT decreased and increased, respectively, across the first 5-min to the last 5-min of the PVT (ps<.001); however, these changes were not influenced by the nature of the preceding intervention (i.e., control, passive, active exercise). **Conclusions:** Active and passive exercise provide a postexercise EF benefit; however, the benefit is transient and does not proactively diminish MF associated with a sustained vigilance task.

Disclosures: G. Jeyarajan: None. **L. Buwadi:** None. **D. Haile:** None. **A. Ayaz:** None. **L. Nagamatsu:** None. **M.D. Heath:** None.

Poster

PSTR186: Executive Function and Cognitive Flexibility

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Topic: H.04. Executive Functions

Support: NSERC Discovery Grant

Title: A single bout of focused attention meditation and aerobic exercise benefit executive function

Authors: *L. BUWADI, C. EDGAR, D. HAILE, M. D. HEATH;
Western Univ., London, ON, Canada

Abstract: Executive function (EF) is a cognitive construct that includes the core components of inhibitory control, working memory and cognitive flexibility. Hence, identifying intervention to improve EF is an important area of inquiry. One intervention shown to consistently benefit EF is a single bout of aerobic exercise (AE) - a benefit linked to an exercise-mediated increase in cerebral blood flow, catecholamines, and brain derived neurotrophic factors (BDNF). Notably, however, accessibility to AE is not universal (e.g., spinal cord injury and paresis) and it has been suggested that focused attention meditation (FAM) may serve as a proxy to support EF by improving the efficiency of the neural generators linked to EF. Accordingly, the present study investigated whether a single session of FAM elicits a post-intervention EF benefit comparable to a single bout of AE. Participants were healthy young adults (n= 16, 18 - 26 y/o, 9 female) who completed three 15-minute conditions consisting of: (1) a non-AE and non-FAM control, (2) FAM (i.e., guided breathing along with a visual stimuli), and (3) moderate intensity AE (60% heart rate reserve via cycle ergometer). EF was examined before immediately after each condition via the antisaccades task. For the control condition, results showed that pre- and post-intervention, reaction times (RT) did not differ ($p=0.620$), whereas AE and FAM produced reliable pre- to post-intervention reductions in antisaccade RTs ($ps<0.05$). In conclusion, the findings demonstrate the potential of both AE and FAM as viable interventions for improving EF, offering promising avenues for individuals with varying accessibility to traditional exercise modalities.

Disclosures: L. Buwadi: None. **C. Edgar:** None. **D. Haile:** None. **M.D. Heath:** None.

Poster

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Topic: H.04. Executive Functions

Support: JSPS 23KJ1169
JSPS 20J20893
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JSPS 18H04081

Title: Pupil dynamics decodes very light exercise benefits to prefrontal executive function: possible involvement of locus coeruleus arousal system

Authors: *R. KUWAMIZU[1], Y. YAMAZAKI[1], H. SOYA[1,2];
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Abstract: Proper physical activity, even very light intensity such as slow running or walking, improves brain and cognitive health. Our previous work has shown that very light aerobic exercise acutely elicits increased dorsolateral prefrontal cortex (DLPFC) activation and improves executive function. The exact neurobiological mechanism behind the positive effects of very light exercise remains unclear. Based on our animal evidence (Hiraga et al., bioRxiv, 2023), we hypothesized that exercise-induced activation of the locus coeruleus (LC) arousal system is the thus far undetermined brain mechanisms. Recently, pupil dynamics has attracted attention as a readout of the brain's ascending arousal systems, especially LC activation. We have focused on pupillometry during aerobic exercise to identify the brain dynamics mechanism of the effects of very light exercise. Initially, it showcased pupil dilation during even very light-intensity exercise (< 37% V̇o2peak, below the ventilatory threshold) by graded exercise test. Secondly, inter-individual differences in exercise-induced pupil dilation were correlated with inter-individual differences in arousal-related mood enhancement during exercise and LC contrast, a marker of LC integrity measured by neuromelanin-weighted MRI scans. This suggests that the LC-catecholaminergic system is a potential mechanism for very light exercise-induced pupil dilation. Finally, we examined whether pupil dilation induced by very light exercise predicts postexercise benefits on executive function. A Stroop task to assess executive function (inhibitory control) was performed before and after the exercise, and prefrontal cortical activation during the task was measured using multichannel fNIRS. As a result, very light exercise elicited pupil dilation, improvement in Stroop task performance, and task-related left DLPFC activation. In addition, the change in pupil dilation predicted the magnitude of improvement in Stroop task performance, and mediation analysis showed that pupil dilation during very light exercise determined postexercise enhancement of executive function. These findings support our hypothesis that the LC arousal system works during very light exercise and is a possible underlying mechanism of enhanced prefrontal executive function. It also suggests that pupillometry may be a valuable tool to interpret the positive impact of aerobic exercise on improving brain and cognitive health.

Disclosures: R. Kuwamizu: None. **Y. Yamazaki:** None. **H. Soya:** None.

Poster

PSTR186: Executive Function and Cognitive Flexibility

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.14/S3

Topic: H.04. Executive Functions

Support: Asahi Soft Drinks Co., Ltd.

Title: Anti-cognitive fatigue effects of carbonated water during prolonged esports play: A randomized crossover trial

Authors: *S. TAKAHASHI[1], W. KOSUGI[2], S. MIZUNO[2], T. MATSUI[1];
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Abstract: Mitigating and recovering from a decline in executive function (EF), known as cognitive fatigue, is crucial for maintaining a balance between health and high human performance in modern society. Drinking carbonated water, which stimulates the brainstem through both transient receptor potential vanilloid (TRPV1) and transient receptor potential ankyrin 1 (TRPA1) to form sensations of heat and cold in the pharynx, enhances cognitive performance in simple tasks and poses low health risk. Esports, competitive video gaming, is a significant digital activity with complex cognitive demands that represents the convergence of cyberspace and physical world in modern society. We thus hypothesized that carbonated water alleviates cognitive fatigue during prolonged esports play and promotes subsequent recovery. To test this hypothesis, we conducted a randomized crossover trial involving 15 healthy adults (14 males and 1 female, 22.9 ± 2.5 years) under conditions of fresh water (FW) and carbonated water (CW). Participants competed in a virtual football game against standard-level gaming AI for 180 minutes while consuming the assigned water type. Psychological parameters (sense of fatigue, enjoyment, sleepiness, and EF assessed by Flanker task) were measured before, during (at 60, 120, and 180 minutes), and 30 minutes after playing (Post 30 min). Physiological parameters such as heart rate, blood glucose, and pupil diameter were also monitored throughout the session. Statistical analysis was performed using two-way ANOVA. Sense of fatigue in the FW condition increased over time and persisted at Post 30 min, whereas it did not increase in the CW condition. Sleepiness did not increase at any measurement point, and enjoyment levels were higher during and following play in CW than in FW. EF, as assessed by interference and correct response rates in the Flanker task, declined after 120 and 180 minutes of play in FW, with no significant decrease observed in CW. At Post 30 min, Flanker interference recovered in both conditions, with more pronounced recovery in CW. Pupil diameters as an indicator for prefrontal activity in FW decreased with playing duration, correlating with decreased Flanker interference, a trend not observed in CW. Maximum heart rates were higher in CW than in FW. Blood glucose levels decreased slightly depending on playing duration, regardless of water type. These findings suggest that drinking carbonated water not only mitigates cognitive fatigue during prolonged esports play but also enhances recovery afterward. Beyond esports, carbonated water could serve as a low-risk ergogenic aid for sustained cognitive digital activities integral to modern human life.

Disclosures: S. Takahashi: None. **T. Matsui:** None.

Poster

PSTR186: Executive Function and Cognitive Flexibility

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.15/S4

Topic: H.04. Executive Functions

Support: NIH Grant R01AG078266
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NIH Grant R01NR015452

Title: Motor inhibition in people with mild cognitive impairment: a stop signal study

Authors: *A. GORI[1], R. PAUL[1], K. HEFFNER[2], C.-S. R. LI[3], S. HU[1];
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Abstract: Motor inhibition involves the cancellation of a prepotent movement prior to its execution, a function that typically decreases with age. Previous research has reported impaired stopping efficiency during inhibition in healthy older adults as well as in patients with mild cognitive impairment (MCI). The present study aimed to evaluate the neural activations in motor inhibition in healthy controls (HC) and MCI patients. Participants completed three 8-minute sessions of the stop signal task (SST) during functional magnetic resonance imaging (fMRI). In the SST, participants were instructed to press a button to a frequent “go” stimulus but withhold their response when the go was followed by a “stop” stimulus, which appeared in approximately 1/3 of all trials. A button press in a go trial is a Go Success (GS), and no button press in a stop trial is a stop success (SS). Stop signal reaction time (SSRT) was computed as an index of inhibitory control. Brain images were analyzed using a generalized linear model built on the go signal onsets of individual trial types, and the results were evaluated at voxel p=0.005 whole brain with p=0.05 FWE at the cluster level. Behavioral results showed longer go trial reaction time (goRT) and SSRT in MCI as compared to HC, indicating a generally slower processing speed both in motor execution and inhibition in MCI. In the brain, the contrast of SS>GS that measures response inhibition showed greater left insula activation in MCI than in HC, perhaps suggesting an inefficient compensatory process. These results together provided new insights into the neural mechanisms of inhibitory control in older adults at risk for dementia.

Disclosures: A. Gori: None. **R. Paul:** None. **K. Heffner:** None. **C.R. Li:** None. **S. Hu:** None.

Poster

PSTR186: Executive Function and Cognitive Flexibility

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR186.16/S5

Topic: H.04. Executive Functions

Title: Human Neural Correlates of Successful and Unsuccessful Action Cancellation

Authors: *M. E. ALARIE[1], D. A. DIESBURG[2], W. F. ASAAD[3];
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Abstract: The stop-signal task (SST) is the gold standard paradigm for investigating response inhibition, or the ability to halt ongoing actions. In traditional SST designs, participants are instructed to respond to a go signal with a button press or, on ~30% of the trials, withhold their response when a delayed stop signal appears following the go signal. One significant challenge in SST paradigms is identifying and addressing proactive control behaviors, where participants preemptively slow down or withhold responses in anticipation of the stop signal (failures to launch). This is exacerbated by the binary nature of SST paradigms, where the absence of a response on a stop trial is interpreted as successful action cancellation. Therefore, the extent to which successful stops are truly suppressed actions or withheld responses due to proactive mechanisms is unclear. We aimed to disentangle proactive and reactive control strategies in SST performance and their underlying neural-behavioral dynamics in 1 patient undergoing intracranial monitoring for intractable epilepsy. We used joystick movements as a continuous metric of SST performance, where movements from center fixation to left- and right-hand panel thresholds indicated action initiation and completion, respectively. In line with previous work, we observed increased beta activity (~8.42 dB) in the right inferior frontal gyrus (rIFG) after the stop signal on successful response inhibition (SRI) trials. Trials where actions were initiated but not completed demonstrated higher rIFG beta activity (~3.77 dB) after the stop signal compared to SRI trials with no movement. Further, examining the time period before the stop signal revealed higher rIFG beta power (~6.70 dB) on no movement SRI trials, suggesting the role of proactive mechanisms during failures to launch.

Disclosures: M.E. Alarie: None. **D.A. Diesburg:** None. **W.F. Asaad:** None.

Poster

PSTR187: Animal Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.01/S6

Topic: H.06. Social Cognition

Support: NRF-2022R1A2C3008991

Title: A blend of innovation and imitation for sociocultural evolution

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Abstract: Sociocultural evolution, which describes how cultural behaviors develop over time in populations, has been documented in both human and nonhuman animal groups (Whiten, 2007; Clutton-Brock, 2021). For this, innovators, displaying novel behaviors previously unseen in their populations, have been considered to play an important role in evolution by exploring new ecological niches, diversifying actions and traits, and ultimately enhancing overall fitness (Tebbich, 2016). However, it has been reported that imitators, who replicate the actions of

others, are frequently dominant in various social groups (Reader, 2016). This raises questions about the conditions and mechanisms under which innovators and imitators can induce social evolution. Here, using a computational approach with a multi-agent system, we investigated the effect of innovators and imitators on the evolution of population behaviors. To test this, we designed a model simulating the survival of multi-agents. Each agent in this model has a single trait parameter that determines its shape, affecting energy acquisition and consumption efficiency. Firstly, we tested whether innovators alone could induce social evolution. We found that only innovators, whose traits evolve randomly and substantially differ from others', cannot increase the lifespan of individual agents, as their traits cannot reach an optimal value for survival. Next, we investigated the possibility of inducing social evolution by introducing imitators into a group consisting solely of innovators. Notably, when we introduced imitators who even randomly copied the traits of others, lifespan increased and converged to the optimal level. This implies that while social evolution is hard to induce in innovators alone, it can be induced where innovators and imitators, even in a random manner, coexist. In addition, when the ratio of innovators to imitators was altered, the converged lifespan was maximized when a small number of innovators and the majority of imitators were present in the group. Overall, our findings suggest a symbiotic relationship between innovators and imitators, with the innovator serving as the effect's seeder and the imitators as its propagators. Consequently, the coexistence of the minority of innovators and the majority of imitators is demonstrated to be a fundamental configuration requisite for population evolution.

Disclosures: S. Baek: None. **M. Kang:** None. **S. Paik:** None.

Poster

PSTR187: Animal Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.02/T1

Topic: H.06. Social Cognition

Support: NRF-2022R1A2C3008991

Title: Random decision-making in a multi-agent system for dynamic adaptation

Authors: *C. OCK[1], S. BAEK[2], S.-B. PAIK[1];
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Abstract: In a multi-agent system, a dynamic environment can bring critical challenges in system adaptation. In particular, interaction caused by each agent's action (e.g., crowding) can make single-agent-level optimal solutions become suboptimal (Nguyen 2020). These are commonly seen in real-world circumstances such as traffic jams (Sugiyama 2008), which occur when multiple drivers follow the shortest route without considering other drivers' actions. The field of reinforcement learning tried to tackle this issue by each agent updating the solution

(Gronauer 2022), but it still suffers from computational cost when applied to large-scale multi-agent systems. While from ant data (Imirzian 2019), we found that the ant that chose a longer path was as fast as the ant that followed the shortest path, suggesting the possible benefits of incorporating behavioral suboptimality in the system. Moreover, the ant experiment suggested that ants might choose suboptimal behavior randomly (Dussutour 2009). Inspired by ant, we introduce simple strategy that helps to adapt to dynamic environment by allowing agents to choose suboptimal solution. We implemented a multi-agent road traffic simulation, in which agents travel along the grid-shaped road toward the destination. We compared the performance by the average time spent by each agent. To confirm the effect of the single-agent level optimal solution in a multi-agent system, the greedy group, which follows the optimal path at the single-agent level was presented. We found that as the group size increased, the average time dramatically increased. Surprisingly when a random group, which randomly chooses suboptimal paths with a certain probability, was introduced, the average time considerably decreased compared to the greedy group, as the group size increased over a certain extent. This implies that random strategy can have equivalent or even better capability without extensive learning. Moreover, to find the effect of randomness, we changed the degree of randomness by the group size, and it was found that higher randomness is most advantageous as the group size grows. When randomness by group size was introduced, we found that the average time spent was minimized or equivalent to either group which spent a shorter time for each group size. This implies that merely modulating the randomness by the size of the group is feasible for real-world systems, where the number of agents participating in the system dynamically changes. Overall, our results demonstrate the effectiveness of including randomness in dynamic systems, thus providing insight into the potential benefits of stochastic behaviors in multi-agent conditions.

Disclosures: C. Ock: None. **S. Baek:** None. **S. Paik:** None.

Poster

PSTR187: Animal Behavior

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Program #/Poster #: PSTR187.03/T2

Topic: H.06. Social Cognition

Support: Tohoku University Research Program “Frontier Research in Duo” No.2101
MEXT KAKENHI JP24H01218
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Title: A marker-based motion tracking system for small animals enabling objective inference of the cognition of social signals.

Authors: *M. FUJIBAYASHI, K. ABE;
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Abstract: In naturalistic communications of animals, the reactions of each individual mutually influence the other's behavior. The detailed analysis of these behavior has been a key challenge in the field of behavioral neuroscience. However, the deficiency of sophisticated tools for behavioral analysis has prevented the understanding of such behavioral aspects, especially in free-moving conditions. In this study, we introduced a marker-based motion capture technique, which was previously confined to large animals, for the study of small animals' behavior. Employing lightweight color markers and a novel algorithm including color feature extraction and silhouette tracking, this system demonstrated precise tracking of markers and body locations of freely moving subjects across diverse environments and among different individuals. This expands its potential applications to multiple subject tracking of directly interacting animals. With this system, we have quantitively analyzed the behaviors of zebra finches (*Taeniopygia guttata*) in the response to various stimulus, including male and female conspecifics in live or virtual formats, auditory stimuli, and assessed their differences in social or individual discrimination as well as in the process of learning under conditioning procedure. We thoroughly analyzed the utilization of vision in conspecific recognition and revealed variations in the usage of monocular and binocular sight, and further the left and right eyes, as well as the duration of sight among the presented individuals. These analyses further enabled us to evaluate the visual attention of songbirds, providing an objective method to infer the cognition of stimuli. In addition, we examined the behavioral reactions of directly interacting subjects and revealed more naturalistic aspects of social interactions. We also verified that our motion capture system can be utilized in the analysis of mouse (*Mus musculus*) behavior in the context of interactive engagements with familiar and unfamiliar conspecifics. Our methodology presents an efficient and easy-to-use tool to perform advanced behavioral analysis in small animals. Moreover, our system provides an objective method to evaluate their cognition of communicative signals and to analyze the involved neural mechanisms through the combination with general neural activity manipulation methods.

Disclosures: M. Fujibayashi: None. **K. Abe:** None.

Poster

PSTR187: Animal Behavior

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Program #/Poster #: PSTR187.04/T3

Topic: H.06. Social Cognition

Support: NSERC, RGPIN-2018-04699

Title: Sex hormones' rapid effects on social and non-social short-term memory and neuronal plasticity in the dorsal hippocampus of male mice

Authors: *K. E. LADOUCEUR[1], C. D. BAILEY[2], E. CHOLERIS[3];
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Abstract: It has been established that sex-steroid hormones can rapidly influence memory via either delayed genomic or rapid mechanisms that are independent of gene transcription. Previous studies have shown that estradiol (E2) facilitates social and non-social short-term memory (STM) in the dorsal hippocampus (HPC) of ovariectomized female mice. Although it is known that androgens and estrogens also affect the brain and behavior in males, their rapid effects on STM in the dorsal HPC of male mice have yet to be elucidated. Therefore, we investigated the rapid effects of E2 and dihydrotestosterone (DHT) on three STM paradigms, object placement, object recognition, and social recognition, in the dorsal HPC of castrated (CX) CD1 male mice. The mice were bilaterally administered into the dorsal hippocampus via implanted cannulae either E2 (vehicle, 10, 25, 50, 100, 150, 200, or 300 nM) or DHT (vehicle, 0.0625, 0.25, 0.5, or 0.75 μg/μL) and their short-term memory performance was assessed in one of the three tasks. Each task was completed within a time frame consistent with the rapid effects of hormones (40 minutes since treatment administration). Furthermore, the rapid effects of E2 and DHT on neuronal plasticity were also examined through changes in dendritic spine density in the CA1 subregion of the dorsal HPC. Results show that E2 and DHT rapidly facilitate social and non-social STM in the dorsal HPC of males and increase CA1 hippocampal dendritic spine density. These findings demonstrate a crucial role for both estrogens and androgens in the rapid regulation of male cognition and neuronal plasticity.

Disclosures: K.E. LaDouceur: None. **C.D. Bailey:** None. **E. Choleris:** None.

Poster

PSTR187: Animal Behavior

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Program #/Poster #: PSTR187.05/T4

Topic: H.06. Social Cognition

Support: CIHR 179866
FRQNT 326838

Title: Social valence dictates sex differences in identity recognition

Authors: *Q. XU, A. LAROSA, A. WONG, J. Q. LEE, M. P. BRANDON, T. WONG;
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Abstract: Social interactions often carry emotional meaning, where negative experiences lead to behaviors that aim to prevent conflict and playful or nurturing interactions are generally rewarding. The emotional affect attached to social interactions is termed social valence. Maladaptive negative social valence processing has been implicated in enhancing mood disorder susceptibility, such as in the higher vulnerability to depression in women. Sex differences in

social valence processing could be related to the hippocampus, whose activity was shown to be modulated by negative and positive socially valenced experiences. However, this hypothesis remains untested. We used the approach of social identify recognition and developed positive social valence (PSV) and negative social valence (NSV) tasks, where the identity of conspecifics was associated with distinct positive (food reward) or negative (foot shocks or social defeat) valences. Exhibiting an increase or decrease in interaction time with PSV or NSV mice, respectively, versus a familiar neutral mouse represented identity recognition. We found that in the PSV task, both male (n=13) and female (n=10) mice demonstrated identity recognition. However, only male (n=31) but not female mice (n=32) differentiated between a neutral and a shock or social defeat-associated mouse in the NSV task. Identity recognition in the NSV task in female mice was not improved by increasing training sessions and was not influenced by estrous status. No sex differences were found in the positive and negative version of the tasks for object recognition, suggesting specific sex differences in social valence processing. Finally, using in vivo calcium imaging, we revealed reduced hippocampal representation of social information in female mice when compared to male mice, suggesting imprecise encoding of NSV. Our data demonstrate that while both male and female mice are capable of identify recognition, negative social valence has a stronger detrimental effect on identity recognition in female than in male mice. Importantly, this observed sex difference cannot be attributed to impaired social memory capacity or deficits in associative learning in female mice. Sex differences in social valence processing may contribute to the heightened vulnerability to social stress-related mood disorders in women.

Disclosures: Q. Xu: None. **A. Larosa:** None. **A. Wong:** None. **J.Q. Lee:** None. **M.P. Brandon:** None. **T. Wong:** None.

Poster

PSTR187: Animal Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.06/T5

Topic: H.06. Social Cognition

Title: Manipulation of cerebellar nuclei activity modulates cognition, locomotion, and repetitive behaviors in mice

Authors: *H. VIEIRA[1,2], T. LYLE[2], J. L. VERPEUT[3];
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Abstract: Although previously considered exclusively a motor area, the cerebellum is now implicated in cognitive, social, and repetitive behaviors (Badura et al., 2018; Verpeut et al., 2023). Furthermore, both preclinical models of autism spectrum disorder (ASD) and human patients are found to have atypical cerebellar activity and long-distance connections (Stoodley, 2014; Stoodley et al., 2017). Additionally, preclinical models exhibit repetitive behaviors (e.g., self-grooming, jumping, circling, and marble burying (Kim et al., 2016). Repetitive behaviors are

increased in animals with reduced Purkinje cell activity (Benarroch, 2017) but may also be modulated by connections between the cerebellar nuclei (CN) and ventral tegmental area (VTA) (Carta et al., 2019). We hypothesized that decreasing CN-VTA activity would decrease reward-mediated cognition and repetitive behaviors, while increasing CN-VTA activity would increase cognition and repetitive behaviors.
To manipulate CN activity, we used Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to increase local excitation (AAV2-hSyn-hM3D(Gq)-mCherry) or inhibition (AAV2-hSyn-hM4D(Gi)-mCherry). Clozapine-N-oxide (CNO; 10 mg/kg), a synthetic DREADD ligand, was administered via drinking water from postnatal day 21-35. Learning and reversal cognition were investigated in an operant conditioning pairwise visual discrimination task. Locomotion, time spent, and repetitive behaviors (i.e., grooming, rearing, and climbing) were examined by hand-scoring and the machine learning software SLEAP (Social LEAP Estimates Animal Poses). Neural activation across multiple brain areas was quantified via c-Fos, an immediate early gene.
CN manipulation did not affect learning, but CN excitation improved reversal performance ($p < 0.05$) in females, while CN inhibition improved reversal performance ($p < 0.01$) in males. DREADD activation (both inhibition and excitation) revealed a significant reduction in grooming ($p = 0.012$), rearing ($p = 0.009$), and climbing ($p = 0.003$). CN excitation increased time near the touchscreen and reward compared to the untreated group ($p < 0.05$). However, compared to untreated animals, CN inhibition reduced time near the touchscreen ($p < 0.05$). Results suggest CN manipulations alter cognitive performance in sex-specific ways, reduce repetitive behaviors, and may impact reward processing and attention. Future experiments will examine how CN-VTA activity influences local and long-distance cerebellar connections. These findings suggest potential avenues for exploring novel pathways in neurodevelopmental disorders like ASD.

Disclosures: H. Vieira: None. **T. Lyle:** None. **J.L. Verpeut:** None.

Poster

PSTR187: Animal Behavior

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Program #/Poster #: PSTR187.07/T6

Topic: H.06. Social Cognition

Support: ANR-21-CE37-0016

Title: Subcortical correlates of macaques' social tolerance scale

Authors: *S. SILVERE[1], J. LAMY[2], C. PO[2], M. LEGRAND[1], J. SALLET[3], S. BALLESTA[1]; [1]Primate Ctr. - Silabe (LNCA - UMR 7364), Univ. of Strasbourg, STRASBOURG, France; [2]ICube, Univ. de Strasbourg-CNRS, STRASBOURG, France; [3]Stem Cell and Brain Res. Inst. (SBRI, U1208), Univ. of Lyon 1, INSERM, BRON, France

Abstract: Relying on years of ethological studies of monkeys belonging to the *Macaca* genus, 25 species of macaques have been ordered on a 4-grade social tolerance scale. This scale is based on 18 covariant behavioral traits such as counter-aggression and reconciliation rates of each species that are ordered from low (Grade 1, such as *Macaca mulatta* - rhesus macaque) to high social tolerance (Grade 4, such as *M. tonkeana* - Tonkean macaque). However, the neuroanatomical correlates of these interspecific differences in macaques' social tolerance remain unknown. Previous studies of the social brain in *M. mulatta* have shown that the volume of subcortical regions (e.g. amygdala, striatum, or raphe nucleus) correlates with social variables (e.g. individual's hierarchical rank or group size). We thus hypothesize that volumetric variations of subcortical regions belonging to the social brain, should reflect the level of tolerance of the species studied. To bring an evolutionary perspective to the social brain hypothesis, we fostered a unique collection of 36 *post mortem* brain samples from 12 macaque species, combining Strasbourg-based 7T MRI scans with data from already existing databases (PRIMate Data Exchange, Japan Monkey Centre, Oxford University). Furthermore, we also conducted *in vivo* brain scans on 3T MRI on individuals from two species (*M. mulatta* and *M. tonkeana*) from well-studied semi-free ranging macaques' social groups of 13 to 26 individuals. Based on recent insights into the primates' social brain, we focused on the subcortical anatomy using a semi-automatic atlas (SARM atlas). Our main result identified that amygdala's volume can predict macaque social grades, with tolerant macaques exhibiting larger amygdala nuclei than intolerant ones. Our analysis also pinpointed that this effect is particularly noticeable during early life stages as the amygdala became, proportionally to the rest of the brain, bigger with age in intolerant species and smaller in tolerant species. These findings provide unprecedented evolutionary insights into cross-species neuroanatomical correlates of naturalistic social behaviors in non-human primates. Further analysis will focus on *in vivo* anatomical and functional neural networks, connected to the amygdala, that will provide more detailed explanations to the cognitive and behavioral differences of macaques along the social tolerance scale.

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Poster

PSTR187: Animal Behavior

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Program #/Poster #: PSTR187.08/T8

Topic: H.06. Social Cognition

Support: KAKENHI JP 22K06480
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KAKENHI JP 20K21562

Title: Subordinate macaque monkeys exhibit altruistic behaviors in reward/aversive stimulus-dependent decision making

Authors: *K. ENOMOTO[1], K. WATANABE[2], M. HARUNO[1];
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Abstract: Most primate species live in groups and constitute hierarchical societies. To avoid risks and conflicts and acquire resource efficiently, it is essential to make appropriate decisions considering the social ranking of the self and others. Since behavioral adjustments by social ranking are widely observed not only in humans but also in apes and monkeys, neural mechanisms for the rank-dependent behavioral adjustment are likely common in most primates. To elucidate the effects of social rank on behavior in monkeys, we examined reward/aversive stimulus-dependent social behavior of macaque monkeys. Using three female Japanese monkeys, we first estimated their dominance indices based on a food-sharing test between two monkeys. Next, for implementing our decision-making task, we developed a novel device: a transparent display placed between two transparent touch panels, allowing monkeys sitting on either side to monitor each other's actions and facial expressions while making behavioral choices. Two monkeys learned a social choice task in which they chose one of two alternatives, each consisting of a pair of varying amounts of their own reward (juice) and aversive stimuli (air puffs to the face) to themselves or their partner. When rewards and aversive stimuli were given to themselves, the monkeys could appropriately avoid risk and choose the higher reward option (over 80%). On the other hand, when aversive stimuli were given to the partner with the higher dominance rank, the subordinate monkeys exhibited prosocial behaviors. More specifically, in cases where the amount of reward between two options was the same and the strength of the air puff was different, the lower-ranked monkeys chose the option with less aversive events for the partner significantly more often (over 50%), even though there was no aversive stimulus for themselves for choosing either option. This prosocial behavior was observed significantly more often than in the non-social control condition, in which aversive stimulus was given for an empty chair. Furthermore, even if their own reward was less, the subordinate monkey also chose the option with less aversive events to the partner (5-50%). These results clearly demonstrate that macaque monkeys can adopt altruistic behaviors like humans.

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Poster

PSTR187: Animal Behavior

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Topic: H.06. Social Cognition

Title: Intrinsic Curiosity as a Driver of Rule Learning in Marmosets

Authors: X. SHI[1], *X. WANG[2];
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Abstract: Curiosity is a fundamental motivator for humans, but its underlying biological mechanisms are not well understood. A significant challenge in this research has been a lack of behavioral paradigms that genuinely evoke animals' intrinsic curiosity, as previous studies often depend on extrinsic motivators like food rewards in behavioral training. To bridge this methodological gap, we have developed a behavioral paradigm to explore the curiosity-driven learning behaviors in marmosets, a highly social New World monkey species. An apparatus with touchscreen was attached to a marmoset's home cage, allowing the animal to autonomously choose whether to enter and participate in rule learning tasks. These tasks involved learning to discriminate between different images or sound sequences by touching specific areas on the screen, with correct choices resulting in the presentation of a novel video stimulus. No other external rewards were provided to the marmosets. The experimental subjects had free access to water and foods in their home cages. Our results showed that the pursuit of novel stimuli led marmosets to enter the apparatus and engage in the learning tasks. Although there was considerable individual variability in learning efficiency, most marmosets (n=4, total 6 marmosets) achieved the proficiency in an image discrimination task with a correct response rate exceeding 75%. Furthermore, a subset of the subjects (2 of 4 marmosets) successfully transferred to new rules after the novel video stimulus associations were reversed. Additionally, one marmoset was able to finish a complex 4-syllable sound sequence discrimination task, motivated solely by its own curiosity. Through these efforts, we have established a behavioral paradigm to use curiosity as a potent motivator for learning in a non-human primate species. This work enriches our understanding of animal curiosity and may eventually shed light on neural mechanisms underlying human curiosity.

Disclosures: X. Shi: None. **X. Wang:** None.

Poster

PSTR187: Animal Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.10/T10

Topic: H.06. Social Cognition

Support: NSF-AIDMM-NRT (DGE-2152014 to J.R.)
NIMH (R01-MH124387 to F.F.)
Helen Lyng White fellowship (Neuroscience Center, UNC Chapel Hill)

Title: Topological data analysis characterizes rich behavior dynamics in naturalistic social interaction in ferrets

Authors: *J. REILING[1], F. FROHLICH[2,3], M. ZHANG[1];
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Abstract: A key challenge in analyzing naturalistic social interaction is to fully capture the rich, dynamic behaviors without predefined task-based paradigms. Traditional data analysis techniques designed for task-based experiments are not well-equipped to handle the complexity and flexibility of naturalistic behavioral dynamics. New methods better tailored for naturalistic dynamics are in demand. Topological data analysis (TDA), a family of applied mathematics methods that computationally characterize the "shape" of complex data, is increasingly employed to characterize neural activity. Temporal Mapper is a recently developed TDA method to capture stable states (attractors) and transitions in complex neural dynamics in both theoretical models and fMRI data. Temporal Mapper represents neural dynamics as a directed graph, where nodes represent unique brain states, and edges represent transitions between states. The main objective of the present study is to generalize the use of this computational approach from characterizing brain dynamics to behavioral dynamics, in particular, in naturalistic social interaction between animals. More specifically, we developed a computational pipeline to construct a transition network of social-behavioral states from video recordings of naturalistic social interaction. The pipeline consists of four steps: (1) tracking the movement of animals' body segment motion from videos using the DeepLabCut (DLC), a computer vision tool; (2) cleaning decoded time series generated by DLC with automatic interpolation of missing segments (e.g., due to brief occlusion) and correction of swapped ferret identity labels; (3) transforming absolute body segment coordinates to relative body positions between animals, more relevant to depicting social interaction; (4) constructing transition networks from times series of animals' relative body positions with Temporal Mapper. We successfully applied the pipeline to video recordings of ferret naturalistic social interaction and obtained rich transition networks of behavioral states. Further examination of the nodes of transition networks demonstrates that social states are associated with richer relative body positions compared to non-social states. This project provides an initial step towards a unified computational-experimental framework for brain-behavioral dynamics in naturalistic settings.

Disclosures: J. Reiling: None. **F. Frohlich:** D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Insel Spital, Academic Press. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of North Carolina, Chapel Hill. F. Consulting Fees (e.g., advisory boards); Electromedical Products International. **M. Zhang:** None.

Poster

PSTR187: Animal Behavior

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Topic: H.06. Social Cognition

Support: NIH R01 5TfR01ActfMH126035
NIH DP2

T32MH065214
Allen Institute

Title: Mapping behavioral and neural correlates of territory discrimination and exploration

Authors: *D. ALLEN, A. L. FALKNER;
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Abstract: Throughout the mammalian kingdom animals use chemosensory marks to delineate boundaries between conspecifics in the wild. These cues convey information about an animal's identity, physical fitness, and current affective state which, in conjunction with their spatial location, can form representations of territories in the mammalian brain. Additionally, the presence of urine marks can drive adaptive behavioral responses. In particular, male mice predominantly avoid urine cues when detected, and will actively patrol and defend chemosensory boundaries from other males. Male's also preferentially mark locations in the environment where conspecific cues are detected, potentially as a form of competition. While some research has worked to dissociate multiple facets of mouse territories, our work develops an assay to capture marking and pose behavior in real time as mice form and explore territories. We use a novel, automated thermal detection pipeline to extract and quantify urine marks in real time. With this paradigm, we recapitulate findings in the field showing that male mice avoid conspecific territories as a function of how much that conspecific marked. We then used this assay to ask whether activity in the lateral septum (LS) differentiates between territories, and whether it modulates territorial exploration. Decades of work on the function of LS has highlighted diverse functionality spanning spatial coding, social discrimination, and affective regulation, which suggest that it could play a role in mediating territorial behaviors. We show that functional ablation of LS disrupts stereotyped territory avoidance in male mice. Additionally, silicon probe recordings in LS show population level coding of home cage, suggesting that territory identity is encoded in LS to drive these exploration behaviors. Furthermore, when exploring differentially marked locations in the arena, we observed positive correlations of single LS units with distance to urine marks. These findings together demonstrate a potential role of LS to discriminate between territories, and guide chemosensory-dependent exploration behavior as mice navigate their environment.

Disclosures: D. Allen: None. **A.L. Falkner:** None.

Poster

PSTR187: Animal Behavior

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.12/T12

Topic: H.06. Social Cognition

Support: NIH T32 NS096050

Title: The ventral hippocampus encodes social novelty in mice

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Abstract: Prior research has determined that the ventral hippocampus is necessary for mice to preferentially investigate novel conspecifics over familiar conspecifics. However, few studies have recorded endogenous activity in the ventral hippocampus during social interactions so it largely remains unknown what social information the ventral hippocampus encodes. We hypothesize that the ventral hippocampus encodes social recognition information and contains neurons that preferentially fire for novel and familiar conspecifics. To test this hypothesis, we performed one-photon calcium imaging to record endogenous activity in the ventral CA1 and ventral CA3 subfields of the hippocampus in male mice. We then developed a novel behavioral assay called in the linear approach assay in which novel and familiar conspecifics are presented to the imaging mice at the same spatial location. This approach is critical to disentangle social from spatial encoding in the hippocampus. We trained support vector machine classifiers to decode social targets from population neural activity. Support vector machine classifiers were unable to decode two novel conspecifics above chance levels however they were able to decode novel and familiar conspecifics above chance levels. Interestingly, decoding accuracy is significantly higher in the ventral CA3 compared to the ventral CA1. Additionally, individual ventral hippocampus neurons preferentially fired for both novel and familiar conspecifics. Together, these results provide evidence that the ventral hippocampus encodes social novelty. Additionally, this research suggests that there are functional differences in the ventral CA1 and ventral CA3 with the latter encoding social recognition information more robustly.

Disclosures: B. Dykstra: None. **G. Berman:** None. **M. Murugan:** None.

Poster

PSTR187: Animal Behavior

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Program #/Poster #: PSTR187.13/U1

Topic: H.06. Social Cognition

Support: NIH/NIDCD 04845 (LMR)
F30 MH122048 (KKS)
Schmitt Program for Integrative Neuroscience

Title: Extraction of Identity Information From Visual But Not Auditory Communication Information In Primate Prefrontal Cortex

Authors: K. K. SHARMA[1], M. DILTZ[1], M. FUCHS[1], Y. C. CAI[1], ***L. M. ROMANSKI**[2];
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Abstract: Facial gestures and vocalizations combine in many brain regions to form meaningful exchanges during social communication. In the macaque, the VLPFC is a site of multisensory integration that shows high regional activation (via fMRI) and selective single unit responses to

social stimuli. Most recently, our group has shown that populations of neurons, instead of single units, in the VLPFC persistently encode the identity of a conspecific while viewing naturalistic, communicative expressions. Given that approximately 70% of VLPFC neurons are multisensory, frequently combining auditory and visual information non-linearly, we sought to understand if population encoding of identity is a result of multisensory integration or could be supported by a single sensory modality. For the current study we used the same naturalistic audiovisual movie clips of macaques vocalizing as previously employed and compared population responses in VLPFC to multisensory as well as the unimodal auditory and visual components separately. Ensembles of single units were recorded in the VLPFC from multi-electrode arrays in VLPFC in one macaque. We utilized 9 short audiovisual movie clips that consisted of 3 unfamiliar macaques (3 identities) each producing 3 vocalizations accompanied by prototypical facial expressions (3 vocalization/expressions). Each recording session involved the presentation of 3 sets of stimuli: (1) the audiovisual movie clips, (2) the silent movie clips (same movie, with audio track removed) and (3) the audio track/vocalization alone. We recorded the activity of a small population of neurons (39-42 units) during each task and then performed a sliding bin decoding analysis using the population activity to predict the identity of conspecifics shown in the stimuli of each trial (high-dimensional discriminant analysis, LOOCV, typically 107 training trials). We found that decoding accuracy for identity increased quickly after stimulus onset for audiovisual and visual trials, peaking between 60-80% at 300ms post stimulus onset and sustained above 45% at 800ms (chance = 33%, n=10 populations per modality). In contrast, identity decoding accuracy for auditory (vocalization alone) trials ranged between 30-40% and did not vary across the time of stimulus presentation (n = 9). These results suggest that, in nonhuman primates, visual face and contextual features are critical components of identity processing during social communication that may precede or guide accompanying vocal perception.

Disclosures: K.K. Sharma: None. **M. Diltz:** None. **M. Fuchs:** None. **Y.C. Cai:** None. **L.M. Romanski:** None.

Poster

PSTR187: Animal Behavior

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Topic: H.06. Social Cognition

Support: Oxytocin U19 BRAIN Initiative Grant, National Institute of Neurological Disorders and Stroke, National Institutes of Health (2U19NS107616)

Title: Sensory modalities contributing to dam approach toward sick pups

Authors: *G. KAUR[1], A. CASLIN[2], R. C. FROEMKE[3];
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Abstract: Being sick changes our behaviors, and it is useful for animals to be able to detect sickness in others (Eisenberger et al. Neuropsychopharmacol 2017). We are examining how parental animals recognize and respond to sickness in their offspring (Caslin et al. SFN Abstracts 2024), and dams display persistent increased physical contact (i.e., huddling) toward familiar lipopolysaccharides (LPS)-injected pups vs. saline controls. Here we aimed to determine which sensory modalities allow maternal mice to determine and flexibly respond to pup health status. We used three-chambered social preference tests in wild-type mice to determine if vision and olfaction contribute to dam approach towards an LPS-injected vs. saline-injected P14 juvenile. We also measured physiological changes to weight and temperature, and recorded pup vocalizations before and after LPS vs. saline injection. Prior to testing, dams were habituated to the arena for twenty minutes. Dams were tested in several different conditions: in the dark, with a transparent barrier, and with juvenile urine cues. In contrast to approach when sick pups were present, dams avoided olfactory cues from LPS-injected pups compared to saline-injected pups. The juvenile temperature increased in the first 6 hours after LPS injection; after 6 hours, temperature returned to baseline levels. In contrast, pup vocalizations did not significantly differ before and after injections. Thus, somatic (pup temperature) and olfactory signals may be important ways for parental animals to sense sickness in offspring, although olfactory cues alone may lead to avoidance rather than attraction in the healthy adult.

Disclosures: G. Kaur: None. **A. Caslin:** None. **R.C. Froemke:** None.

Poster

PSTR187: Animal Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.15/U3

Topic: H.06. Social Cognition

Support: Intramural Research Program of the NIH, National Institute on Aging

Title: In search of determinants of social cognitive aging in a Long-Evans rat model

Authors: *S. DUTTA GUPTA[1], K. HUILLCA-JARA[2], E. PEREZ[1], J. M. LONG[3], P. R. RAPP[4];
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Abstract: Social connectedness is integral to health and well-being across the lifespan. Weakened social networking during aging is associated with a shorter lifespan in rodents and earlier mortality in humans. However, some social-cognitive domains, including emotion recognition and impression formation also decline with age, raising the question of whether the compromised social dynamics in aging a primary consequence of social capabilities or are

secondary to overall neurocognitive decline. It is crucial to identify the key contributing factors in order to develop effective interventions for social deficits, which benefit overall health in advanced age. The present study aimed to better understand how aging affects social cognition using a Long-Evans rat model. Young (6-7 months old; n = 40) and aged (24-25 months old; n = 75) male rats were behaviorally characterized based on their spatial memory performance in the Morris water maze, and we then assessed sociability and social novelty functions using a three-chambered social interaction test. In the sociability trial, both age groups exhibited a strong preference for social interaction with a trapped unfamiliar conspecific over an inanimate object. In the subsequent social novelty trial, when rats were given a choice between a familiar animal (from the sociability trial), or a novel conspecific, young rats displayed a robust social novelty preference. Aged rats by comparison showed much wider individual differences, on average, distributing their exploration equally between the novel and familiar conspecifics. Strikingly, a substantial subset of aged rats spent more time interacting with the familiar animal, a social phenotype not observed in young rats. Further analysis revealed that the social and spatial learning indices were uncoupled, indicating that some features of social cognition are vulnerable to aging independent of changes in memory. To explore the basis of the individual differences observed, next, we quantified oxytocin immunoreactive neuron number in the hypothalamic paraventricular nucleus and supraoptic nucleus, i.e., a neuropeptide implicated in social cognition. The preliminary results suggest that hypothalamic oxytocin cell number is preserved in aging. Ongoing work is exploring additional cell types, circuits, and interventions to interrogate the underlying networks of social cognition in aging.

Disclosures: S. Dutta Gupta: None. **K. Huillca-Jara:** None. **E. Perez:** None. **J.M. Long:** None. **P.R. Rapp:** None.

Poster

PSTR187: Animal Behavior

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.16/U4

Topic: H.06. Social Cognition

Support: 5R01MH116882-04

Title: Investigating the contribution of the cerebellar dentate nucleus to cognitive and social processing

Authors: *Y. VELAZQUEZ[1], P. TSAI[2];
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Abstract: The function of the cerebellum (CB) has been long thought to be restricted to motor coordination. However, human neuroimaging studies have revealed that it also plays a role in cognitive functions such as working memory, language, and social processing. Discrete regions

of the CB such as the dentate nucleus (DN) have been implicated in cognitive tasks regarding associative learning and goal-directed navigation; however, these learning processes are orchestrated through the complex interaction of several brain regions, including the hippocampus (HPC). Interestingly, DN-HPC anatomical connectivity has been recently established, but to what extent cerebellar DN activity contributes to cognitive processing, and whether DN-HPC connections are functionally relevant remains poorly understood. We hypothesize that DN function is necessary for proper cognitive processing, and activity in this region can modulate the activity of the HPC and therefore, its functions associated to cognition. To study this, we used excitatory and inhibitory 'Designer Receptors Exclusively Activated by Designer Drugs' (DREADDS) to determine the necessity of DN activity for cognitive behaviors in wild-type C57BL/6 male mice. Mice were injected into the DN bilaterally with either a AAV-hSyn-hM3D(Gq)-mCherry excitatory or AAV-hSyn-hM4D(Gi)-mCherry inhibitory DREADD; these receptors can only be activated by the ligand clozapine-N-oxide (CNO). Two weeks after injection, mice were treated with vehicle or CNO before testing for cognitive behavioral deficits in social and object novelty recognition, spatial navigation, and fear **conditioning. In addition, we evaluated the expression of the immediate early gene cFos in the HPC after chemogenetic excitation and inhibition of the DN. Chemogenetic modulation of the DN resulted in social novelty recognition impairments, while motor functions were intact. Moreover, Gq-mediated activation of the DN resulted in increased cFos expression in the dorsal HPC-CA1 subregion, while Gi-mediated inhibition of the DN resulted in a reduction in cFos expression in the same area. These results show that DN activity is necessary for social recognition, and is sufficient to impact HPC firing, highlighting the importance of cerebellar activity for cognitive processing and cognitive relevant circuits.**

Disclosures: Y. Velazquez: None. **P. Tsai:** None.

Poster

PSTR187: Animal Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR187.17/U5

Topic: H.06. Social Cognition

Title: Role of LIR-mediated mATG8 function in neuronal autophagy and behavior

Authors: *S. PARK[1], H. KIM[2], Y.-K. LEE[1], C.-S. LIM[2], J.-A. LEE[1];
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Abstract: Mammalian ATG8 proteins (mATG8s) are crucial for autophagosome biogenesis and cargo recruitment during autophagy. This process is tightly regulated by many mATG8-associated proteins with LC3 interacting regions (LIR). Although many LIR-containing proteins have been identified, their specific physiological relevance in modulating mice behavior is largely unknown. Therefore, in this study, to elucidate potential roles of LIR-mediated mATG8

function in mice behavior, we have generated knock-in mice overexpressing HyD-LIR(TP)-Venus which specifically binds to mATG8s (LC3s, or GABARAPs) using CaMKII-CRE-inducible system known to exhibit selectivity for excitatory glutamatergic neurons in vivo. Indeed, HyD-LIR(TP)-Venus was localized predominantly to LC3 family or GABARAP family-positive autophagosomes in the hippocampal or cortical neurons, leading to an accumulation of p62, a selective autophagy substrate, especially in the hippocampus and cortex but not in the cerebellum. This suggests an impairment in autophagic flux through the sequestration of endogenous LC3s and GABARAPs. To investigate the effect of LIR-mediated mATG8 by HyD-LIR(TP)-Venus on learning and memory, we performed behavioral tests including object location memory, three -chamber, and Morris water-maze tests. Very interestingly, the expression of HyD-LIR(TP)-Venus in excitatory glutamatergic neurons resulted in deficits in object location memory, social recognition memory, without affecting anxiety levels or spatial memory in the Morris water maze test. Proteomic analysis identified several synaptic proteins with LIR motifs as potential mediators of social memory deficits in mice, highlighting the critical role of LIR-containing proteins in neuronal autophagy and cognitive behaviors. This study reveals novel insights into how mATG8s and their interactions with LIR-containing synaptic proteins influence neuronal function and behavior.

Disclosures: S. Park: None. **H. Kim:** None. **Y. Lee:** None. **C. Lim:** None. **J. Lee:** None.

Poster

PSTR188: Striatal and Corticostriatal Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.01/U6

Topic: H.08. Learning and Memory

Support: NIH IRP
Annual report ZIMH 2032

Title: Chemogenetic disruption of projections from perirhinal cortex to rostromedial caudate impairs stimulus-reward association learning in rhesus monkeys

Authors: *S. WEBSTER-BASS[1], W. WANG[1], M. A. ELDRIDGE[1], N. MIYAZAKI[2], J. E. PEARL[3], W. LERCHNER[1], B. LI[1], T. SETOGAWA[4], J. N. TURCHI[1], P. RODRIGUEZ[1], B. J. RICHMOND[1];
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Abstract: Nonhuman primates use learned stimulus-reward associations to guide foraging. We previously showed that both rhinal cortex (Rh) and rostromedial caudate (rmCD) have causal roles in associating value with visual stimuli in rhesus monkeys. Layer 5 neurons in Rh project to rmCD. Here, we interrupted this connection by combining a unilateral Rh lesion with

contralateral expression of an hM$_4$Di DREADD via injection of a retrograde lentivirus (FuG-E) into rmCD of 2 monkeys. To validate successful targeting, we used post-operative MR imaging of a contrast agent (MnCl2) co-infused with the virus, PET imaging of radioligand-receptor binding, and digital reconstruction of the lesion. Monkeys were trained preoperatively to perform the 'reward-size' behavioral task, to demonstrate their knowledge of stimulus-reward associations. For every trial in this behavioral task, the monkey must hold a touch bar to start the trial, wait for 1 of 4 images to appear on the display, and release the bar when the 'go' cue is displayed to receive the unique reward amount associated with each unique image. At baseline, or after vehicle injection, monkeys consistently rejected the 2 smallest reward sizes, and accepted the 2 largest reward sizes. Interruption of projection neurons from Rh to rmCD via systemic injections of DREADD agonist deschloroclozapine (DCZ, 0.1mg/kg, i.m.), or clozapine N-oxide (CNO, 10 mg/kg, i.m.), had little effect on previously learned stimulus-reward associations but impaired the learning of new stimulus-reward associations. We localized this learning impairment by selectively silencing projection neurons from perirhinal cortex (PRh) to rmCD via intracerebral microinfusions of DCZ into PRh, which led to a significant deficit in learning of new stimulus-reward associations. These results suggest that the learning, not retrieval, of visual stimulus-reward associations involves projection neurons from PRh to rmCD.

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Poster

PSTR188: Striatal and Corticostriatal Circuits

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Topic: H.08. Learning and Memory

Support: FAPESP 2021/05022-9
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CAPES/PROEX 001
CAPES-PrInt 88887.576069/2020-00
CNPq 305541/2022-6

Title: Effects of Deep Brain Stimulation on Reversal Learning in Rats: A Striatal Region Analysis

Authors: *I. WAKU, A. E. REIMER, A. R. DE OLIVEIRA;
Dept. of Psychology, Federal Univ. of São Carlos, São Carlos, Brazil

Abstract: Deep brain stimulation (DBS) is a promising neuromodulatory therapy for psychiatric disorders such as obsessive-compulsive disorder. Clinical evidence suggests that DBS targeting the ventral capsule/ventral striatum (VC/VS) may enhance behavioral flexibility, a key trait often

impaired in mental disorders. Behavioral flexibility can be assessed through reversal learning tasks, which explore an individual's ability to adjust their behavior when a response-consequence association rule is reversed. Our study investigates the impact of DBS of striatal subregions on rats' behavioral flexibility during reversal learning. For this, 29 male Wistar rats (CEUA protocol 9343151021) were implanted with bipolar electrodes targeting either the mid-striatum or ventral striatum (VS). A three-phase reversal learning task was used, comprising Learning, Reversal, and Reversal to Baseline (RTB) phases, each consisting of three sessions. During the Learning phase, one side of the experimental chamber was associated with a reward, while the other side was not. In the Reversal phase, the locations of the correct and incorrect responses were switched. Rats received bilateral stimulation (biphasic, 130 Hz, 0.1 ms, 300 μA) or a sham procedure 1 hour before and during the Reversal sessions. Subsequently, the location of the correct response was switched again in the RTB phase, and the animals were tested without stimulation. Accuracy (ACC) and reaction time (RT) were assessed using statistical analysis with generalized models (GLMs and GLMMs). Mid-striatum stimulation exhibited no significant effect on ACC and RT ($p>0.05$), whereas VS DBS increased both ACC and RT during the Reversal phase (ACC: $\Delta=19.59\pm6.87$, $p=0.05$; RT: $\Delta=2.85\pm1.34$, $p<0.05$). Additionally, VS DBS increased ACC ($\Delta=18.76\pm6.46$, $p<0.05$) without affecting RT ($p>0.05$) in the first RTB session. These findings indicate that mid-striatum DBS did not influence parameters associated with reversal learning, whereas VS DBS improved animals' performance by shifting the speed-accuracy trade-off towards greater accuracy. These results suggest that different striatal regions may regulate distinct aspects of behavioral flexibility and decision-making strategies. Our future research will explore the prefrontal cortex's role in mediating VS DBS's effects on behavioral flexibility during the reversal learning task.

Disclosures: I. Waku: None. **A.E. Reimer:** None. **A.R. de Oliveira:** None.

Poster

PSTR188: Striatal and Corticostriatal Circuits

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Topic: H.08. Learning and Memory

Support: EMBO postdoctoral fellowship
NIH Grant 1RF1NS132913-01

Title: Coordinated replay across motor cortex, premotor cortex, and the striatum during skill consolidation

Authors: *A. MIZRAHI-KLIGER[1], C. L. WOODARD[1], K. GANGULY[2];
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Abstract: Adaptive behavior relies on identifying regularities in the environment to guide movement and maximize reward. Widespread cortical inputs to the striatum allow the brain to

identify features that reliably predict reward, and to bind them to the motor routines needed to obtain it. While sleep-dependent processing is known to be crucial for this process, it is not fully understood how sleep promotes cross-region communication between disparate cortical regions and the striatum to drive the onset of skilled behavior. Here, we simultaneously record the activity of hundreds of neurons in M1 (motor cortex), M2 (premotor cortex analog) and the dorsolateral striatum (DLS) as mice acquire a reaching task along days, as well as during subsequent sleep periods. We find that the crystallization of skill is associated with the emergence of strong and long-duration theta (4-10 Hz) activity that is coherent between M1, M2 and DLS, entraining local and cross-region spiking, during sleep. Theta activity also locks the replay of cortical and striatal task-related activity patterns. This might be important for driving synaptic plasticity and supporting next-day performance. We conclude that multiarea theta coherence during sleep may allow the sleep-dependent binding of activity in different nodes of the cortico-striatal system to attain a rich representation of movement context allowing naturalistic skill performance.

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Poster

PSTR188: Striatal and Corticostriatal Circuits

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Program #/Poster #: PSTR188.04/U9

Topic: H.08. Learning and Memory

Support: NIH IRP

Title: Response differences in monkey perirhinal cortex and rostromedial caudate in a reward schedule task

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Abstract: Perirhinal cortex (PRh) and rostromedial caudate (rmCD), two anatomically connected brain regions, both have causal roles in assigning value to visual stimuli in rhesus monkeys. We previously showed that single neurons in PRh are sensitive to associations between the cues and reward states in a reward schedule task, in which the number of remaining trials before reward, i.e., reward states, was indicated by visual cues. Reward was delivered at the end of a schedule. Schedules were, 1, 2, or 3 trials long, and progress through a schedule was indicated by a change in the visual cue. We recorded simultaneously from PRh and rmCD while a monkey performed a visually cued reward schedule task. Error rate and reaction time decreased as the monkey progressed through the schedule to obtain a reward, suggesting that the monkey was sensitive to the cue and reward state. About 32% (49/152) and 16% (44/278) of recorded neurons in PRh and rmCD were significantly modulated by the reward schedule task. To elucidate the nature of these responses, we swapped the cue in one of the states before reward

and the cue in state with reward. For the neurons responded differently to these two reward states before cue-swapping, a subset of them in both PRh (8/25, 32%) and rmCD (22/36, 61%) could not differentiate these two reward states anymore after cue-swapping, which indicated neural responses to associations between cues and reward states. For the neurons that maintained the differentiation after cue-swapping, the majority in PRh (15/17, 88%) responded to visual cues. Conversely, the majority in rmCD (11/14, 79%) responded to reward states. Our results suggest different roles of Prh and rmCD in stimulus-reward associations.

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Poster

PSTR188: Striatal and Corticostriatal Circuits

Location: MCP Hall A

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Program #/Poster #: PSTR188.05/U10

Topic: H.08. Learning and Memory

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Simons Collaboration on the Global Brain (NU)
Harvard Brain Initiative Postdoc Pioneers (RA)

Title: Cell-type specific dopaminergic modulations of sensory responses of striatal neurons at different timescales

Authors: *Z. TIAN, R. AMO, M. G. CAMPBELL, N. UCHIDA, M. WATABE-UCHIDA; Harvard Univ., Cambridge, MA

Abstract: Dopamine is thought to play central roles in regulating learning and modulating ongoing behaviors. Yet it remains unclear how these functions arise from cellular mechanisms *in vivo*. Previous studies have shown that dopamine in the tail of striatum (TS) - the sensory domain of striatum - is modulated by salience of sensory stimuli (Menegas et al., 2018), and promotes avoidance of a threatening stimulus (Tsutsui-Kimura et al., 2022) as well as auditory discrimination (Chen et al., 2022). Although these processes likely involve dopamine's ability to modulate plasticity and excitability of striatal neurons, underlying mechanisms remain unclear. Here, we examined how dopamine modulates sensory responses of neurons in the TS expressing D1 or D2 dopamine receptors. To circumvent several technical problems in using optogenetic stimulation in TS, we carefully designed the experiment. First, we locally stimulated dopamine axons in TS for specificity. Second, because TS is highly sensitive to sensory stimuli, we performed experiments under a strong background light to mask the visual artifacts evoked by the light used for optogenetics. Third, as recent studies indicated potential artifacts caused by supraphysiological dopamine (Long et al., 2022), we calibrated stimulation parameters to match

the natural range of TS dopamine release in response to a strong, multi-modal sensory stimulus. Under these conditions, we then examined calcium signals from D1 or D2 neurons in response to a novel, low-intensity complex sensory stimulus using fiber photometry with or without concurrent optogenetics activation of dopamine axons. We observed that on the first day (Day 1) of experiencing the sensory stimulus, concurrent dopamine axon stimulation enhanced the sensory response of D1 neurons (stimulation vs no stimulation trials, $n = 6$ mice, $p < 0.005$, paired t-test). Interestingly, such enhancement was not observed on Day 2. Further, whereas sensory responses of D1 neurons decreased on Day 2 compared to Day 1 in control mice with no opsin expression, optogenetic activation of dopamine axons prevented such decay of the sensory responses in D1 neurons (decay of control vs opsin animals, $n = 6$ mice for each, $p < 0.05$, t-test). We did not observe effects of optogenetic activation of dopamine axons on D2 neurons. Our results demonstrate that dopamine modulates online activity and plasticity of D1 neurons at different stages of sensory experiences. This dynamical modulation of sensory responses in TS potentially contributes to flexible sensory representation and avoidance behaviors both acutely and throughout learning.

Disclosures: Z. Tian: None. **R. Amo:** None. **M.G. Campbell:** None. **N. Uchida:** None. **M. Watabe-Uchida:** None.

Poster

PSTR188: Striatal and Corticostriatal Circuits

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Simons Collaboration on the Global Brain

Title: Dissecting the circuit basis and global organization of dopamine-driven temporal difference learning in the striatum

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Abstract: Phasic dopamine (DA) release plays a major role in learning by assigning incentive value to associated stimuli, a process that is essential to healthy life but goes awry in numerous diseases including addiction and mood disorders. A leading theory proposes that this process is analogous to a reinforcement learning algorithm called temporal difference (TD) learning, and

that DA acts analogously to the reward prediction error (RPE) term within the TD algorithm. However, a complete picture of whether and how neural circuitry in the basal ganglia implements TD learning remains elusive. Here, we combine multisite optogenetics, photometry, and antidromic optotagging with Neuropixels to demonstrate that the circuitry bidirectionally connecting D1 DA receptor-expressing medium spiny neurons (D1-MSNs) in the lateral nucleus accumbens (lNAc) to lNAc-projecting DA neurons in the ventral tegmental area (VTA) accomplishes key components of TD learning. Specifically, pairing calibrated optogenetic stimulation of lNAc DA axons with an odor cue ("artificial conditioning") generated signatures of TD RPE in DA activity, namely a positive response to odor onset and a timed negative response to omission of predicted stimulation (N=10 experimental mice, N=3 no opsin control mice). Electrophysiological and photometric recordings of striatal activity during the same artificial conditioning paradigm revealed that DA release potentiated the odor-evoked activity of lNAc D1-MSNs (N=7 mice), but not D2-MSNs (N=6 mice). Finally, optogenetic stimulation of lNAc D1-MSNs with diverse temporal patterns drove lNAc DA release according to the temporal derivative of the stimulation pattern (N=10 experimental mice, N=3 no opsin control mice). This temporal derivative computation is a fundamental building block of TD learning and can explain TD RPE-like DA responses during artificial conditioning. Next, we use multisite artificial conditioning tasks with large-scale antidromic optotagging of DA neurons to provide evidence that lNAc plays a privileged role in this process relative to dorsal striatal areas. Specifically, optogenetic stimulation of DA axons in lNAc, but not dorsomedial or dorsolateral striatum, drove the formation of odor responses in DA neurons projecting to all three areas (N=109 optotagged neurons from 8 mice). This result points to lNAc as a source of value learning for the rest of the striatum, consistent with classical actor-critic models. Altogether, these results reveal a minimal circuit capable of accomplishing key components of TD learning and begin to delineate the mesoscale organization of TD learning within the basal ganglia.

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Poster

PSTR188: Striatal and Corticostriatal Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.07/U12

Topic: H.08. Learning and Memory

Title: Interplay of thalamo- and corticostriatal input-timing dependent plasticity in motor flexibility

Authors: E. PERRIN[1], S. PEREZ[1], M. VANDECASTEELE[1], H. BERRY[2], ***N. GERVASI**[3], L. VENANCE[1];
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Abstract: The striatum, the mail input nucleus of basal ganglia, is a major site of procedural memory formation as the acquisition of a behavioral repertoire has been associated with cortico-striatal (CS) plasticity. Striatum integrates inputs from the cortex and some thalamic nuclei, that display concomitant or sequential activity. If a vast literature has focused on CS plasticity, little remains known about thalamo-striatal (TS) plasticity rules, their interplay with CS plasticity and involvement in behavioral flexibility. We recorded in vivo plasticity (opto-evoked LFP) in mice engaged in procedural learning on a horizontal ladder and found CS-LTP upon learning of a rung pattern#1 during 10 days (without modification of TS-evoked-LFP), whereas this plasticity was cancelled upon novelty (sudden change for a rung pattern#2) to the profit of a TS-LTP; here, we focused on the somatosensory cortex and on the parafascicular nucleus of the thalamus. In vivo Neuropixel recording of TS/CS activity during this task revealed activity patterns reminiscent of time-coding plasticity. We thus characterized the input-timing dependent plasticity (ITDP) at CS and TS synapses in brain slices, in supra-and subthreshold regimes by distinguishing striatal projecting neurons belonging to the direct (D1) and indirect (D2) pathways. We found that most temporal combination induced LTP except when thalamus (supra)activity precedes cortical one, where LTD was observed, highlighting the crucial impact of timing in cortical and thalamic activities for the memory engram at striatal synapses. Ex vivo occlusion experiment, after mice subjected to the horizontal ladder and rung pattern learning, showed that ITDP CS-LTP and TS-LTP were engaged during procedural learning and novelty, respectively. Interestingly, the constant modification of the rung patterning induced an increase in TS synaptic weight, while CS transmission decreases. We next disturbed the TS transmission, by opto-stimulated at a low frequency the parafascicular nucleus and observed decreased performance on the ladder, while these later ones were not affected during cortical opto-stimulation. Finally, we observed that mice undergoing opto-evoked LTD at TS synapses, showed delayed learning of the rung pattern#2. Overall, our results show that the ability of TS plasticity to override CS plasticity could allow for flexible behavior, helping the exit of an existing automatism in favor of a new behavioral strategy.

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Poster

PSTR188: Striatal and Corticostriatal Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.08/U13

Topic: H.08. Learning and Memory

Title: Anti-hebbian plasticity drives sequence learning in striatum

Authors: G. VIGNOUD[1], L. VENANCE[1], ***J. TOUBOUL**[2];
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Abstract: Spatio-temporal patterns have been observed in a variety of brain areas in response to stimuli, prior or during action, or even in spontaneous activity. The biological mechanisms endowing neurons with the ability to distinguish between different sequences remain largely unknown. In fact, learning sequences of spikes raises multiple challenges, such as maintaining in memory spike history and discriminating partially overlapping sequences. Striatal output neurons (SPNs) expressing synaptic plasticity with cortex, have been reported to play a critical role in integrating context elements and developing sensorimotor associations. Corticostriatal synapses display anti-Hebbian STDP (Perrin and Venance, 2019 Curr Op Neurobiol) whereby a cortical spike followed by an SPN spike leads to a depression of the associated synaptic weight. While many computational studies have investigated the impact of Hebbian spike-timing dependent plasticity (STDP), only a few studies considered anti-Hebbian STDP. We explore the capacity of anti-Hebbian STDP, observed at cortico-striatal synapses of SPNs, to drive the learning of sequences. To this purpose, we design a spiking model of the SPN receiving spike patterns defined as sequential input from a fixed set of cortical cells. To test for different features separately, our models progress from the simplest to more realistic, which allows an in-depth exploration of the ability of the biological learning rules to support sequence learning and the role played by each biological feature in contributing to sequence learning. We use a simple synaptic plasticity rule that combines anti-Hebbian STDP and non-associative potentiation for a subset of the presented patterns called rewarded patterns. We study, in various situations, the ability of the SPN to discriminate rewarded from non-rewarded patterns by firing only after the presentation of a rewarded pattern. In particular, we show that two biological properties of striatal networks, spiking latency and collateral inhibition, contribute to a significant increase in accuracy, by allowing a better discrimination of partially overlapping sequences. This analysis further proposes a functional role for spike latency and collateral inhibition in the framework of sequence learning, suggesting that they could contribute to a remarkable ability to identify and optimize the learning of sequences of spikes that outperforms some artificial learning algorithms subjected to similar constraints. Altogether, these results argue that the anti-Hebbian STDP observed at cortico-striatal synapses may serve as a biological substrate for learning sequences of spikes.

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Poster

PSTR188: Striatal and Corticostriatal Circuits

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Program #/Poster #: PSTR188.09/U14

Topic: H.08. Learning and Memory

Title: tracking cortico-striatal plasticity during the transition from goal-directed behavior to habitual behavior/strategies

Authors: *M. FABRIZIO, L. VENANCE, N. GERVASI;
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Abstract: Procedural learning allows acquiring skills that enables to adapt to the environment, through the automatization of complex cognitive-motor sequences, as a whole, such as cycling. This form of learning evolves through repeated and successful trials, transitioning from goal-directed behavior to habitual actions. Goal-directed behavior involves conscious and flexible actions, sensitive to the contingencies between actions and outcomes, whereas habits are automatic, less flexible and insensitive to the action-outcome contingency. The dorsal striatum receives inputs form the whole cortex, with the dorsolateral (DLS) and -medial (DMS) striata connected with sensorimotor and associative-limbic cortices, respectively. The cortico-striatal plasticity in DLS and DMS appears as a key element for the procedural learning's engram formation. Previous research, including studies from our laboratory, has demonstrated differential involvement of DLS and DMS during early versus late learning stages (Perez et al., 2020 Cell Reports), but focusing on limited steps. A longitudinal exploration of the plasticity and network dynamics is thus requested to fully understand this implicit memory formation.
To do so, we perform longitudinal in vivo electrophysiological monitoring of cortico-striatal plasticity using complex behavioral tasks on head-fixed mice. We developed a reward-driven training paradigm within the MobileHomeCage system, where food-restricted mice navigate a six-zone cage to receive rewards, over a 30-day period to instill habit formation. Opto-evoked LFP techniques was used to monitor longitudinal changes in synaptic weights in DLS and DMS. After 8 days of training, we observed that mice had learned the task. Devaluation test indicates that mice are not using goal-directed behavior strategy in task execution. Through individual scoring, we observed a consistent enhancement in performance from day-8 to day-30, indicative of mice refining their strategies in alignment with habit formation. Longitudinal opto-evoked local field potential recordings revealed the establishment of bilateral cortico-striatal LTP in both the dorsolateral (DLS) and dorsomedial (DMS) striatum from training day 6 to day 30. Overall, our study utilizing longitudinal electrophysiological monitoring in mice has provided insights into bilateral cortico-striatal plasticity during habit formation. This research represents a critical step towards identifying the neural engram underlying the physiological state of procedural learning.

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Poster

PSTR188: Striatal and Corticostriatal Circuits

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Program #/Poster #: PSTR188.10/U15

Topic: H.08. Learning and Memory

Title: Striatal endocannabinoid long-term potentiation mediates one-shot learning

Authors: C. PIETTE[1], A. HUBERT[2], S. PEREZ[3], H. BERRY[4], J. TOUBOUL[5], ***L. VENANCE**[3];
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Abstract: One-shot learning, the acquisition of memory after a unique and brief experience, is a crucial ability for behavioral adaptation, since salient but rare or brief events may require adjusting behavioral repertoire for survival. Biological mechanisms involved have remained elusive (Piette et al., 2020), and the limited number of action potentials during a one-shot experience generally conflicts with Hebbian paradigms for stable plasticity expression. At cortico-striatal synapses, only a few spikes are however able to evoke *in vitro* an endocannabinoid-mediated LTP (eCB-LTP) involving CB1 and D2 receptors, while more intense stimulation induces a NMDAR-mediated plasticity (Cui et al., 2015; Cui et al., 2016; Xu et al., 2018), suggesting that distinct molecular signaling pathways could mediate synaptic plasticity during one-shot *vs.* incremental learning. We tested the hypothesis of the involvement of cortico-striatal eCB-LTP in one-shot learning. We developed a behavioral test for mice that can be acquired after one brief session. During a one-shot familiarization, mice spontaneously contact a loose piece of sticky tape placed in the openfield and rapidly remove it. Following a 24 h- up to 1 month-interval, we observed that more than half of the mice retained a memory of this one-shot experience, by expressing avoidance of the tape. We then found that a cortico-striatal LTP emerged 24h after familiarization, in mice that had a brief contact (< 20 seconds) with the sticky tape and later showed avoidance signs. Next, we aimed at elucidating the signaling pathways underlying the cortico-striatal LTP induced by one-shot learning. Based on *in vivo* Neuropixel recordings, a detailed computational model of cortico-striatal synapse predicted an increased occurrence of eCB-LTP induction events during the contact with the sticky tape. *Ex vivo* patch-clamp recordings revealed an occlusion of eCB-LTP in mice exposed once to the sticky tape. In addition, we showed that knock-out mice for CB1 or D2 receptors at presynaptic cortical efferents exhibited impaired one-shot learning, while no significant difference was observed between D-AP5 and saline-infused mice. These multiple approaches demonstrate that eCBs underlie one-shot learning. Overall, these findings extend the timescale along which learning occurs in the dorsolateral striatum and outline for the first time the temporal and activity-dependent boundaries delineating the expression of a synaptic plasticity pathway within a learning paradigm. This work also offers novel keys to interpreting the wide array of functions of the eCB system.

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Poster

PSTR188: Striatal and Corticostriatal Circuits

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Simons Collaboration on the Global Brain

Title: Striatum-to-cortex interactions support evidence-guided decisions

Authors: *R. CHO[1], S. S. BOLKAN[1], L. BROWN[2], J. LOPEZ LUNA[3], M. SCHOTTDORF[1], A. G. BONDY[1], B. MCMANNON[1], R. N. FETCHO[4], C. A. ZIMMERMAN[1], A. PAN VAZQUEZ[5], M. SINISCALCHI[1], I. B. WITTEN[6];
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Abstract: Direct and indirect pathway neurons in the dorsomedial striatum (DMS) provide strong, opponent control of decision-making when mice are engaged in a cognitively demanding evidence accumulation task, while the pathways have little effect on movement in the absence of decision-making, or on sensory guided decisions that do not rely on evidence accumulation (Bolkan et al., 2022). How do basal ganglia pathways exert opponent and task-dependent control of decision-making behavior? Here, we explore the hypothesis that feedback onto cortex from the basal ganglia direct and indirect pathways may control decision-making behavior through the striatal-thalamic-cortical loop. The anterior cingulate cortex (ACC), which projects to DMS and receives indirect feedback back from DMS (Foster et al., 2021), is selectively required for tasks with high cognitive demand (Kim et al., 2016; Dias and Aggleton, 2000; Cardinal et al., 2003). Here we have employed high-density Neuropixels 2.0 recordings across DMS and ACC, when a2a-cre or drd1-cre mice performed sensory-guided or evidence-based decision-making tasks in virtual reality (Pinto et al., 2018). These simultaneous, large-scale recordings revealed that neural representation of choice is stronger and arises earlier in the decision process within the ACC compared to DMS, while the opposite is true of the reward outcome. In a subset of trials, indirect or direct pathway neurons in the DMS were unilaterally inhibited during the evidence accumulation epoch, in order to investigate the effect on local and cortical coding of evidence and choice. We observed that inhibition of each pathway shifts choice-related population trajectories in opposite directions in DMS and ACC: neural trajectories were shifted towards contralateral coding with indirect pathway inhibition and towards ipsilateral coding with direct pathway inhibition. This effect on choice neural dynamics in the ACC (but not DMS) was specific to a task that requires evidence accumulation, as we observed minimal effects on choice trajectories with striatal inhibition in a sensory-guided task. Therefore, our results support a model of the feedback loop onto the cortex from the basal ganglia contributing to the task-dependent and opponent control of decision-making behavior by basal ganglia pathways.

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Poster

PSTR188: Striatal and Corticostriatal Circuits

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Title: Pre-existing visual responses in a projection-defined dopamine population explain individual learning trajectories

Authors: *A. PAN VAZQUEZ[1], Y. SANCHEZ ARAUJO[1], B. MCMANNON[1], M. LOUKA[1], A. BANDI[2], L. HAETZEL[3], I. BRAIN LABORATORY[4], J. W. PILLOW[1], N. D. DAW[1], I. B. WITTEN[1];
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Abstract: Learning a new task is challenging because the world is high dimensional, with only a subset of task features being reward-relevant. What neural mechanisms allow us to learn the relevant dimensions, and why do some individuals learn these faster than others? To address these questions, we recorded longitudinally from dopamine (DA) axon terminals in the nucleus accumbens (NAc), dorsomedial (DMS) and dorsolateral striatum (DLS) of mice learning a de novo visual stimulus discrimination task. Mice presented a variety of learning strategies, while some mice weighted stimulus on both sides of the screen equally, many preferentially weighted the stimulus on one side of the screen versus the other. Across DA projections, responses to the visual stimuli tracked these idiosyncratic learning strategies. However, even before training started and any rewards were delivered, contralateral visual responses were present in DA terminals only in the DMS. These pre-existing responses predicted the extent of later behavioral learning for contralateral stimuli. Moreover, in a separate optogenetic stimulation experiment, activation of these terminals at the time of stimulus presentation improved contralateral performance. Thus, the initial conditions of a projection-specific and feature-specific DA signal help explain individual learning trajectories. More broadly, this work supports a model where functional heterogeneity across DA projections serves to bias target regions towards learning about different features of the task environment, providing a mechanism to address the dimensionality of the initial task learning problem.

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Poster

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Topic: H.08. Learning and Memory

Support: NIH Grant R01MH125824

Title: Chemogenetic inhibition of amygdala inputs to striatum modulates reinforcement learning

Authors: *K. M. ROTHENHOEFER, M. STOCKER, V. D. COSTA;
Psychiatry and Div. of Developmental and Cognitive Neurosci. at Emory NPRC, Emory Univ., Atlanta, GA

Abstract: Managing the explore-exploit tradeoff requires decision makers to explore options with unknown consequences rather than exploit options whose consequences are known. Explore-exploit decision making is typically studied in the context of maximizing gains, but is understudied in the context of minimizing losses—especially in rhesus macaques. We developed a three-arm bandit task that induces explore-exploit tradeoffs by introducing novel choice options that are associated with the gain or loss of virtual tokens. These virtual tokens are secondary reinforcers that were cashed out for primary juice rewards based on the task performance. Using tokens as a secondary reinforcer allowed us to include aversive conditions where macaques could lose tokens. This provides a route to examine monkeys' exploratory decision making in different valence contexts. The use of a fixed maximum threshold for cashing out tokens for primary reward also allowed us to determine if monkeys use directed exploration to manage explore-exploit tradeoffs. Prior studies have implicated the amygdala and ventral striatum in encoding decisions to explore or exploit. We used pathway-specific chemogenetics to evaluate the effects of inhibiting excitatory amygdala inputs to the ventral striatum on exploratory decision-making. We injected a retrograde AAV into the ventral striatum to transfect the axon terminals of amygdala neurons projecting to the ventral striatum and injected a second AAV to express an inhibitory Cre-dependent chemogenetic receptor in the same amygdala neurons. We then examined how inhibiting this glutamatergic amygdala pathway affected the monkeys' reinforcement learning and novelty seeking. Consistent with prior research in rodents, inhibiting amygdala projections to striatum impeded the monkeys' ability to discriminate novel cues associated with magnitude gains in tokens while learning of aversive associations remained intact. We also found that chemogenetic inhibition of amygdala projections to striatum reduced exploration of novel, uncertain choice options and increased selection of familiar, well-learned options. Chemogenetic inhibition of novelty seeking was observed across valence contexts. These results indicate glutamatergic amygdala projections to ventral striatum is an inherently appetitive neural circuit that mediates exploration in uncertain decision making contexts.

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Poster

PSTR188: Striatal and Corticostriatal Circuits

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Topic: H.03. Decision Making

Support: NIMH 1F31MH135646-01
Army Research Office 78259-NS-MUR

Title: The role of astrocytes in probabilistic decision-making

Authors: *J. PAI[1], F. SOGUKPINAR[1], T. PAPOUIN[1], S. CHING[2], N. HIRATANI[1], K. OGASAWARA[1], I. E. MONOSOV[1];
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Abstract: In a changing uncertain world, it is crucial for animals to be able to store and flexibly update value representations to guide subsequent behavior. This updating is often called reinforcement learning (RL). RL is thought to depend on basal ganglia circuitry, particularly dopamine-dependent synaptic plasticity in the striatum. Even as the field gains insight into how different neural cell types in striatum contribute to RL, the role of astrocytes remains unclear. Astrocytes are known to have strong calcium responses and to release gliotransmitters capable of modulating synaptic plasticity and behavior. In the striatum, astrocytes respond to dopamine and are responsible for mediating some forms of dopamine-dependent synaptic plasticity (Corkrum et al, 2020). However, whether and how astrocyte function is important for RL is not yet known. To investigate this, we attenuated astrocyte calcium signaling across different regions of striatum while mice performed a probabilistic bandit task. We programmed in-home cage operant devices (FED3s) with right and left nose poke ports to deliver food pellets with 80% and 20% probabilities, respectively. Side-reward contingencies switched after 20-30 rewarded trials, un-cued to the mouse. Agents performing in this task must continuously update their value representations based on the history of outcomes they receive. After mice were trained to perform the bandit task, we delivered bilateral virus injections into ventral striatum (VS, n=9 mice), dorsomedial striatum (DMS, n=9 mice), and dorsolateral striatum (DLS, n=6 mice). We used a viral construct that attenuates astrocyte calcium signaling without inducing astrocyte or neuron death (AAV5-GfaABC1D-mCherry-PMCA; Yu et al, 2018). To compare behavioral parameters of RL before and after virus injection, we fit mice's behavior with a Q-learning model with separate learning rate parameters for rewarded (rew+) and non-rewarded (rew-) outcomes. We found that VS injected mice show significantly increased learning rates to rew- but not rew+ outcomes. DMS- and DLS-injected mice did not show significant changes in either rew+ or rew- learning rates. We also examined how non-RL measures of behavior changed after virus injection, including the number of trials mice initiated each session and the number pokes mice made during time-out periods. We found that VS- and DLS-injected mice initiated more trials per session. In contrast, DMS-injected mice made fewer timeout pokes. These results suggest that astrocytes play a role in mediating outcome learning specifically in VS and are likely involved in non-RL mechanisms of motivated behavior across striatum.

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Poster

PSTR188: Striatal and Corticostriatal Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.15/U20

Topic: H.08. Learning and Memory

Support: NIH DA042889
NIH R01NS108151
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McKnight Endowment for Neuroscience 048240
European Research Council 268795

Title: Dopamine D1 receptor activation in the striatum is sufficient to drive reinforcement of anteceding cortical patterns

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Abstract: Timed dopamine signals underlie reinforcement learning, favoring neural activity patterns that drive behaviors with positive outcomes. In the striatum, dopamine activates five dopamine receptors (D1R-D5R), which are differentially expressed in striatal neurons. However, the role of specific dopamine receptors in reinforcement is poorly understood. Using our cell-specific D1R photo-agonist, we find that D1R activation in D1-expressing neurons in the dorsomedial striatum is sufficient to reinforce preceding neural firing patterns in defined ensembles of layer 5 cortico-striatal neurons of the motor cortex. The reinforcement is cumulative and time-dependent, with optimal effect when D1R activation follows the selected neural pattern after a short interval. Our results show that D1R activation in striatal neurons can selectively reinforce cortical activity patterns, independent of a behavioral outcome or a reward, crucially contributing to the fundamental mechanisms that support cognitive functions like learning, memory, and decision-making.

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Poster

PSTR188: Striatal and Corticostriatal Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.16/U21

Topic: H.08. Learning and Memory

Support: NIDA Z1A DA000587
NINDS U01NS120824

Title: Acetylcholine in the nucleus accumbens signals the salient cue rather than reward prediction error.

Authors: *Z. ZHANG[1], K. M. COSTA[1], Y. ZHUO[2,3], G. LI[2,3], Y. LI[2,3,4,5,6], G. SCHOENBAUM[1];
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Abstract: Acetylcholine (Ach) is critical for learning, and its function varies across different brain regions. The role of cholinergic signaling in the nucleus accumbens core (NAcc), a region vital for learning, remains unclear. In previous work, we found that NAcc cholinergic signals dipped while rats experienced reward or performed reward-associated actions, anticorrelated to dopamine (DA) reward prediction error (RPE) signaling. We hypothesized that this dip could be encoding RPEs itself, or signaling motivationally salient cues, compounding with DA's role in learning. To test this, we simultaneously recorded Ach and DA using new generation genetically encoded sensors (gACh4h and rDA3m, respectively) in freely moving rats. Combining rats' behavior and DA response, known to represent RPEs, enables us to identify different learning stages. In the Experiment 1, 9 rats were trained with a GO-NOGO task. In each trial, one of two odors was randomly selected and delivered. One odor indicated a water reward in the fluid well, while the other indicated no reward. Initially, rats were unaware that odor cues predicted reward outcomes, and the novel cues did not significantly alter ACh levels. As the learning progressed, rats started avoiding the fluid well after sampling the cue indicating no reward, suggesting that rats began to associate odors with reward outcomes. Meanwhile, the DA responses to the two cues differed, while ACh developed a dip to both cues with no significant difference in magnitude, suggesting that ACh in NAcc signaled the motivational salience of the cues instead of RPEs as DA did. After rats achieved 90% accuracy in 20 trials, the Ach response was different for each cue. This was unlikely to be due to RPEs or critical for learning, given that the rats had learned the value of cues and the selectivity in DA had been established earlier. To provide further evidence for this interpretation, we conducted Experiment 2, where 6 rats were initially trained on the same GO-NOGO task and stopped 20 trials after achieving 90% accuracy in 20 trials. Then, we reversed the contingency between cues and reward availability and trained rats until the same behavioral criterion was met again. Consistent with the signaling of RPEs, the DA response evoked by two cues reversed after the contingency change. In contrast, the ACh lost its original preference for cues and failed to establish a new preference after the reversal. All these results support that the cholinergic system in the NAcc signal diverges from RPE signaling and instead aligns well with what is predicted for salience.

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Poster

PSTR188: Striatal and Corticostriatal Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.17/

Topic: H.08. Learning and Memory

Title: Neuronal cilia in dorsomedial striatum mediate flexible goal-directed learning

Authors: Z. YANG, ***S. PHUA**;
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Abstract: Insistence on sameness is a trademark behavior in autism spectrum disorder (ASD) individuals. ASD-associated behavioral rigidity is attributed to deficits in cognitive flexibility influenced by the basal ganglia, especially the dorsomedial striatum (DMS) that mediates action-outcome (A-O) associative learning. Within the DMS, dopamine (DA) and serotonin (5HT) signaling are thought to play inverse modulatory roles in associative learning, yet how DMS spiny projection neurons (SPNs) interpret these neuromodulators is not fully comprehended. Our research group has recently focused on illuminating antenna-like structures known as primary cilia in DMS-SPNs. While primary cilia have remained largely elusive in the adult brain, a growing number of G-protein coupled receptors have been discovered in the neuronal cilia, including dopamine receptor 1 and serotonin receptor 6. Therefore, we hypothesized that primary cilia in DMS-SPNs mediate associative learning through interpreting DA-5HT signals. We employed AAV-CRISPRko to ablate *Ift88* gene in SPNs of adult mouse DMS. *Ift88* loss induced robust cilia ablation as compared with *Rosa26*-CRISPRko control. When we subjected *Ift88*-CRISPRko and *Rosa26*-CRISPRko mice through a multi-phase goal-directed training paradigm that required learning and update of A-O contingencies, we were surprised that Ift88-CRISPRko mice outperformed Rosa26-CRISPRko mice in associative learning. These results suggest that cilia ablation in DMS-SPNs altered the balance in interpreting learning-associated DA-5HT signals. We are currently investigating whether neuronal cilia sense DA-5HT signals via recently reported axon-cilium synapse-like contacts. By mapping the spatial proximity between neuronal cilia and serotonergic axons, we discovered that A-O learning experience increased the distance between neuronal cilia and serotonergic axons; this could repress axon-cilium contact frequency and the ability of neuronal cilia to sense 5HT signals in DMS. We further hypothesized that axon-cilium contacts might be regulated by a mechanism similar to synaptic plasticity and we detected robust accumulation of syndromic ASD-associated Shank3 in over 50% of DMS neuronal cilia at basal state. To our knowledge, this constitutes the most definitive molecular intersection between primary cilia and ASD signaling to-date and is reinforced by the identification of cilia-associated genes in ASD gene interaction networks. Further investigation will unravel the emerging connection between primary cilia and ASD and promote novel interest in illuminating the functions of ciliary-enriched GPCRs in cognition and learning.

Disclosures: Z. Yang: None. **S. Phua:** None.

Poster

PSTR188: Striatal and Corticostriatal Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.18/U22

Topic: H.10. Human Learning and Cognition

Support: NIDCD Grant R21DC010576

Title: Nucleus accumbens contributes to perceptual facilitation during statistical learning of temporal structures embedded in sequential input

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Abstract: Statistical learning (SL) is an implicit cognitive process in which the brain extracts regularities from the environment through repeated exposure. The brain mechanisms that support SL are important for understanding its contribution to complex behavior such as categorization and language. Here we demonstrate the contribution of nucleus accumbens (NAcc), a structure associated with error-driven learning, to perceptual facilitation as a result of SL. We investigated the temporal dynamics of blood-oxygenation-level-dependent (BOLD) activity in healthy human subjects performing a visual SL task. Twenty-three adults (age = 20.79 +/- 2.89 years, 7 males) participated with written consent. Participants viewed sequentially presented images while responding to target images embedded in each sequence, whereby stimuli were either temporally arranged into deterministic triplets ("S-block") or presented in random order ("R-block"). The target location followed no systematic pattern and the participants were not informed of the embedded temporal structure. To characterize spatiotemporal patterns of the multivoxel BOLD activity in each trial, we built hidden Markov models (HMMs) by assuming that a finite set of latent brain states drove the activity. Each state was linked to the observed activity via a multivariate Gaussian distribution with dimensions matching the number of voxels in the NAcc. We identified the optimal number of states that provided the best fit by model comparison. In each subject, we located the state that occurred the most frequently during target presentation (referred to as "critical state"). Reaction time (RT) analysis showed a facilitation effect of structured sequences on target detection: RT was significantly lower in S-blocks than in R-blocks (Wilcoxon signed-rank $W = 23.0$, $p < 0.00028$). The critical state in the NAcc was associated with this facilitation effect. Consistently across individuals, RT was significantly lower when the critical state was present in the NAcc than when the state was absent (observed difference greater than 1000 out of 1000 state-permuted trials). Moreover, this state occurred more frequently in R-blocks than in S-blocks. Any other latent state alone in the NAcc, or any state in the hippocampal formation and V1, did not differentiate the RT. The finding of a critical state in the NAcc, whose presence corresponds to perceptual facilitation and is more frequent in non-predictive sequences, suggests a potential error-driven feedback signal for training the

internal prediction during SL. These findings open the door to further elucidating learning-related brain dynamics in healthy human subjects.

Disclosures: W. Tang: None. **P. Lanka:** None. **Z. Qi:** None.

Poster

PSTR188: Striatal and Corticostriatal Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR188.19/U23

Topic: H.03. Decision Making

Title: Biphasic Modulation of Striatal Cholinergic Activity by Direct-Pathway Medium Spiny Neurons

Authors: *R. CHEN[1], H. GANGAL[2], X. XIE[3], J. WANG[4];
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Abstract: Acetylcholine (ACh), released by striatal cholinergic interneurons (CINs), is crucial for cognitive flexibility. Previous studies have shown a special pattern of striatal ACh changes following extensive training, characterized by a dip and then a rebound. Likewise, ex vivo investigations have indicated that GABAergic inputs can trigger a pause-rebound sequence in CIN firing activity. Our study explores whether instrumental learning strengthens the connection between GABAergic direct-pathway medium spiny neurons (dMSNs) and CINs, contributing to action-outcome contingency learning in instrumental conditioning. Using slice electrophysiology, we found that optogenetic stimulation of dMSNs in D1-Cre;Ai32 mice induced a pause-rebound pattern in CIN firing, with stronger stimulation resulting in a more pronounced rebound. Consistently, our confocal imaging studies revealed that optogenetic dMSN stimulation increased striatal ACh release in D1-Cre;Ai32 mice infused with red ACh sensor (rACh1.7). However, optogenetic stimulation of indirect-pathway medium spiny neurons (iMSNs) in A2A-Cre;Ai32 mice did not induce rebound in CIN firing activity or striatal ACh release. In free-moving of D1-Cre; Ai167 mice infused with a green ACh sensor gACh4m to monitor striatal ACh release we found that optogenetic stimulation of dMSNs induced a dip followed by a strong rebound in ACh release, suggesting that optogenetic dMSN excitation *in vivo* also triggers a bi-directional regulation of striatal ACh activity . To explore how operant conditioning activate dMSN activity and ACh release, we infused AAV-Flex-jGCaMP7f and AAV-gACh4m into the dorsomedial striatum of D1-Cre rats, which were then trained with instrumental learning. We discovered that dMSN activity surged after lever presses or reward delivery, while ACh release dipped transiently and then rebounded during reward delivery after extensive training but not initial training. Subsequent reversal learning training revealed a prolonged rebound in ACh release, consistent with our prior studies indicating that ACh

facilitates reversal learning. In conclusion, our findings support the notion that during instrumental training, reward delivery induces an increase in dMSN activity, associated with a dip-rebound pattern of striatal ACh release. These results improve our understanding of the neural mechanisms underlying the roles of ACh and dMSNs in regulating instrumental learning.

Disclosures: R. Chen: None. **H. Gangal:** None. **X. Xie:** None. **J. Wang:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

Location: MCP Hall A

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Program #/Poster #: PSTR189.01/U24

Topic: H.09. Spatial Navigation

Support: National Key R&D Program of China (No. 2019YFA0709502)

Title: The spatial inputs to the human hippocampus for navigation and memory

Authors: *E. T. ROLLS[1], X. YAN[2], G. DECO[3], Y. ZHANG[2], J. FENG[4];
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Abstract: Hippocampal and parahippocampal gyrus spatial view neurons in primates respond to the spatial location being looked at. The representation is allocentric, in that the responses are to locations “out there” in the world, and are relatively invariant with respect to retinal position, eye position, head direction, and the place where the individual is located. The pathway for this spatial view information was analysed in humans. Whole-brain effective connectivity was measured with 1 ms TR magnetoencephalography between 30 visual cortical regions and 150 other cortical regions using the Human Connectome Project Multimodal Parcellation atlas in 21 participants performing a 0-back scene memory task. In a ventromedial visual stream, V1-V4 connect to the ProStriate region where the retrosplenial scene area is located. The ProStriate region has connectivity to ventromedial visual cortex regions VMV1-3 and VVC. VMV1-3 and VVC connect to the medial parahippocampal gyrus PHA1-3, which, with the VMV regions, include the parahippocampal scene area. The medial parahippocampal PHA1-3 regions have effective connectivity to the hippocampus, entorhinal cortex, and perirhinal cortex. Diffusion topography in 171 HCP participants at 7T supported this hierarchical organisation. An implication is that spatial view information is computed by feature combinations for parts of viewed scenes, very different to the local place representations in rodents. It is proposed that hippocampal spatial view cells provide a basis for the ‘where’ component of human episodic memory, and for navigation using viewed landmarks, with the orbitofrontal cortex reward inputs to the human hippocampus providing the goals for navigation.
Rolls, ET. 2024. Two What, Two Where, Visual Cortical Streams in Humans. Neuroscience and Biobehavioral Reviews 160: 105650.

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Rolls ET. 2023. Brain Computations and Connectivity. Oxford: Oxford University Press. Open Access: https://www.oxcns.org
Rolls ET. 2022. The hippocampus and reward systems. Prog Neurobiol 217: 102334.
Rolls, ET, Treves, A. 2024. A theory of hippocampal function: new developments. Prog Neurobiol.

Disclosures: E.T. Rolls: None. **X. Yan:** None. **G. Deco:** None. **Y. Zhang:** None. **J. Feng:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.02/U25

Topic: H.09. Spatial Navigation

Support: NSF/IOS 1924732
NIH R01MH123260-01

Title: Retrosplenial control of environmental cues used for spatial reorientation

Authors: *M. E. NORMANDIN, *M. E. NORMANDIN, C. M. GAGLIARDI, I. A. MUZZIO; Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA

Abstract: Reorientation, the process by which lost navigators regain their bearings, is fundamental for navigation. Under oriented conditions, navigators use both external and internal cues to navigate. However, during disorientation, the internal sense of direction is unreliable and lost navigators must rely on external cues to reorient. Geometry plays a prominent role under these conditions. However, the origin of geometric representations has not been clearly established. The retrosplenial cortex (RSC) has been shown to be important for coding environmental shape. Notably, the activity of its principal cells can be inhibited by long-range GABAergic projections originating from hippocampal area CA1 (H-RSC), suggesting a loop through which the hippocampus could silence RSC inputs. Here, we tested the involvement of RSC during reorientation using chemogenetics, optogenetics, single unit electrophysiological recordings, and calcium imaging. Behavioral results show that disoriented mice use geometry to reorient early in training, but incorporate features (i.e., nongeometric visual cues) as they learn their directional value, which serves to minimize geometric errors. Chemogenetic inhibition of RSC principal cells prevents the use of geometry during early reorientation, having minimal effects during late training after animals switch to featural strategies. Optogenetic activation of H-RSC long-range inhibitory projections shows similar results, corroborating that RSC inputs are only critical for geometry-based reorientation. We proceeded by assessing the alignment of RSC cells and the activity of RSC head direction cells—neurons that activate when an animal's

head is oriented in a specific direction—throughout the task. Electrophysiological recordings revealed that the RSC population quickly aligns with environmental geometry at the onset of training but display stability as animals integrate featural cues for reorientation. Similarly, head direction (HD) cells initially adjust based on the enclosure's geometry during early reorientation but gradually align with non-geometric featural cues as time progresses. To examine HD population dynamics, we devised techniques to analyze HD cells using calcium signals. Initial analysis characterizing unidirectional and bi-directional (i.e., cells exhibiting preferred firing directions 180° apart) HD cells showed that percentages of these cells remain constant throughout learning. We are currently examining if the tuning of these cells is altered by learning and how they correlate with behavior. These findings shed light underlying circuits involved in the reorientation processes.

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Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

Location: MCP Hall A

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Program #/Poster #: PSTR189.03/U26

Topic: H.09. Spatial Navigation

Support: NIH R01MH123260-01
NSF/IOS 1924732

Title: Reorienting behavior and retrosplenial circuitry in complex environments

Authors: *P. M. OGALLAR, C. M. GAGLIARDI, I. A. MUZZIO;
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Abstract: Reorientation, the process by which lost navigators regain their bearings, is fundamental for navigation. Under oriented conditions, navigators use both external sensory information and internal, self-motion cues to navigate. However, during disorientation, the internal sense of direction is unreliable and lost navigators must only rely on external cues to reorient. Studies have shown that geometry is especially important for reorientation across species. However, these studies did not evaluate reorientation in ethologically relevant tasks, as the usual experimental setups were not typically found in nature (e.g., simple rectangular chambers with clear geometric cues). Additionally, although reorientation results supported geometry-based reorientation, animals usually perceive the entire enclosure before making choices. This suggests that certain geometric subcomponents (i.e., long wall on the left and short wall in front) might have guided behavior rather than a comprehensive representation of the shape of the enclosure. Here, we aim to study reorienting behavior in a complex, large environment to evaluate the generalizability of geometry-based strategies in more natural scenarios. Further, we look at the functional role of the retrosplenial cortex (RSC), an area

involved in layout representations, during reorientation in a complex environment. We trained both female and male mice in a large, complex environment, which contained local and global geometric cues to assess reorientation in disoriented mice. The task consisted in searching for a buried reward in one out of 4 cups placed in each of the inlets of a large, double H maze, randomizing the entry point between the two central inlets. We found that mice used global geometry to reorient, confusing similar inlets, even when the global layout of the chamber could not be seen from the current point of view of the animal. Moreover, the use of geometry persisted in the presence of visual cues. However, when GABAergic projections from hippocampal area CA1 to RSC were activated, a manipulation that inhibits cells in RSC, mice performed at random, suggesting that the RSC is critical for the use of geometry. We are currently conducting calcium recordings during these tasks and evaluating the impact of other navigational cues. Our data suggest that the use of geometry during reorientation is robust, even in complex environments, generalizing the predominance of these cues to more natural scenarios. Further, results demonstrate that the RSC is crucial for discriminating geometrically similar locations even in large enclosures.

Disclosures: P.M. Ogallar: None. **C.M. Gagliardi:** None. **I.A. Muzzio:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

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Program #/Poster #: PSTR189.04/U27

Topic: H.09. Spatial Navigation

Support: NSFC Grant 31872775
NSFC Grant 32200836

Title: Border cells without theta rhythmicity in the medial prefrontal cortex

Authors: X. LONG, B. DENG, ***R. SHEN**, L. YANG, L. CHEN, Q. RAN, X. DU, S.-J. ZHANG;
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Abstract: The medial prefrontal cortex (mPFC) is a key brain structure for higher cognitive functions such as decision-making and goal-directed behavior, many of which require awareness of spatial variables including one’s current position within the surrounding environment. Although previous studies have reported spatially tuned activities in mPFC during memory-related trajectory, the spatial tuning of mPFC network during freely foraging behavior remains elusive. Here, we reveal geometric border or border-proximal representations from the neural activity of mPFC ensembles during naturally exploring behavior, with both allocentric and egocentric boundary responses. Unlike most classical border cells in the medial entorhinal cortex (MEC) discharging along a single wall, a large majority of border cells in mPFC fire particularly along four walls. mPFC border cells generate new firing fields to external insert, and remain

stable under darkness, across distinct shapes and in novel environments. In contrast to hippocampal theta entrainment during spatial working memory tasks, mPFC border cells rarely exhibited theta rhythmicity during spontaneous locomotion behavior. These findings reveal spatially modulated activity in mPFC, supporting local computation for cognitive functions involving spatial context and contributing to a broad spatial tuning property of cortical circuits.

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Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

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Topic: H.09. Spatial Navigation

Support: National Institute of Health Grant R01NS129874
Alzheimer's Association Research Grant 21-850571

Title: Encoding and reactivation of navigation-associated multisensory information

Authors: *U. KAYA[1], M. KUMAR[1], G. ROTHSCHILD[1,2];
[1]Psychology, [2]Kresge Hearing Res. Inst. and Dept. of Otolaryngology - Head and Neck Surgery, Univ. of Michigan, Ann Arbor, MI

Abstract: Navigation relies on the utilization of sensory information from multiple modalities to form a coherent representation of space and the location of the subject within it. Furthermore, navigation can shift from reliance on one sensory modality to another, based on the availability of sensory information. However, whether and how information processing in sensory pathways of different modalities dynamically support navigation remains largely unknown. In the hippocampus, locomotion trajectories through space are represented via sequential firing of hippocampal place cells. Furthermore, hippocampal replay is proposed to facilitate the consolidation of these experiences into memory. Recent studies have shown that navigation-relevant information, such as locomotion speed, head orientation, and position, are also encoded in sensory cortical regions, including the auditory cortex and visual cortex. Moreover, both the auditory cortex and the visual cortex exhibit forms of reactivation that are coordinated with hippocampal replay. These findings suggest that neural representations of navigation-related information may be coordinated across the auditory cortex, visual cortex, and hippocampus. However, whether this is the case and, if so, how this coordination is modulated by changes in available sensory input is unknown. To address this gap, we simultaneously recorded single-unit population activity across the auditory cortex, visual cortex, and the dorsal CA1 region of the hippocampus of rats that performed a reward-guided spatial navigation task in a novel track design. In this track, the animals were exposed to distinct visual and auditory cues in different locations and could use them to navigate to the reward location. The animals' location, speed and

head direction, as well as the sounds that the animals were exposed to, were continuously recorded in synchrony with the neural recordings. Our preliminary findings reveal patterns of spatially-selective visual information coding in the visual cortex, spatially-selective auditory information coding in the auditory cortex, in parallel with explicit spatial coding by dorsal CA1 populations in the hippocampus. We used computational analyses to identify patterns of coordination between these regions during navigation. To determine whether changes in sensory input modulate the strength and pattern of neural representations within and across these regions, we carried out similar recordings in complete darkness and quantified changes in neural response patterns. Together, these findings shed new light on the neural mechanisms underlying navigation using sensory cues of varying modalities.

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Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

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Program #/Poster #: PSTR189.06/U29

Topic: H.09. Spatial Navigation

Support: CIHR Postdoctoral Fellowship 8401179
FRQS Postdoctoral Fellowship
NSERC Discovery Grant
CIHR Project Grant

Title: Population dynamics of CA1 spatial coding during reorientation to a visual landmark

Authors: *J. LEE[1], T. XU[2], M. P. BRANDON[3];
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Abstract: To flexibly navigate environments the brain must continuously update its sense of direction and position through the integration of visual landmarks and self-motion cues. Prior work has shown that movement of visual landmarks is sufficient to drive reorientation in the brain's mapping of direction, position, and even self-motion. Yet, it remains unclear how visual cues are integrated with spatial codes across distributed neural populations to update the sense of position in real time. Here we record large neural populations in hippocampal subregion CA1 while mice freely navigate in a recently developed augmented reality behavioral task shown to induce reorientation in the internal representation of heading direction in the anterior dorsal nucleus (ADN) of the thalamus. With this approach, we ask how displacement of a polarizing visual cue impacts spatial coding of large neural populations in CA1 while mice freely navigate. We find that displacement of a polarizing cue causes reorientation of an internal representation for both position and direction in CA1. However, the reorientation dynamics observed were heterogeneous, both in time-course and degree of reorientation. We thus explore the

heterogeneity and network-level determinants of reorientation in position and directional coding from the population- to single-cell level in CA1, and compare the temporal dynamics and heterogeneity of reorientation to model predictions in the distributed hippocampal-cortical system. The similarities and differences across regions and models will help uncover how population dynamics determine the stability of spatial representations and their updating across diverse neural systems.

Disclosures: J. Lee: None. **T. Xu:** None. **M.P. Brandon:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.07/U30

Topic: H.09. Spatial Navigation

Support: CIHR Project Grant #367017
CIHR Project Grant # 377074
NSERC Discovery Grant # 74105
Canada Research Chairs Program

Title: Evolution of hippocampal neuronal dynamics during learning a reward-based navigation task

Authors: *M. YAGHOUBI[1], C.-A. MOSSER[2], S. WILLIAMS[3], M. P. BRANDON[2];
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Abstract: The hippocampus plays a crucial role in constructing a cognitive map of the environment, which aids in navigation and memory-dependent behaviors. On the other hand, accumulating research has revealed that once reward locations are learned the hippocampal representation undergoes important changes to better facilitate the computation and prediction of reward. However, how different aspects of a reward-based navigation task are represented in the hippocampus, and how this representation evolves throughout learning remain incompletely understood. To address this, we image large CA1 populations as the mouse is learning to solve a cognitively demanding reward-based navigation task. We find that the representation of the reward in both single cell and population levels decreases over time, while the representation of the cues to the reward increases over the course of learning. Our finding suggests that hippocampal representation of reward could serve as a teaching signal that supports learning when reality fails to match expectation -- so as the animal progresses in learning the task, the difference between expectation and reality reduces and accordingly reward response that acts as a teaching signal decreases. This is further supported by observing many individual cells that

encode reward in early days and gradually shift back to encode the cues to the reward over the course of learning.

Disclosures: M. Yaghoubi: None. **C. Mosser:** None. **S. Williams:** None. **M.P. Brandon:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

Location: MCP Hall A

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Program #/Poster #: PSTR189.08/U31

Topic: H.09. Spatial Navigation

Support: CIHR Project Grant #367017
CIHR Project Grant # 377074
NSERC Discovery Grant # 74105
Canada Research Chairs Program

Title: The representation of spatial coding in the retrosplenial cortex during drift and reorientation

Authors: *J. KARIMI ABADCHI[1], J. LEE[2], T. XU[3], A. FOK[4], F. LIN[4], M. P. BRANDON[2,5]; [1]Douglas Res. Centre/McGill Univ., Montréal, QC, Canada; [2]Psychiatry, McGill Univ., Montreal, QC, Canada; [3]McGill Univ., Dollard-des-Ormeaux, QC, Canada; [4]McGill Univ., Montreal, QC, Canada; [5]Psychiatry, Douglas Hospital research centre, Montreal, QC, Canada

Abstract: Successful navigation requires the accurate estimation of one's position and orientation within an environment. The brain is thought to achieve this by combining external sensory, particularly visual, and self-motion cues. Research has shown that the displacement of visual landmarks leads to the reorientation of the brain's internal representation of the animal's position in the hippocampus and head direction in the anterodorsal nucleus of the thalamus (ADN). Yet, how the visual information reaches ADN from the visual cortex remains poorly understood. An important candidate region for bridging the visual cortex and ADN is the retrosplenial cortex (RSC) as it is shown that a) it integrates visual and spatial information, and b) it sends axonal projections to ADN. To shed light on the role of RSC in the brain estimation of the position and head direction, we recorded calcium transients from a large population of RSC neurons using the UCLA miniscope while mice freely navigated within an augmented reality behavioral apparatus known to induce reorientation of the head-direction representation in the ADN. Our goal is to determine, (1) how position and head direction are encoded in the population-level activity of RSC neurons in the presence and absence of a polarizing visual cue, and (2) how the RSC position and head direction representation reorients in response to visual cue displacement.

Disclosures: J. Karimi Abadchi: None. **J. Lee:** None. **T. Xu:** None. **A. Fok:** None. **F. Lin:** None. **M.P. Brandon:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

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Topic: H.09. Spatial Navigation

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NSERC Discovery Grant #74105
Canada Research Chairs Program
Tomlinson Doctoral Fellowships

Title: Investigating the source of network gain in the thalamic head direction system

Authors: *F. LIN[1], M. P. BRANDON[2];
[1]Integrated Program in Neurosci., McGill Univ., Montréal, QC, Canada; [2]Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: Head direction (HD) cells represent an animal's head direction during spatial navigation, serving as the 'internal compass' of the brain. A growing body of evidence suggests that the tuning of HD cells is tightly controlled by visual inputs. Previous work in the lab has reported that the HD neural activity in mouse anterodorsal thalamic nucleus (ADN) is constantly reset by a moving visual cue (Ajabi et al., 2023). This work also revealed that 'network gain' is reduced during realignment of the HD system, and the amount of this reduction is larger for faster realignments. In our current work, we aim to understand how visual information regulates ADN neural activity at the circuit level. To this end, we performed anatomical circuit tracing with retrograde AAV virus to identify the upstream regions sending projections to ADN in wild-type adult mice. Consistent with previous reports, ADN received projections mainly from the lateral mammillary nucleus (LM), the anterodorsal sector of the reticular thalamic nucleus (TRN), the retrosplenial cortex (RSC), and the postsubiculumn (POST). Additionally, we characterized the distribution of presynaptic RSC neurons along the anterior-posterior axis. We also identified the types of presynaptic cells in these regions, which sheds light on their functional roles in regulating ADN HD neurons. Second, we performed fiber photometry recordings to study how the activity in the TRN-ADN circuit changes during visual cue rotation. Preliminary evidence suggests that the TRN-ADN circuit is engaged during realignment of the ADN head direction system. Overall, these results provide evidence that visual input is one of the main regulators of the head direction system in ADN.

Disclosures: F. Lin: A. Employment/Salary (full or part-time):; Integrated Program in Neuroscience, McGill University, Douglas Hospital Research Centre. **M.P. Brandon:** A. Employment/Salary (full or part-time):; Department of Psychiatry, McGill University, Douglas Hospital Research Centre. 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.; CIHR Project Grant #367017, CIHR Project Grant #377074, NSERC Discovery Grant #74105, Canada Research Chairs Program.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.10/U33

Topic: H.09. Spatial Navigation

Support: CIHR Project Grant 367017
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NSERC Discovery Grant 74105
Canada Research Chairs Program

Title: Neural correlates of network gain in the dynamics of reorientation: the uncertainty hypothesis

Authors: *H. NAGARAJ[1,2], M. P. BRANDON[3,2];
[1]McGill Univ., Montreal, QC, Canada; [2]Douglas Hospital Research Centre, Montreal, QC, Canada; [3]Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: The internal compass of the brain, also known as the head direction (HD) system, forms a crucial component of the neural circuit necessary for effective spatial orientation, and includes regions such as the anterodorsal thalamic nucleus (ADN). The HD system maintains a sense of orientation by iteratively tracking self-motion information with local cues used as an anchoring mechanism. In the absence of these cues, the system tends to exhibit a drift in representation. A recent study from our lab in mice showed that the population activity within the ADN exhibited a decrease in network gain, which is a measure of global activity levels, during reorientations induced by changes in visual cues. The extent of this reduction in gain influences the dynamics of reorientation, with lower gain leading to faster reset events. We propose that network gain is a measure that reflects the uncertainty of HD representation, and that manipulating the degree to which a visual cue predicts the orientation accurately would influence the network gain. We use 1P Ca2+-recordings in the ADN of freely behaving mice along with visual cue manipulations to examine this idea and test the hypothesis that cue uncertainty would affect the network gain, and thus, the dynamics of reorientation. We employ computational modelling to explain how visual cue certainty can impact network gain as well. Thus, our findings would help further our understanding of the dynamics of reorientation in the head-direction system, and how extrinsic visual cues could have an impact on the same. These findings might have implications on examining how extrinsic cues, network gain and reorientation dynamics could impact spatial navigation strategies.

Disclosures: H. Nagaraj: None. **M.P. Brandon:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

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Support: CIHR Project Grant #367017
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Canada Research Chairs Program

Title: Modelling an uncertain head direction system - mechanisms of network gain

Authors: *S. LA ROSA[1], M. P. BRANDON[2];
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Abstract: Tracking head direction (HD) is an important part of the broader spatial navigation system in the rodent brain. HD cells are found throughout the brain, including in the anterior-dorsal thalamic nucleus (ADN), a region which integrates information from the vestibular and visual systems. When provided with a reliable and polarizing visual cue, the HD system will orient to it. When this cue is moved, the system will reorient. These reorientations can occur at different speeds, mediated by an additional factor, network gain, which changes the population activity of the HD system. During situations of high gain, the network is slower to reorient, while during low gain, the network reorients faster. This fact leads us to believe that gain is a measure of the uncertainty of the input into the HD system. We propose a model of the head direction system which integrates angular velocity and visual input, as well as network gain. The model is inspired by the known connections between the ADN and regions such as the lateral mammillary nucleus and the thalamic reticular nucleus. It provides a flexible means by which to explore how the system will react to different environments and different stimuli. In addition, modification of the model architecture leads to a change in how the HD system reorients to a visual cue. The model also provides us with a means to predict the activity of the HD system when novel cues are provided, as well as when an agent is performing path integration tasks in which visual cues are unavailable.

Disclosures: S. La Rosa: None. **M.P. Brandon:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

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Support: CIHR Project Grant #367017
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Canada Research Chairs Program
NSERC PGS D

Title: Contextual tuning predicts task learning in the rodent hippocampus

Authors: *Z. HAQQEE[1], S. LA ROSA[2], S. WILLIAMS[3], M. P. BRANDON[4];
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Abstract: Hippocampal place cells demonstrate context-dependent spatial representations of their environment in subjects that have been well-trained on a navigation task. How these representations evolve with learning is seldom studied, particularly in more complex tasks that are gradually learned over weeks. Using one-photon calcium imaging with microendoscopes (UCLA Miniscope), we tracked hundreds of cells in dorsal CA1 of the hippocampus of mice gradually learning a paired-associate learning touchscreen task. Using generalized linear models, we identify specific ensembles of cells that dynamically evolve their tuning to spatial and contextual features over more than a month of daily training on the touchscreen task, from habituation to overtraining. Overall, hippocampal cells in individual mice became increasingly specific in their tuning to the trial context with task learning, particularly during the most dynamic portion of their learning curves. Notably, a subset of context-selective cells became significantly less likely to fire during trial-specific reward collection phases of the task at rates that also closely followed trial-specific task performance metrics. We found that non-place-cells were more likely to be tuned to task context than place cells. However, cells that became increasingly selective to task context during learning did not sacrifice their spatial tuning strength over time. The data suggests that learning might gradually refine the cognitive map of the hippocampus, with hippocampal cells becoming increasingly sparse in their tuning, showcasing a structure of task representation emerging with rewarded experience.

Disclosures: Z. Haqqee: None. **S. La Rosa:** None. **S. Williams:** None. **M.P. Brandon:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.13/V1

Topic: H.09. Spatial Navigation

Support: R21MH135321

Title: Subiculum multi-directional tuning matches path-structure to encode trajectories.

Authors: *R. PLACE[1], D. A. NITZ[2];
[1]UCSD, La Jolla, CA; [2]Univ. of California San Diego, San Diego, CA

Abstract: Activity within the Subiculum (SUB) has typically been defined as an extension of CA1, with SUB firing patterns appearing as multiplexed “place fields” that demonstrate stability across changing environments[1,2]; however, SUB neurons have recently been found to track topographic structures, including environmental axes or spatial positions defined at the point of intersecting boundaries[3,4].To expand upon these results, we recorded simultaneous SUB and CA1 activity in rats as they traversed a series of interconnected paths. A novel task required rats to learn routes defined amongst a network of three path axes that intersect to form a triangular grid. For each trial, rats were cued to start at 1 of 12 positions along the network’s perimeter (i.e. start points occur at 30-degree increments along the maze boundary), with the starting location dictating the given trial’s rewarded route. We found maze-room orientation served to guide behavior, and the path arrangement allowed us to test orientational and positional impacts on neural activity.While place activity was observed in both SUB and CA1 neuron spiking patterns, SUB responses contained a strong directional signal that was absent from CA1. Further, SUB directional tuning could be divided into neurons expressing unimodal versus multi-modal activity peaks, with multi-directional tuned neurons displaying a range of offsets defined by the maze structure. During task navigational demands, SUB neurons displayed strong intra-network coordination, which largely depended upon relationships to the variety of directionally tuned neurons. Intra-SUB coordination, as well as multi-directional tuning in general, was largely lost during post-task open-field recordings. Interestingly, we found the variety of SUB directional responses to decode and discriminate amongst route progressions with greater accuracy than classic spatially tuned neurons. SUB’s directional activity, in fact, formed a contextually relevant spatial code.These findings highlight that SUB’s unique responses might serve to produce an aspect of the cognitive map that is distinct from CA1 and is optimized to guide complex behavior in learned environments. Notably, SUB’s anatomically positioned to transform information between CA1, anterior thalamus (AT) and cortical areas (e.g. retrosplenial, entorhinal), for which multiplexed spatial and movement features have been described[5].(1)Kim & Frank. (2012). J. Neurosci. 34, 11539-11558.(2)Sharp. (2006). Beh. Brain Res. 2, 206-214.(3)Olson et al. (2017). Nat. Neurosci. 20, 170-172.(4)Sun et al. (2024). Nature 627, 821-829.(5)Alexander et al. (2015). Nat. Neurosci. 18, 1143-1151.

Disclosures: R. Place: None. **D.A. Nitz:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.14/V2

Topic: H.09. Spatial Navigation

Title: Spatial periodicity of firing in retrosplenial cortex follows path shape and sub-structure

Authors: *Y. XU[1], D. A. NITZ[2];
[1]UCSD, San Diego, CA; [2]Univ. of California San Diego, San Diego, CA

Abstract: Traversing complex routes is often facilitated by considering the route as a set of related sub-spaces. However, the neural mechanisms underlying this compartmentalization process are not well understood. We investigated whether neurons in retrosplenial cortex (RSC, N=265), a hub for multiple forms of spatial and directional processing, exhibit firing patterns that reflect encoding of route sub-spaces. Rats (N=3) traversed a variety of path shapes on a ring-shaped track, a plus-shaped track, a large and small hexagonal track, and an irregularly shaped track. Single-unit recordings revealed that while RSC ensembles generated distinct representations for every track location, a significant proportion of RSC neurons exhibited periodic activation patterns that repeated across route segments of the same shape. Recurring activation patterns were observed at the scale of the full route and at scales capturing route sub-spaces (e.g. half-routes and quarter-routes). This periodic encoding was maintained even in track configurations (circle and irregular tracks) that are devoid of repeating action sequences, indicating independence from motor patterns. Strikingly, these spatially periodic firing patterns adapted to the specific structure of each maze. In the plus maze, quarter-route periodicity dominated, while in the hexagonal maze, sixth-route periodicity was most prevalent. These findings suggest RSC neurons exhibit periodicity at many spatial frequencies, but that the distribution of spatial periodicities adapts to the unique sub-space structure of different route shapes. This adaptable periodic encoding may constitute a fundamental mechanism for decomposing navigational problems into tractable segments across diverse environments and/or for population encoding of path shape.

Disclosures: Y. Xu: None. **D.A. Nitz:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.15/V3

Topic: H.09. Spatial Navigation

Title: Encoding of task, object, and environmental locations in hippocampus and subiculum during landmarks-based navigation

Authors: *J. C. TUNG[1], E. XU[2], D. A. NITZ[3];
[1]Cognitive Sci., UCSD, La Jolla, CA; [2]UCSD, San Diego, CA; [3]Univ. of California San Diego, San Diego, CA

Abstract: Objects in an environment, such as terrain features and botanic and other matter, may possess navigational or goal-relevant salience to animals in the wild. A rock or bush, for example, might mark the entrance to a den or signal some distance and orientation to a food source. Consistent with this observation, object-sensitive spatial encoding has been reported in neurons in areas with known involvement in spatial mapping and navigation, including the entorhinal cortex, hippocampus, and subiculum in rodents running freely in an environment. Object sensitivity has been reported at both the single neuron and population level, in the form of object vector firing, in which neurons are attuned to the distance and orientation of the animal to the object (relative to the environment). Prior work has focused largely on animals moving freely in a closed environment that contains one or more stationary objects. In this work, we investigate the encoding of objects in the rat hippocampus and subiculum while route-running in an environment designed to provide multiple spatial frameworks including an object-based subspace created by the use of an asymmetrical object as the route terminus. Rodents are trained to run structured routes around an external path before entering into an inner arena through one of four entrances and then travel directly to the interior of an L-shaped object placed in one of nine locations, and arranged in one of several orientations, within the inner arena. This paradigm enables spatial encoding to be viewed within multiple spatial frameworks, including frames of reference based on: route; allocentric environment; object-within-environment; object-within-route; rat-centered object location; and object-orientation subspaces. Neurons exhibit different firing patterns during the route running task than during periods of free foraging.

Disclosures: J.C. Tung: None. **E. Xu:** None. **D.A. Nitz:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.16/V4

Topic: H.09. Spatial Navigation

Title: Multidirectional tuning in subiculum follows both environmental and task-defined reference frames

Authors: *S. KIM[1], R. J. PLACE[2], D. A. NITZ[3];
[2]Cognitive Sci., [1]UCSD, La Jolla, CA; [3]Univ. of California San Diego, San Diego, CA

Abstract: The dorsal subiculum (SUB) is most often considered as a hippocampal output complementary to hippocampal sub-region CA1. For this reasons, SUB neuron activity profiles have classically been described according to spatial tuning and suggesting it functions as a more compressed yet less spatially detailed “cognitive map”. While this is true when constraining analysis to open-field navigation, several studies have now reported SUB firing patterns to encode topographical features of the environment, such as boundaries, corners, and maze axes. SUB’s unique connectivity with regions containing head-direction cells (e.g. anterior thalamus and retrosplenial cortex), in addition to dense CA1 afferents, may position it to generate an

altogether different type of cognitive map, which learns to relate orientations between notable environment and task features. To further investigate the characteristics of directional and multi-directional tuning of SUB neurons and their role in spatial cognition, we recorded single unit activity in SUB and CA1 simultaneously while animals traversed within an environment constrained to a network of interconnected pathways. The set of interconnected pathways (i.e., the "path network"), was organized as a set of tessellated triangles in the overall shape of a hexagon. The navigational task linked 5 starting positions at the perimeter to goal positions also at the perimeter. Travel from the former to the latter demanded movement through the path network's center. In this way, task performance yielded unique directional transitions with the full set of routes covering all possible transitions in increments of 60 degrees. This allowed us to test the rules that govern multidirectional tuning offsets, as were recently reported to occur for SUB neurons recording during performance of a different task in the same environment. Further, we examined SUB and CA1 activity in rats following rotations of the full task structure, as achieved by rotating the start and goal positions by 60-, 120-, or 180-degrees mid-session. This allowed us to test the extent that directional versus spatial aspects of SUB versus CA1 cognitive maps would re-organize to changes in task orientational demands and to determine whether directional tuning in SUB would take reference to the environment, to the space defined by the task structure, or both. We observed robust tuning of individual SUB neurons to multiple directions of movement during task performance and encoding of path network locations affording transitions between different movement direction combinations.

Disclosures: S. Kim: None. **R.J. Place:** None. **D.A. Nitz:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

Location: MCP Hall A

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Program #/Poster #: PSTR189.17/V5

Topic: H.09. Spatial Navigation

Title: Anterior thalamic, subicular, V2, and M2 tuning of route space, angular velocity, and orientation across multiple transition angles on a 12-arm radial maze

Authors: *E. GALANG, M. DING, D. A. NITZ;
UC San Diego, La Jolla, CA

Abstract: Navigation in both humans and animals is often constrained to movement along paths. In addition to salient boundaries and landmarks, many environments are spatially defined by the organization of pathways and their intersections and orientations to each other as well as the shape of repeatedly used complex routes. In such environments, effective movement to goal locations demands an interface between neurons encoding environmental locations and orientations, neurons with tuning to route progress, and motor control systems capable of encoding turning behavior and/or angular velocity. In the present work, we examine the tuning properties of neurons in anterior thalamus, subiculum, secondary motor cortex (M2), and

secondary visual cortex (V2) during performance of a route-running task on a 12-arm radial maze. The task demanded movement of animals along inverted U-shaped paths between the end points of each arm. By varying the number of arms (0-5) between goal locations, we were able to continuously vary the turning angle and the pairings of heading associated with the two arms of any given trajectory. Despite gaining strong input from hippocampal sub-region CA1, our early results indicate that many subiculum neurons were tuned primarily to one or more environmental orientations. Anterior thalamic neurons exhibit high-fidelity tuning to individual orientations. Both V2 and M2 neurons exhibit tuning to angular velocity. Further analyses are expected to reveal the dynamic interplay between neurons encoding orientation, transitions between orientations, actions, and route locations.

Disclosures: E. Galang: None. **M. Ding:** None. **D.A. Nitz:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.18/V6

Topic: H.09. Spatial Navigation

Title: Neurons of posterior parietal cortex encode the meta-structure shared by routes demanding opposite action sequences

Authors: *A. B. JOHNSON[1], D. A. NITZ[2];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Univ. of California San Diego, San Diego, CA

Abstract: Environments for which movement is constrained to networks of interconnected pathways often exhibit an organization, or meta-structure, that can be learned and used in navigational reasoning. However, how emergent properties of meta-structure, such as topological similarity, are represented in the brain remain largely unstudied. To reveal meta-structural representation in the brain we studied posterior parietal cortex (PPC) neurons while animals navigated on a ‘triple-T’ environment with 6 distinct, but interconnected routes: four internal routes which overlapped to varying degrees, and 2 return routes which flanked the internal routes. Previous work has described PPC neurons encoding progress through routes that: is independent of the action tuning of some PPC neurons; is independent of the location and orientation of a route in the environment; and is not dependent on a view of the full route. Here, PPC single-unit neuron activity was compared across route-pairs that were structurally equivalent but were opposite with respect to sequencing of L versus R turns. We observed two distinct subpopulations of neurons. Positional rate vectors for the first exhibited large negative correlation values across oppositely shaped routes. The second subpopulation exhibited large positive correlation values across all topologically similar routes despite the opposite action sequences they demanded To test the contributions of self-motion for these two populations of neurons, we modeled the firing rate of both populations using linear and angular velocity. A leave-one-out approach was used to determine the relative contribution of linear and angular

velocity for each of these populations. We found that while angular velocity responses may underlie the large negative correlations, both angular and linear velocity fail to explain the large positive correlations. This suggests that while self-motion representation is important for neurons that discriminate across differently shaped routes, there exists a never before described population of PPC neurons capable of responding not only to progress along a route-centered frame of reference but also across route pairs whose shapes are mirror-images of each other. We submit that PPC neuron populations can generalize their firing patterns according to meta-structural features such as route topography. This suggests that PPC neuron populations are capable of learning and responding to emergent features within a single spatial context.

Disclosures: A.B. Johnson: None. **D.A. Nitz:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

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Topic: H.09. Spatial Navigation

Support: Norman H. Anderson Fund for Research by the University of Chicago

Title: Strategy-specific correlates of beta oscillations in the dorsal CA1 of rats

Authors: *P. TOPTAS, G. WANG, A. E. KAYE, Z. LEVERONI, J. PROCTOR-BONBRIGHT, U. MANI, B. GARCIA CORADO, Y. WON, L. CHEN, J. Y. YU;
Univ. of Chicago, Chicago, IL

Abstract: Beta oscillations (12-30 Hz) are found in sensory, premotor, frontal cortical, and hippocampal regions, and are thought to support sensory perception, motor planning and decision-making. During the cue presentation period of olfactory decision-making tasks, coordinated beta oscillations in olfactory and frontal cortical regions, and the hippocampus are hypothesized to support the decision-making process. It remains unknown whether beta oscillations are associated specifically with sensory-based decisions or generally with decision-making. Here, we determined the relationship between beta oscillations in the rat hippocampus under cue-based and non-cue-based decision-making strategies. We trained rats in two-alternative forced-choice olfactory decision-making tasks and recorded local field potential from the dorsal CA1 of the hippocampus. We found that the rats' choices in the tasks can be classified as cue- or non-cue-based. Choices made under a cue-based strategy were consistent with odor-action associations. Choices made under the non-cue-based strategy were instead strongly biased towards one of the two alternative choices irrespective of the odor cue. We found that the power of beta oscillations in the dorsal CA1 were higher on trials consistent with the cue-based compared with the non-cue-based strategy. Our results suggest that cue- versus non-cue-based decision strategies are associated with distinct network states, and beta oscillations in the dorsal CA1 are prominent during cue-based sensory decisions.

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Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.20/V8

Topic: H.09. Spatial Navigation

Support: BRFSG-2021-11 Brain Research Foundation
Norman H. Anderson Fund

Title: Prior experience influences cortical representations during novel task learning

Authors: *S. SHRIDHAR[1], Z. LEVERONI[1], B. FENG[1], K. KHANNA[2], J. GUTIERREZ[1], J. Y. YU[1];
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Abstract: Experience can shape future behaviors, but how specific experiences influence learning in a new environment remains unclear. Generalization of shared features between experiences is thought to affect learning. Prefrontal cortical networks are hypothesized to support generalization between experiences but how activity in these networks is influenced by prior experience is unknown. We ask how prefrontal cortical activity during spatial learning depends on the animal's prior experience. We quantified whether behavioral and neural correlates of learning in a new task differed between rats with distinct prior experiences, which involved task rules that were applicable or non-applicable to the new environment. To create a distinct prior experience, we trained two groups of rats where one group initially learned a probabilistic spatial rule while the other group learned an alternation rule. Both groups then learned an alternation rule on a maze with a different geometry in a new environment. The task rule from the prior experience was either applicable (alternation) or non-applicable (probability) in the new task (alternation). We found that the learning rate in the novel task was similar irrespective of prior experience and all animals reached similar final levels of performance. To examine prefrontal cortical representations in the novel task, we recorded spiking activity from populations of medial prefrontal cortex neurons using multi-tetrode recording devices. We asked how the neural population activity between the novel and familiar tasks depended on prior experience. To do this, we used principal component analysis to extract the neural trajectories corresponding to trials across sessions and measured the similarity between session pairs. We found that animals with applicable prior experiences had a higher cosine similarity across sessions compared with the animals with non-applicable experiences. Our results demonstrate that similar behavioral performance can be associated with distinct cortical representations that reflect shared features between the experiences, and broadly builds upon our understanding of experience representations in the brain.

Disclosures: S. Shridhar: None. **Z. Leveroni:** None. **B. Feng:** None. **K. Khanna:** None. **J. Gutierrez:** None. **J.Y. Yu:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.21/V9

Topic: H.09. Spatial Navigation

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Title: Awake sharp-wave ripple rates decrease with familiarity irrespective of learning state

Authors: *N. ZHOU[1,2], Z. LEVERONI[1], H. XU[1], B. FENG[1], K. KHANNA[3], J. Y. YU[1]; [1]Univ. of Chicago, Chicago, IL; [2]Institute for Mind and Biology, Chicago, IL; [3]Univ. of Chicago Lab. High Sch., Chicago, IL

Abstract: Hippocampal sharp-wave ripples (SWRs) are required for learning and memory. Two models describe the function of SWRs during learning. In the 'storage hypothesis', SWRs serve to consolidate memories of new information during novel experiences. This model predicts SWR rate is high during a new experience and decreases with familiarity as the amount of new information decreases with time. In the 'planning hypothesis', SWRs support the recall of memory for deliberation and evaluation of future options for decision making. This model predicts that SWR rate correlates with cognitive demand. Early learning stages require higher cognitive demand compared with later stages and SWR rate has an inverse correlation with performance. Given that familiarity and performance are correlated in many tasks, and both models predict a decrease in SWR rates, it is challenging to determine the contribution of these two proposed functions during learning. To determine how each model can explain the contribution of SWRs to learning, we designed an experiment where rats learned two mazes that differ in difficulty. The two mazes have different geometries and share the same alternation rule. This design controls for familiarity but results in differences in performance, which dissociates familiarity from learning state. This allows us to determine the relationship between SWR rate and familiarity or learning state. We recorded local field potential in the hippocampal dorsal CA1 region over 5 days of learning and quantified the rate of SWRs. To estimate the learning state, we used a state-space model to estimate the probability of making a correct choice at each trial. For one maze, the probability of a correct choice reached 80% whereas on the other, reached 50% at the end of 5 days. We found SWR rates were similar across the two mazes and saw a similar decrease between the first and last day of training. Our experiment demonstrates a dissociation between familiarity and learning state, indicating that SWR rate decreases even when the learning state remains at chance. Our results support the hypothesis that SWRs contribute to learning by consolidating new information.

Disclosures: N. Zhou: None. **Z. Leveroni:** None. **H. Xu:** None. **B. Feng:** None. **K. Khanna:** None. **J.Y. Yu:** None.

Poster

PSTR189: Cortico-Hippocampal Interactions Underlying Spatial Navigation II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR189.22/V10

Topic: H.09. Spatial Navigation

Title: Coordinated Hippocampal-Cortical Beta Oscillations in Spatial and Non-Spatial Learning

Authors: *G. WANG, P. TOPTAS, N. ZHOU, S. SHRIDHAR, Z. LEVERONI, A. YANG, H. XU, M. LIUFU, U. MANI, L. CHEN, J. PROCTOR-BONBRIGHT, J. Y. YU;
Univ. of Chicago, Chicago, IL

Abstract: Rhythmic coordination between hippocampal-cortical networks is hypothesized to support diverse aspects of cognition. Beta frequency oscillations (~12–30 Hz in rodents) are implicated in memory-guided decision-making. Beta oscillations in olfactory and hippocampal-cortical networks are hypothesized to support olfactory sensory perception and processing. Beta oscillations in motor cortical areas are hypothesized to support movement planning and execution. We report coordinated beta oscillations occur in the rat hippocampal-cortical networks during reward consumption periods. We recorded neural activity in the prelimbic cortex and dorsal hippocampus CA1 in two behavior tasks. One group of rats performed a two-choice odor discrimination task, while another group performed spatial navigation tasks. Bursts of beta oscillations were found in both the prelimbic cortex and dorsal hippocampus CA1 during reward consumption periods in both tasks. These beta bursts exhibited distinct temporal dynamics: in the prelimbic cortex, they occurred within 0.5 seconds of the reward, whereas in CA1, they were more distributed throughout the reward period. The bursts had a mean rate of 0.7 Hz, and each burst contained an average of 4 cycles. Bursts are coordinated between the prelimbic cortex and dorsal CA1, with an average phase lag of 1/8 of a cycle. We observed that beta burst rate decreases with improving performance. Importantly, we show beta bursts occur independently of hippocampal sharp wave ripples. Our findings support the hypothesis that coordinated hippocampal-cortical activity in the beta frequency signal cross-region coordination during learning and decision making beyond sensory perception and processing.

Disclosures: G. Wang: None. **P. Toptas:** None. **N. Zhou:** None. **S. Shridhar:** None. **Z. Leveroni:** None. **A. Yang:** None. **H. Xu:** None. **M. Liufu:** None. **U. Mani:** None. **L. Chen:** None. **J. Proctor-Bonbright:** None. **J.Y. Yu:** None.

Poster

PSTR190: Grid Cells and Spatially Modulated Cells I

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Title: Left-right-alternating theta sweeps in entorhinal grid cells and beyond

Authors: *A. VOLLAN[1], R. GARDNER[2], M.-B. MOSER[3], E. I. MOSER[3];
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Abstract: Place cells in the hippocampus and grid cells in the entorhinal cortex are elements of a neural map of self-position. To benefit navigation, the representation of self-position in these cells must be dynamically related to surrounding locations. A candidate mechanism for linking places along an animal's path has been described in place cells, where the sequence of spikes within each cycle of the theta oscillation encodes a trajectory from the animal's current location towards upcoming locations. However, to bridge the animal's path with the wider environment, beyond places previously or subsequently visited, an experience-independent spatial sampling mechanism might be required. Here we used Neuropixels probes to record neural activity in freely moving rats and show, that within individual theta cycles, ensembles of grid cells and place cells encode a position signal that sweeps linearly outwards from the animal's location into the ambient environment, with sweep direction alternating stereotypically between left and right across successive theta cycles. These sweeps were accompanied by, and aligned with, a similarly alternating signal in a separate population of direction-modulated cells with putative connections to grid cells via conjunctive grid×direction cells. Sweeps extended into never-visited space inaccessible to the animal and persisted during REM sleep. Sweep directions could be explained by an algorithm that maximizes cumulative coverage of surrounding space. The sustained and unconditional expression of theta-patterned left-right-alternating sweeps in the entorhinal-hippocampal positioning system provides an efficient 'look-around' mechanism for sampling locations beyond the travelled path. In ongoing work, we record neural activity in multiple cortical and sub-cortical structures of the limbic system while decoding sweeps in entorhinal grid cells. Bilateral recordings in the anteroventral thalamus revealed antiphasic theta cycle skipping across hemispheres, such that the anteroventral thalamus in each hemisphere was more active when sweeps were directed to the contralateral side. This observation indicates that left-right-

alternating sweeps permeate population activity throughout the limbic system and opens possibilities to study their mechanistic origin and functional role in cognition.

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Poster

PSTR190: Grid Cells and Spatially Modulated Cells I

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Centre for Algorithms in the Cortex 332640
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Title: Multi-module grid cells in the medial entorhinal cortex

Authors: *Y. GRONICH[1], V. A. NORMAND[2], M.-B. MOSER[2], E. I. MOSER[2], Y. BURAK[1,3]; [1]Edmond and Lily Safra Ctr. for Brain Sci., Hebrew Univ. of Jerusalem, Jerusalem, Israel; [2]Kavli Inst. for Syst. Neurosci., Trondheim, Norway; [3]Racah Institute of Physics, Hebrew University of Jerusalem, Jerusalem, Israel

Abstract: Grid cells in the medial entorhinal cortex are functionally organized in subgroups (called grid modules), each characterized by a distinct spatial scale. Population analysis of individual grid modules has shown that each module internally represents a latent two-dimensional variable, manifested in the position of the joint activity on a manifold with toroidal topology. The existence of separate toroidal manifolds suggests that modules constitute distinct sub-networks that are largely independent of each other. It is unknown, however, whether there are mechanisms that link the activity in different modules. In this study, we used simultaneous recordings from thousands of entorhinal cells to investigate the tuning of individual cells to the internally represented latent variables. We developed a method based on Hidden Markov Models to identify the toroidal tuning of each cell, and to subsequently decode the dynamics of the latent variables based on the neural recordings. Surprisingly, we next identified previously unknown sub-populations of neurons that are sharply tuned to two modules, a phenomenon we refer to as ‘co-tuning’. This co-tuning property of the cells is manifested in their spatial firing patterns, which could otherwise be misinterpreted as a distorted grid pattern. Intriguingly, co-tuned cells are predominantly observed in modules with consecutive grid spacings, and are anatomically distributed near the boundary between the two modules. We speculate that the co-tuned cells may be involved in an interaction between grid modules.

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Poster

PSTR190: Grid Cells and Spatially Modulated Cells I

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Centre of Neural Computation 223262
Centre for Algorithms in the Cortex 332640
Kavli Foundation
Ministry of Science and Education, Norway

Title: Phase-dependence of functional connectivity in grid cell networks

Authors: *N. DE JONG[1], R. VALE[1], R. R. NAIR[1], Y. BURAK[2], W. ZONG[1], M.-B. MOSER[1], E. I. MOSER[1];
[1]Kavli Inst. for Systems Neuroscience, NTNU, Trondheim, Norway; [2]Edmond and Lily Safra Ctr. for Brain Sci., Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Grid cells in the medial entorhinal cortex are thought to create an internal map of self-position that animals use for spatial navigation. Neurons within a module (defined as a sub-population of grid cells with common periodicity of the spatial response pattern) are hypothesized to participate in a continuous attractor network (CAN), whose recurrent connectivity constrains the joint neural activity to a low-dimensional toroidal manifold. While increasing evidence supports this theoretical proposal, a fundamental aspect of grid cell CAN models remains untested, namely the assumption that for neural activity to be constrained to the attractor manifold, grid cells within the same module must be connected - monosynaptically or polysynaptically - via synaptic weights that depend on the cells' grid phases (the similarity of their firing fields). Many CAN models propose a Mexican hat-like connectivity profile with excitation between functionally similar cells and inhibition of more dissimilar neurons.
To experimentally test this prediction, we have developed an all-optical recording and stimulation approach allowing us to investigate functional connectivity between grid cells with known phase differences. Our procedure consists of: i) using a miniature two-photon (2P) microscope (MINI2P) to identify grid cells in freely-moving mice, ii) transferring the mice to a benchtop 2P photostimulation system where the field-of-view is aligned to the field-of-view recorded with the MINI2P, iii) photoactivating rsChRmine expressing grid cells with known grid phases identified in step i, and iv) measuring stimulation triggered responses in non-stimulated grid cells and relating these responses to the functional properties of the stimulated cells, such as their grid phase.
We can record well over 100 grid cells from a single module using the MINI2P miniscope and

on average more than 75% of these neurons can be identified and photostimulated in the benchtop 2P system. Preliminary observations point to the existence of excitatory functional connections between grid cells with similar phases, with excitation decreasing as phase differences increase. If further validated by additional experiments, these initial findings may provide direct evidence for the Mexican hat connectivity profile suggested for grid cell connectivity in CAN models.

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Poster

PSTR190: Grid Cells and Spatially Modulated Cells I

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Program #/Poster #: PSTR190.04/V14

Topic: H.09. Spatial Navigation

Support: Centre of Neural Computation 223262
Centre for Algorithms in the Cortex 332640
Kavli Foundation
Ministry of Science and Education, Norway

Title: Independence of grid cell modules during hippocampal remapping

Authors: C. LYKKEN, B. R. KANTER, A. NAGELHUS, M. GUARDAMAGNA, J. CARPENTER, M.-B. MOSER, **E. I. MOSER**;
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Abstract: Hippocampal place cells randomly reorganize their activity patterns (‘remap’) in response to environmental change. The high dimensionality of the place code enables the network to store large numbers of uncorrelated activity patterns, a fundamental requirement of an episodic memory system. This orthogonality is not present upstream in the medial entorhinal cortex (MEC), where the neural activity of grid and head direction cells is constrained to low-dimensional manifolds. It is still unclear how grid cell input, which is confined to a set of fixed states, could generate uncorrelated hippocampal representations downstream. Given that grid cells are organized into modules, it has been proposed that independent realignment of grid modules may create orthogonal place representations downstream. To understand the transformation that occurs in this circuit, we used Neuropixels probes to simultaneously record from large numbers of grid cells spanning multiple modules as well as hippocampal place cells. Here, we show that grid modules shifted their spatial firing patterns independently between distinct, familiar environments, even though the rotation of the grid pattern was coherent. Critically, this functional independence coincided with the global remapping of downstream place cells, which formed orthogonal place representations in each environment. In a subset of experiments where the orthogonalization of the place code was incomplete (‘partial remapping’),

the degree of module independence was reduced, often with pairs of modules maintaining some coherence. This demonstration of the differential translation of module phases illustrates a mechanism for transforming a low-dimensional representation of space in the entorhinal cortex into a high-dimensional representation of place in the hippocampus.

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Poster

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Centre of Neural Computation 223262
Centre for Algorithms in the Cortex 332640
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Ministry of Science and Education, Norway

Title: Event structure sculpts neural population dynamics in the lateral entorhinal cortex

Authors: *B. R. KANTER[1,2], C. M. LYKKEN[1,2], M.-B. MOSER[1,2], E. I. MOSER[1,2];
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Abstract: Our experience of the world is a stream of events which must be segmented and organized in time. The neural mechanisms underlying event segmentation and the encoding of information across multiple timescales remain unknown. Here, we chronically implanted Neuropixels 2.0 silicon probes to simultaneously record many hundreds of neurons in the lateral entorhinal cortex (LEC) of freely behaving rats as we manipulated event structure at multiple timescales. During foraging and even during sleep, population activity drifted continuously along a one-dimensional manifold without reversing direction, potentially serving as a scaffold for the temporal organization of events. Event boundaries, in contrast, caused discrete shifts in the neural state space, suggesting that LEC dynamics directly reflect event segmentation. During tasks with predictable boundaries, activity traveled in additional orthogonal directions to multiplex event information across several timescales. The mechanisms underlying drift and shifts are distinct. Population drift can arise due to minute-scale variability in the firing rates of individual neurons, irrespective of behavioral state. Shifts in state space instead arise from synchronous responses of groups of neurons (in)activated at event boundaries. Together, these results provide a dynamical systems explanation of how events are encoded in time.

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Poster

PSTR190: Grid Cells and Spatially Modulated Cells I

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Centre for Algorithms in the Cortex 332640
Centre of Neural Computation 223262
Ministry of Science and Education, Norway

Title: Interrogation of entorhinal ultraslow periodic sequences across behavioral conditions

Authors: *S. GONZALO COGNO[1], A. LAUTRUP[1], L. BRAUN[1], C. CLOPATH[2], M.-B. MOSER[1], E. I. MOSER[1];
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Abstract: The medial entorhinal cortex (MEC) hosts many of the brain's circuit elements for spatial navigation and episodic memory, operations that require neural activity to be organized across long durations of experience. We have previously reported that entorhinal cells can organize their activity into minute-scale oscillations that manifest as periodic sequences of activity in the neural population [1]. These ultraslow periodic sequences were recorded while mice ran at free pace on a rotating wheel in darkness, with no change in running direction and no scheduled rewards. It remains unknown, however, whether the sequences also occur during more naturalistic behaviours, for example while mice run in an open field arena. Moreover, the functional role of the ultraslow periodic sequences is yet to be determined. Here we show that in free foraging conditions, MEC neuronal activity can organize into sequences. However, the sequential activity is now characterized by resettings and interruptions. By creating a computational model, we investigate the conditions under which the sequences reset. We further illustrate the potential role of the periodic sequences in facilitating, in downstream structures, patterns of neuronal activation that unfold at behavioural time scales. Finally, by analyzing Neuropixels recordings we are currently investigating the participation of grid cells and other entorhinal functional cell types in the ultraslow periodic sequences.
References:
1. Gonzalo Cogno, S., Obenhaus, H.A., Lautrup, A. *et al.* Minute-scale oscillatory sequences in medial entorhinal cortex. *Nature* **625**, 338-344 (2024). https://doi.org/10.1038/s41586-023-06864-1

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Poster

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Centre of Neural Computation 223262
Centre for Algorithms in the Cortex 332640
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Ministry of Science and Education, Norway

Title: Experience-independent emergence of toroidal and ring manifolds in the entorhinal cortex

Authors: *M. GUARDAMAGNA[1], E. HERMANSEN[2], J. CARPENTER[1], C. LYKKEN[1], B. DUNN[2], E. I. MOSER[1], M.-B. MOSER[1];
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[2]Dept. of Mathematics, Norwegian Univ. of Sci. and Technol., Trondheim, Norway

Abstract: The ability to navigate in space appears early on in life, as it is essential for the survival of the individual. The medial entorhinal cortex (MEC) is a crucial hub for the coding of animals' experiences in space. Grid cells and head direction (HD) cells are key components of the spatial navigation system, and, at the population level, these cells organize on low-dimensional manifolds - torus and ring attractors, respectively - in agreement with continuous attractor networks (CAN) theories. While the emergence of spatial coding has been studied in mechanistic detail at the single-cell level, knowledge on how large neural populations organize during development is critically missing. Here we focused on the origin and development of the grid and head direction networks. Using Neuropixels 2.0 probes, we obtained high-density population recordings (from 600 to 1200 simultaneously recorded units) in the entorhinal cortex of developing rat pups as early as post-natal day P8. Neural activity rapidly shifts from highly coordinated (P8), driven by external stimuli, to sparse and desynchronized (P10-11). We uncovered, and quantified, the presence of toroidal manifolds as early P10, before eye and ear-canal opening and before active exploration outside the nest. Toroidal manifolds abruptly emerged around P10, rapidly exhibiting adult-like characteristics. From P11 onward, distinct toroidal modules gradually emerged along the dorso-ventral axis of the MEC decorrelating from a single, coherent module. From P15 onward we observed the gradual emergence of grid fields in individual cells - within the same population from which the toroidal manifold was initially detected. Similarly, we consistently detected ring attractors in pre-/para-subiculum as early as P9, before cells in this area display their characteristic tuning to head direction. The emergence of MEC's low-dimensional manifolds mirrors the development of local excitatory (E-E) and inhibitory (E-I) connections, which appear around P10. Preliminary cross-correlation and molecular analyses suggest that toroidal and ring manifolds are detectable as soon as synaptic connections appear internally in the circuit. We show that neural networks for the coding of space have strong experience-independent components. The data raise the possibility of a general

developmental rule for networks in higher order associative cortices, like the MEC, where the intrinsic organization of the network precedes the individual cells' tuning to variables in the external world.

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Poster

PSTR190: Grid Cells and Spatially Modulated Cells I

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Topic: H.09. Spatial Navigation

Support: Centre of Neural Computation 223262
Centre for Algorithms in the Cortex 332640
Kavli Foundation
Ministry of Science and Education, Norway

Title: Dimensionality of neural populations in the subiculum

Authors: *J. CARPENTER[1], M. GUARDAMAGNA[1], V. A. NORMAND[1], C. LYKKEN[1], B. DUNN[2], M.-B. MOSER[1], E. I. MOSER[1];
[1]Kavli Inst. for Systems Neurosci., Trondheim, Norway; [2]Dept. of Mathematics, Dept. of Mathematics, Trondheim, Norway

Abstract: The subiculum is part of a hippocampal-entorhinal network involved in the encoding and retrieval of distinct spatial environments and episodes. To understand how maps are generated and maintained in this network, we explored the dimensionality of neural activity data recorded from the subiculum, a key intermediary between the high-dimensional inputs from the hippocampus and the low-dimensional inputs from the medial entorhinal cortex (MEC). We used high-density (Neuropixels 2.0 probes to record simultaneously from hundreds to thousands of neurons across the MEC, hippocampal CA1/CA3, and SUB as rats explored several different environments, both familiar and novel, under conditions that lead to global remapping of place cells in the hippocampus while phase relationships are maintained in grid and head direction cells in the MEC. We identify sub-populations and examine how subiculum neurons align with hippocampal and entorhinal dynamics in simultaneously recorded neurons.
Our findings reveal mixed spatial response across the subiculum during spatial navigation and hippocampal remapping. We observe two main types of neuronal responses to remapping within simultaneously recorded data from the subiculum: One population mirrors the MEC by maintaining a low-dimensional, stable spatial representation, where fields shift coherently across recording rooms . These shifts are coherent with grid, head direction cells, and border cells in the MEC. A second population exhibits strong remapping across environments, similar to remapping patterns observed in CA1, though with less orthogonalization between environments. In

experiments where the animal explores 5 distinct environments in separate recording rooms, the remapping population in subiculum creates distinct spatial maps for each room, although in a slightly less sparse way than we see in CA1 or CA3. These variations highlight a gradient of spatial representations across the hippocampal-entorhinal network, with the subiculum acting as a pivotal mediator that accommodates both the highly distinct spatial maps of CA1 and the generalized, stable mappings of the MEC, possibly in distinct subpopulations.

Disclosures: J. Carpenter: None. **M. Guardamagna:** None. **V.A. Normand:** None. **C. Lykken:** None. **B. Dunn:** None. **M. Moser:** None. **E.I. Moser:** None.

Poster

PSTR190: Grid Cells and Spatially Modulated Cells I

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Topic: H.09. Spatial Navigation

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Centre of Neural Computation 223262
Centre for Algorithms in the Cortex 332640
Kavli Foundation
Ministry of Science and Education, Norway

Title: Spatial landmarks attenuate population drift in the medial entorhinal cortex and hippocampus

Authors: *A. LAUTRUP, E. R. SKYTØEN, S. GONZALO COGNO, E. I. MOSER, M.-B. MOSER;
Norwegian Univ. of Sci. and Technol., Trondheim, Norway

Abstract: Extracting spatiotemporal information from experience is essential for episodic memory. Temporal information can be extracted from the activity of neural populations in the lateral entorhinal cortex (LEC). However, whether or how this code for episodic time is integrated with the spatial codes in the entorhinal-hippocampal circuit remains to be determined. To answer this question, we implanted high-density silicon probes in the hippocampus and LEC or the medial entorhinal cortex (MEC) in mice. We recorded neural activity while mice were either freely foraging in an open field arena or head-fixed running on a wheel in sensory-minimized conditions, i.e., in darkness and with neither external stimuli informative for navigation nor scheduled rewards. To determine the amount of temporal information encoded by neural activity in either region, we trained a linear classifier to decode temporal epochs from neural population activity. As expected, the strongest decoding of time was found in the LEC in both tasks, consistent with its population activity drifting over session time and enabling a readout of episodic time (Tsao et al., 2018). During running on the wheel in sensory-minimized conditions, we observed an improvement in decoding accuracy in all regions. However, the

improvement was more pronounced in MEC and hippocampus compared to LEC. Our results suggest that temporal information can be extracted from neural population activity not only in LEC but also in the MEC and hippocampus, but that drift of hippocampal and MEC activity is attenuated by spatial landmarks when these are present.

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Poster

PSTR190: Grid Cells and Spatially Modulated Cells I

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Title: Topographical organization of functional cell types in the medial entorhinal cortex

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Abstract: The medial entorhinal cortex (MEC) forms a map of local space as well as local landmarks. This is achieved through canonical feature cells like grid, object vector (OV), head direction (HD) and border cells. These functional cell types are all located near each other in the superficial layers of MEC but their exact anatomical organization remains to be determined. Previous work has shown that the grid cell network consists of discrete modules of increasing scale along the dorsal-ventral axis of MEC. In addition, imaging studies have hinted at a clustering of grid cells towards the dorsomedial part of MEC while OV, HD and border cells showed a more uniform spatial distribution. However, due to technical limitations former studies were restricted to limited subdivisions of the MEC in individual animals, precluding the imaging of all cell types simultaneously in the same animal. Here, we combine miniaturized 2 photon microscopy (MINI2P) in freely moving mice with stitching of multiple field-of-views to image activity in layer II of nearly the entire dorsomedial area of MEC as well as parasubiculum (PaS). The preparation allowed us to map the canonical features of cells over several hundreds of

micrometers in both directions mediolaterally from the PaS-MEC border as well as dorsal-ventrally within individual animals. Cells were also imaged across multiple planes through the 200 um thick cell layer.Our data show that each grid cell module forms an anatomically organized cluster at the very dorsomedial part of MEC close to the PaS border. While other cell types do not show such a clustering, we find an anticorrelation of their localization to the grid cell cluster.These findings can have implications not only for where the grid signal is generated but also on our understanding of the functional interplay within the grid cell network.

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Poster

PSTR190: Grid Cells and Spatially Modulated Cells I

Location: MCP Hall A

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Program #/Poster #: PSTR190.11/V21

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Title: Spatial representation in the retrosplenial cortex and (para)hippocampal formation of freely moving mice

Authors: *H. ENEQVIST, J. CARPENTER, W. ZONG, E. I. MOSER, M.-B. MOSER; Kavli Inst. for Systems Neurosci., NTNU, Trondheim, Norway

Abstract: For decades, the study of rodent navigation primarily focused on the hippocampal and parahippocampal regions in isolation. However, recent studies suggest that a broader cortical network contributes to the generation of our sense of space. Within this network, the retrosplenial cortex (RSC) appears to serve as a convergence zone in linking the neocortex and the hippocampal formation. The RSC integrates multi-modal sensory information from the cortex whilst also receiving extensive feedback projections from the hippocampal formation. We sought to understand the retrosplenial contribution to spatial coding and navigation by recording simultaneously from this region as well as the hippocampus and the subiculum of adult mice. We used Neuropixels 2.0 probes, or a miniature portable two-photon microscope (MINI2P), to identify hundreds of simultaneously active cells in the agranular and granular RSC, subiculum, and the hippocampus. Utilising both methods, in separate animals, allowed us to study the topographical organisation of spatial coding in RSC, without losing the precise temporal organisation of its population dynamics. Our standard experimental set-up involved foraging for

treats in one or several 80 x 80 cm open field arenas. We investigated how novelty, object interaction, and spatial remapping between environments influenced retrosplenial activity. Additionally, in animals implanted with Neuropixels probes, we conducted sleep recordings to investigate interregional communication between RSC and hippocampus in the absence of external sensory input.
Our data reveal clear spatial tuning in the granular RSC, characterised by a wide variety of firing patterns in two-dimensional arenas. These include egocentric border cells, corner/vertex cells, head direction cells, and cells that respond preferentially to visually salient objects. A small subset of cells in the granular RSC exhibited confined spatial tuning without an egocentric element. Preliminary data indicate that the egocentric activity in the RSC rotates coherently with co-recorded head-direction activity. When moving between two different environments high-firing, non-spatial retrosplenial cells showed signs of rate remapping, whilst a small number of cells in the granular RSC showed classic global remapping responses similar to what is observed in the hippocampus. We are currently quantifying the extent and distribution of global remapping in the RSC circuits and its relationship to simultaneously recorded population activity in the subiculum and hippocampus.

Disclosures: H. Eneqvist: None. **J. Carpenter:** None. **W. Zong:** None. **E.I. Moser:** None. **M. Moser:** None.

Poster

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Topic: H.09. Spatial Navigation

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Title: A subset of egocentric boundary cells in the retrosplenial cortex show preferential tuning to circumventable barriers in an environment.

Authors: *S. MALMBERG[1], G. MATTESSICH[1], D. EVERETT[1], P. A. LACHANCE[2], A. GODDARD[3], L. C. CARSTENSEN[4], M. PATEL[1], A. S. ALEXANDER[5], M. E. HASSELMO[6]; [1]Boston Univ., Boston, MA; [2]Psychological and Brain Sci., Boston Univ., Boston, MA; [3]Rochester Inst. of Technol., Rochester, NY; [4]Psychological and Brain Sci., Boston Univ., Cambridge, MA; [5]Psychological and Brain Sci., UC Santa Barbara, Santa Barbara, CA; [6]Ctr. for Systems Neurosci., Boston Univ., Boston, MA

Abstract: The retrosplenial cortex (RSC) is involved in cognitive processes including spatial navigation and episodic memory. Numerous studies have shown that some neurons in the RSC are egocentrically tuned to the outer boundaries of an arena (e.g. Alexander et al., 2020), responding when boundaries are at a specific angle and distance from the animal's current head direction. However, it is unknown whether these responses are isolated to outer boundaries that

define the physical limits of an environment, or whether cells show egocentric tuning to circumventable walls as well. To understand how we guide navigation through our environment, it is important to understand how the brain encodes obstacles guiding our paths. In this current work, we used 1-photon calcium imaging to record large populations of neurons in the agranular RSC in freely-moving mice as they explored open field environments with a series of inserted barrier presentations in different locations and orientations. Through a series of these experiments, we recorded classically defined egocentric boundary cells (EBCs) as an animal explores an empty open field environment. When a barrier was presented in the same environment, these EBCs showed egocentric tuning to inserted walls in addition to outer boundaries. A significant percentage continued to show the same egocentric directional and distance tuning to the walls that have been inserted at different orientations in space. These results help to differentiate models of egocentric boundary cell responses that can be based on matching of retinotopic input, matching of head-centered coding of boundaries with memory updating, or coding of boundaries based on forward trajectory planning. Examining how the RSC encodes defined limits and obstructions in an environment enhances our understanding of the neural mechanisms underlying adaptive behavior.

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Poster

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Topic: H.09. Spatial Navigation

Support: NIH Grant MH120073
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Title: Dissociation between postrhinal and medial entorhinal spatial reference frames during goal-directed navigation

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Abstract: Navigating to a learned goal location from varied starting points requires animals to process their surrounding environment in an allocentric, or world-centered, reference frame. In contrast, incoming sensory information is egocentric, or observer-centered, requiring a

transformation of this egocentric information into an allocentric reference frame to allow flexible navigation. Neurons in the rat postrhinal cortex (POR), such as egocentric bearing/boundary cells, have been shown to encode the geometric structure and visual landmarks of the surrounding environment in primarily egocentric coordinates. In contrast, neurons immediately downstream from POR in the medial entorhinal cortex (MEC), such as grid and head direction (HD) cells, appear to represent an animal's allocentric location and orientation. While POR and MEC cells have been compared during random foraging, it is not yet known how they differentially represent space during goal-directed navigation. We performed tetrode recordings from spatial cell types in the POR and MEC of freely moving rats as they performed a goal-directed navigation task. Animals were trained to approach an uncued goal location in the northwest quadrant of a square open field environment. Entry to the goal location would result in a randomly scattered food reward, promoting full sampling of the environment, with a 10-second timeout period preventing animals from repeatedly triggering food delivery during individual entries. A single white cardboard sheet ('cue card') along the south wall provided the only orienting cue. Animals learned to reliably approach the goal location within ten training sessions, averaging 1-2 entries per minute. Following training, daily recording sessions with the standard cue location were followed by a session in which the cue card was either moved to a different wall or duplicated along the opposite wall while the goal location remained static, placing the visual scene in conflict with the learned goal. The animals were able to ignore this egocentric-allocentric conflict in order to approach the correct goal location. This maintained behavior was mirrored by grid and HD cells in MEC, whose preferred locations and directions remained in register with the true allocentric reference frame. In contrast, POR neurons shifted their representations to follow the visual cue configuration, reinforcing the primarily egocentric nature of POR cell firing. Further studies will be necessary to elucidate the specific interactions between these conflicting spatial signals that support flexible navigation.

Disclosures: P.A. LaChance: None. **S. Sankaranarayanan:** None. **E. Gross:** None. **M.E. Hasselmo:** None.

Poster

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Topic: H.09. Spatial Navigation

Support: MH120073

Title: Novelty coding in the retrosplenial cortex

Authors: *G. MATTESSICH[1], D. EVERETT[1], S. MALMBERG[1], P. A. LACHANCE[2], J. H. WILMOT[3], A. GODDARD[4], M. PATEL[1], M. E. HASSELMO[5];
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Abstract: The retrosplenial cortex (RSC) has been widely studied in the field of spatial navigation as a potential center for the transformation between egocentric and allocentric spatial reference frames. Understanding the arrangement of physical landmarks in a given environment is fundamental for guiding spatial navigation. Because the RSC receives inputs from sensory, motor and memory-related brain regions, the RSC is positioned to integrate this information to generate appropriate behavioral responses to environmental changes and navigational obstructions in space. In this current work, we used calcium imaging to track large populations of RSC neurons in the agranular RSC as animals explored environments with novel objects and barriers. Using a generalized linear model, we identified a subset of retrosplenial neurons that showed increased firing rates when an animal explores a novel object. Additionally, we used fiber photometry to monitor acetylcholine during novel object exploration. When an animal approaches a novel object, increased cholinergic activity is observed indicating that acetylcholine may play a role in novelty responses. These novelty responses are consistent with the timing of detection events in models of forward trajectory scanning. Novelty can be represented as a mismatch between current sensory input and memory-based retrieval of previous egocentric viewpoints of an environment. Consistent with previous literature, we report that the changes in neuronal firing activity and cholinergic modulation in the retrosplenial cortex are possible mechanisms by which the brain encodes novel changes in an environment as essential elements of memory guided behavior.

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Poster

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Title: Neuroai model of hippocampal-striatal interaction during spatial navigation

Authors: *A. EFREMOV[1], D. LEVENSTEIN[2], A. PEYRACHE[3], B. A. RICHARDS[4];
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Abstract: Navigation in mammals is primarily dependent on the hippocampus (HPC), which constructs a cognitive map of the environment, and the striatum, which supports goal-oriented behavior. However, the nature of information they exchange and its processing is unknown. To investigate this, we developed a model that integrates the functionalities of two regions. HPC is simulated with a sequential predictive recurrent neural network (spRNN) that learns to predict sequences of sensory observations. We have recently shown that this network develops spatially-tuned neurons and a cognitive map that captures the environment geometry. However, it remains unknown whether information contained in the cognitive map is enough for solving navigational tasks, and what aspects of cognitive map provide for efficient learning of such tasks. To model the interaction between the hippocampus and the striatum, we have combined spRNN with an actor-critic reinforcement learning algorithm, which takes as input the spRNN activations and/or visual observations. We then trained the model to solve a navigational task with ambiguities in visual observations. While agents with a limited view of the environment showed poor performance, agents relying on spRNN activations successfully learned the task, although more slowly than agents with a full view of the environment. When representations from the spRNNs were combined with visual input, it improved the agent's performance to the same level as with a fully observable environment. These results show that representations provided by the spRNNs convey more information than just the visual inputs. We also introduced an addition to the learning algorithm that utilizes the structure of spRNN's representational manifold - the intrinsic reward. This reward is positive when spRNN activity gets closer to the memorized activity at the goal location and negative otherwise. With intrinsic rewards, agents achieved learning speeds faster than those with full observability of the environment. Together, our results indicate that hippocampal cognitive maps provide informative representations of navigational tasks features and a valuable metric to act as a learning signal in such tasks.

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Poster

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Topic: H.09. Spatial Navigation

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Title: Sharp waves of the head-direction system

Authors: *S. SKROMNE CARRASCO[1], G. VIEJO[3], R. MANNARELLI[4], Y. WANG[5], A. J. DUSZKIEWICZ[6], A. PEYRACHE[2];
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Abstract: The head-direction (HD) system is an essential component of the spatial navigation system and is believed to provide key signals during sleep to support memory formation in the downstream parahippocampal regions. The HD signal is processed by a mammillary-thalamic-subicular loop within the Papez circuits. Specifically, the lateral mammillary nucleus (LMN) receives an angular velocity signal from the brainstem and is believed to form an attractor network supporting the generation of the HD signal. By recording from ensembles of LMN neurons in freely moving mice during wakefulness and sleep, we reveal the existence of a brief sharp wave (<20ms) generated in the LMN. These “HD-waves” occurred mainly, but not only, during sleep at an average occurrence rate of about 1Hz. It recruited strong firing within the LMN and activated neurons in the thalamic and subicular stages of the HD system. During non-Rapid Eye Movement sleep, it was negatively coupled with hippocampal sharp waves-ripples (SWRs), suggesting that HD-waves and SWRs are orchestrated by antagonist neuromodulatory systems. Finally, HD-waves can be triggered by auditory stimuli during both wake and sleep.Overall, like SWRs that emerge from recurrent connectivity within the CA3 area of the hippocampus, HD-waves provide further evidence for the existence of an attractor network in the LMN. These findings also suggest that the HD circuit generates strong population bursts with potential implication for sleep-dependent memory formation.

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Poster

PSTR190: Grid Cells and Spatially Modulated Cells I

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Title: Long-term spatial representations in the retrosplenial cortex

Authors: *N. CHAHINE[1], A. BERGEL[1], Y. WANG[1], A. PEYRACHE[2];
[2]Montreal Neurolog. Inst., [1]McGill Univ., Montreal, QC, Canada

Abstract: Mammals form long-term memories of their spatial surroundings, allowing them to successfully navigate and survive without re-learning their environment at every visit. The retrosplenial cortex (RSC) is believed to be a crucial node of the brain network supporting spatial memories. Specifically, it occupies a central anatomical position for spatial cognitive processing, receiving direct inputs from the hippocampus, the visual system, and the head-direction (HD) system. Hence, it is an ideal candidate structure to support long-term spatial memories, yet the underlying neuronal correlates of these memories are still largely unclear. Here, we longitudinally tracked the activity of neuronal ensembles in the granular RSC (gRSC) of freely moving rats. To this end, we transfected gRSC neurons with the genetically encoded calcium indicator GCAmP6s and used portable miniscopes to monitor calcium levels as a proxy of neuronal activity. We further asked the question of how a neuronal representation of an environment was affected by small changes in spatial layout. To address this issue, some details of the environment (i.e. color and shape of objects) were frequently changed. We first focused our analysis on the HD signal, an essential component of spatial navigation, which can be tracked and decoded in neuronal ensembles of the brain's navigation system. Although individual neurons were only loosely modulated by HD, gRSC neuronal ensembles accurately coded for animal's HD in both allocentric and egocentric coordinates. Furthermore, the allocentric code was stable across days and independent of environment manipulation, whereas the egocentric code was stable within environments. In conclusion, these findings show how the RSC builds a stable representation of spatial features, allowing animals to form long-term memories of their environments.

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Poster

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Title: Altered hippocampal sharp-wave ripples contribute to impaired spatial memory in a mouse model of Christianson Syndrome

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[1]Integrated Program in Neurosci., Montreal Neurolog. Inst., Montreal, QC, Canada; [2]Dept. of Pharmacol. and Therapeut., [3]Montreal Neurolog. Inst., McGill Univ., Montreal, QC, Canada

Abstract: Christianson syndrome (CS) is a monogenic condition involving X-linked intellectual disability and sleep disturbances. CS arises from mutations in the *SLC9A6* gene which encodes

the endosomal pH (Na^+, K^+)/H^+ exchanger isoform 6 (NHE6). NHE6 regulates the pH of intracellular membrane vesicles to facilitate cargo trafficking needed for proper function and development of neuronal circuitry. However, the relation between loss of NHE6, sleep disturbances and cognitive deficits is currently unknown. The hippocampus is the seat of long-term memory formation and spatial cognition in the brain. During sleep, hippocampal place cells replay previously encountered spatial trajectories **associated with population bursts orchestrated by "sharp wave-ripples" (SWRs)**, which are crucial for **memory consolidation.** In a CS murine model, we found impaired spatial cognition with seemingly unaffected place cell activity. However, we observed increased ripple rates in CS. In addition, we found decreased power in the ripple band, but increased gamma power in CS across brain states. Interestingly, increased gamma power is also associated with altered theta-gamma coupling in CS. These findings suggest that CS is indeed a disorder of impaired oscillatory activity in the brain, with potential implications for memory consolidation. Furthermore, preliminary analysis of single unit activity reveals that when animals are introduced into a new environment, hippocampal remapping is altered compared to WT mice, suggesting that while spatial representations remain intact in CS, deficits in pattern separation could lead to impaired spatial cognition. Our results provide novel insights into understanding impaired cognition during sleep in CS, which in turn will contribute to further characterization of the disease and future therapeutic interventions.

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Poster

PSTR190: Grid Cells and Spatially Modulated Cells I

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Topic: H.09. Spatial Navigation

Title: Abrupt changes in head-direction cell dynamics as a marker of the transition from wakefulness to sleep

Authors: *Y. WANG;
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Abstract: As the body transitions from wakefulness to sleep, the electroencephalogram (EEG) shows major changes. Yet, specific markers precisely determining this transition during the sleep onset process (SOP) are still missing. Here, we have investigated the neuronal markers of SOP based on the neuronal dynamics of the anterior thalamic nucleus in mice. Specifically, we investigated how this transition correspond to changes in the dynamics of head-direction (HD) cells in the anterodorsal nucleus of the thalamus. The HD system is a circuit involved in the navigation system of the mammalian brain, with the anterior dorsal nucleus of the thalamus being a central hub of this circuit. During sleep, the HD cell population fires coherently relative to wake, that is a direction can be accurately decoded at any time, but codes for a randomly

drifting direction. We made the hypothesis that the exact moment when the HD cell population stops coding for the animal's actual direction is a marker of the SOP. We show that this change in dynamics correspond to decrease of electromyogram (EMG) signals. Furthermore, by simultaneously recording neuronal dynamics in the hippocampus, a brain structure showing some of the largest changes in neuronal dynamics between wakefulness and sleep, we found that HD cell drift was progressively coupled to hippocampal dynamics during SOP. In conclusion, our findings provide an operational definition of sleep onset in mammals, opening new avenues for the characterization of the physiological and cognitive processes at play at the transition between wakefulness and sleep.

Disclosures: Y. Wang: None.

Poster

PSTR190: Grid Cells and Spatially Modulated Cells I

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Topic: H.09. Spatial Navigation

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Title: Consistent coordination between medial entorhinal cortex and thalamic head direction cells during wake and sleep

Authors: *G. R. VITE[1], Q. DING[2], A. PEYRACHE[3];
[1]MNI, McGill Univ., Montreal, QC, Canada; [2]McGill Univ., Montreal, QC, Canada; [3]Montreal Neurolog. Inst., McGill Univ., Montreal, QC, Canada

Abstract: Successful navigation requires the generation of signals that remain consistent, regardless of environmental changes. This can be achieved by constraining neuronal activity to low-dimensional subspaces that map spatial aspects of an animal's behavior. Notably, during sleep—a period of diminished external input—pairwise coordination persists within different regions of the spatial navigation system. This is particularly evident in grid cells in the medial entorhinal cortex (MEC) and head-direction (HD) cells in the anterodorsal nucleus (ADn) of the thalamus, supporting the notion that network activity remains organized across brain states. Given the essential role of the ADn in grid cell and HD cell representations, we hypothesized that the organization of neuronal activity within the MEC depends on coherent HD signal input. To investigate this, we conducted simultaneous electrophysiological recordings in the ADn and MEC during wakefulness and sleep. Our results demonstrate that the coordination between HD cell pairs in the ADn and MEC is maintained post-environmental changes, as shown in a cue rotation experiment. Furthermore, the angular offset of preferred directions in ADn-MEC HD cell pairs predicts pairwise correlations during sleep. Additionally, the coordination of MEC HD cell pairs appears to be partially driven by common inputs from thalamic HD cells. In

conclusion, our findings indicate that organized activity in the MEC is, at least in part, regulated by coherent HD signals from the ADn across various brain states.

Disclosures: G.R. Vite: None. **Q. Ding:** None. **A. Peyrache:** None.

Poster

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FRQNT Strategic Clusters Program (2020- RS4-265502 - Centre UNIQUE - Union Neurosciences & Artificial Intelligence - Quebec)
Richard and Edith Strauss Postdoctoral Fellowship in Medicine

Title: Sequential predictive learning is a unifying theory for hippocampal representation and replay

Authors: *D. LEVENSTEIN[1], A. EFREMOV[1], R. HENHA EYONO[1], B. A. RICHARDS[2], A. PEYRACHE[3];
[1]McGill Univ., Montreal, QC, Canada; [2]Neurol. and Neurosurg., McGill Univ., Montreal, QC, Canada; [3]Montreal Neurolog. Inst., McGill Univ., Montreal, QC, Canada

Abstract: The mammalian hippocampus contains a cognitive map that represents an animal's position in the environment and generates offline "replay" for the purposes of recall planning, and forming long term memories. Recently, it's been found that artificial neural networks trained to predict sensory inputs develop spatially tuned cells, aligning with predictive theories of hippocampal function. However, whether predictive learning can also account for the ability to produce offline replay is unknown. Here, we find that spatially tuned cells, which robustly emerge from all forms of predictive learning, do not guarantee the presence of a cognitive map with the ability to generate replay. Offline simulations only emerged in networks that used recurrent connections and head-direction information to predict multi-step observation sequences, which promoted the formation of a continuous attractor reflecting the geometry of the environment. These offline trajectories were able to show wake-like statistics, autonomously replay recently experienced locations, and could be directed by a virtual head direction signal.

Further, we found that networks trained to make cyclical predictions of future observation sequences were able to rapidly learn a cognitive map and produced sweeping representations of future positions reminiscent of hippocampal theta sweeps. These results demonstrate how hippocampal-like representation and replay can emerge in neural networks engaged in predictive learning, and suggest that hippocampal theta sequences reflect a circuit that implements a data-efficient algorithm for sequential predictive learning. Together, this framework provides a unifying theory for hippocampal functions and hippocampal-inspired approaches to artificial intelligence.

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Poster

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Support: SFARI Autism Rat Models Consortium grant 903332

Title: Impaired multisensory integration within the head-direction circuit in a rat model of Fragile X Syndrome

Authors: *A. J. DUSZKIEWICZ[1], A. RÅSTEDT[2], A. VADHER[3], E. R. WOOD[2], A. PEYRACHE[4], P. A. DUDCHENKO[1];
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Abstract: Fragile X Syndrome (FXS) is the most common monogenic form of autism spectrum disorder (ASD), caused by a loss-of-function mutation in the *FMR1* gene. ASD is a neurodevelopmental condition characterized by aberrations in sensory processing, such as hypersensitivity to sensory stimuli and impaired multisensory integration. While research using rodent models of ASD has greatly enhanced our understanding of the neural basis of sensory hypersensitivity, the systems-level causes of multisensory integration deficits associated with ASD are still unknown. Mammalian brains make use of multisensory integration to create representations of space and the head-direction (HD) signal is one of the fundamental building blocks of spatial representations. HD cells are each tuned to a specific direction faced by the animal, and as a network they form the basis of the internal sense of orientation. During reorientation, the HD signal relies on weighted integration of two main sensory streams: vestibular signals conveying angular head velocity (AHV) and visual information about distal landmarks. We leveraged the experimental tractability of the HD signal to quantify how AHV

and vision integrate to form a stable representation of current direction in a rat model of FXS. To that end, we used silicon probes to simultaneously record populations of neurons in two cortical nodes of the HD system involved in vestibulo-visual integration, postsubiculum (PoSub, 30-115 cells per session) and retrosplenial cortex (RSC, 52-171 cells per session), in adult *Fmr1*$^{-/y}$ rats (n = 7) and their wild-type (WT) littermates (n = 8). We quantified the relative influence of vestibular and visual information on PoSub-HD cell tuning using a visual-vestibular mismatch task in which animals explored an elevated platform while a prominent visual landmark was displayed on a 360-degree LED screen. When the landmark was rotated by 45 or 90 degrees, HD cells in WT rats only partially realigned their receptive fields towards the landmark - a hallmark of weighted integration of visual and vestibular information. In contrast, rotation of HD cell receptive fields in *Fmr1*$^{-/y}$ rats precisely matched the rotation of the landmark, indicating that vestibular information was not integrated during reorientation. This phenotype was associated with decreased tuning of individual cells to AHV in both PoSub and RSC as well as higher drift of the HD signal in darkness, underscoring the lack of functional integration of AHV information into the cortical HD system in *Fmr1*$^{-/y}$ rats. Further experiments will determine whether this bias translates to impairments in behavioural tasks that critically rely on self-motion signals.

Disclosures: A.J. Duszkiewicz: None. **A. Råstedt:** None. **A. Vadher:** None. **E.R. Wood:** None. **A. Peyrache:** None. **P.A. Dudchenko:** None.

Poster

PSTR190: Grid Cells and Spatially Modulated Cells I

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR190.23/V33

Topic: H.09. Spatial Navigation

Support: SFARI Autism Rat Models Consortium grant 903332

Title: Characterization of visual and self-motion cue-based navigation in a rat model of Fragile X Syndrome

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Abstract: Fragile X Syndrome (FXS) is a common form of inherited intellectual disability caused by silencing of the Fragile X messenger ribonucleoprotein 1 (*FMR1*) gene, resulting in a loss of its protein product. FXS has a high comorbidity with autism spectrum disorder (ASD), a neurodevelopmental condition whose symptoms include sensory hypersensitivity and impaired multisensory integration. Still, how such sensory deficits in ASD affect complex behaviours that critically rely on a particular sensory stream is unclear. Mammalian brains utilise multisensory

integration to create spatial representations and solve navigational problems. One such example is the head direction (HD) circuit consisting of HD cells that are selectively active when the animal is facing the preferred direction of the cell. The HD circuit integrates two main sensory streams, the visual (external) from landmarks and the vestibular (internal) from angular head velocity, to maintain or reorient the directional preference of HD cells. Recent experiments in our laboratory show that *Fmr1*$^{-/y}$ rats (rat model of FXS) differ from wild-type (WT) rats in how they integrate visual and vestibular (self-motion) inputs in the HD circuit. Specifically, HD circuit in *Fmr1*$^{-/y}$ rats exhibits impaired integration of self-motion inputs with more weight given to visual inputs. Based on these findings, we expect *Fmr1*$^{-/y}$ rats to be capable of landmark navigation but impaired at self-motion based navigation. Thus, we aimed to test *Fmr1*$^{-/y}$ rats on the two types of navigation by using a cue-controlled spatial reference memory water maze task for landmark navigation, and a path integration (PI) task for self-motion based navigation. For the water maze experiment, we used a curtained circular pool divided into eight wedges (octants) by submerged barriers. Positions of distal landmarks and the submerged platform were changed daily while maintaining their relative orientation. As predicted, *Fmr1*$^{-/y}$ rats (n=8) performed just as well as WT rats (n=8) in unrewarded probe trials by showing a preference for the octant in which the platform was predicted to be relative to the visual landmarks . We are now in the process of testing *Fmr1*$^{-/y}$ rats on a PI task using a variant of homing task (Najafian et al, Nat Commun 14:7373, 2023) in which the rats are trained to look for and press a lever on a circular arena and are rewarded with a food pellet in the home box located on the periphery of the arena. After being trained in the light condition, we will test the rats in darkness to test their PI ability. Future recording experiments will also assess the relationship between PI performance and the drift in the HD signal.

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Poster

PSTR191: Navigation Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.01/V34

Topic: H.09. Spatial Navigation

Title: Neurobiology of navigation in the real world: Head-direction cells serve as a neural compass in bats navigating outdoors on a remote oceanic island

Authors: *S. PALGI, S. RAY, S. R. MAIMON, T. ELIAV, A. TUVAL, C. COHEN, L. LAS, N. ULANOVSKY;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Navigation is crucial for both animals and humans. Historically, studies of navigation followed two distinct approaches: On one hand, ethologists and ecologists tracked animals navigating in the wild, focusing on sensory cues and navigational strategies. On the other hand,

neuroscientists studied neural activity in animals moving indoors in small laboratory enclosures, and discovered neurons involved in navigation such as place cells, grid cells, and head-direction cells. While these neurons have been studied extensively indoors, they were never recorded during real-world navigation, outdoors. To bridge this gap, we conducted the first study of neurons in the brain's "navigation circuit" during outdoors navigation. We focused on head-direction cells, which represent the animal's orientation, often referred to as "neural compasses". However, it is unclear whether head-direction cells function as local compasses, which remap between environments, or as global compasses, which maintain a stable representation over time and space. Experiments in laboratory setups were inconclusive, with some supporting the local-compass hypothesis - where the head-direction tuning rotates when the animal is passively moved to a new enclosure, or when a cue card is rotated or removed; while other studies supported the global-compass hypothesis, by showing that when an animal is actively moving between two enclosures - and can use path integration mechanisms - head-direction cells maintain their tuning. The ultimate test of these hypotheses would be during real-world navigation outdoors - where these hypotheses have substantially different predictions. To this end, we developed methods for wireless electrophysiology and high-accuracy positional tracking, and recorded hundreds of single neurons in the presubiculum - a key hub of head-direction cells - while bats were navigating outdoors on a remote oceanic island. Results showed that, first, head-direction cells were abundant in outdoors setting. Second, these neurons showed remarkably stable directional tuning across the island's geography, suggesting that they are anchored globally and not locally. Third, we tested the stability of head-direction cells to the natural dynamics of celestial cues - such as the moon - which are the most prominent distal visual cues outdoors: we found that the cells maintained the same preferred direction regardless of the moon's appearance, disappearance, or rotation. Together, these first ever single-unit recordings in animals navigating outdoors suggest that head-direction cells maintain a global sense of direction, and can serve as the brain's global neural compass.

Disclosures: S. Palgi: None. **S. Ray:** None. **S.R. Maimon:** None. **T. Eliav:** None. **A. Tuval:** None. **C. Cohen:** None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

PSTR191: Navigation Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.02/V35

Topic: H.09. Spatial Navigation

Support: ERC
NIH R01
NSF-BSF CRCNS

Title: Sparse versus dense coding of very large environments in hippocampal subregions CA3 and CA1

Authors: *S. R. MAIMON, T. ELIAV, L. LAS, N. ULANOVSKY;
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Abstract: The hippocampus is comprised of distinct subregions, including areas CA1 and CA3, which differ markedly in their anatomical connectivity. CA1 is largely a feedforward network with almost no intrinsic excitatory connections, whereas CA3 is a recurrent network of densely interconnected pyramidal neurons - which also project to CA1. While the anatomical connectivity differs substantially between these two subregions, the basic firing properties of spatially-modulated place cells in CA1 and CA3 were found by previous studies to be surprisingly similar, consisting mainly of single place-fields with similar field-sizes and spatial information. We hypothesized here that the similar coding properties reported for CA1 and CA3 neurons stem from the small laboratory environment sizes that were used - and that perhaps under more naturalistic spatial scales of hundreds of meters or kilometers the coding schemes of these two areas might differ. In our previously published paper (Eliav*, Maimon* et al., Science 2021) we showed that CA1 place cells recorded in bats flying in a 200-meter long tunnel, exhibit multiple place fields with very different field-sizes for different fields. This multifield multiscale spatial code is fundamentally different from the single fields observed in small laboratory boxes. Here we compared these findings from CA1 to place cells in CA3 that we recorded in bats flying in the same long tunnel. These analyses revealed a dramatic difference between these two subregions: Unlike CA1 place-cells, CA3 place-cells mostly had only single place-fields; however, the sizes of individual place-fields were similar between the two areas. Consequently, the spatial information was significantly higher for place-cells in CA3 versus CA1. These results suggest a fundamental functional difference in neural-coding between these two anatomical subregions of the hippocampus: Sparse coding in CA3 versus dense coding in CA1.

Disclosures: S.R. Maimon: None. **T. Eliav:** None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

PSTR191: Navigation Circuits

Location: MCP Hall A

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Program #/Poster #: PSTR191.03/V36

Topic: H.09. Spatial Navigation

Title: Neurobiology of navigation in the real world: Hippocampal spatial codes in bats navigating outdoors on a remote oceanic island

Authors: *S. RAY, S. PALGI, S. R. MAIMON, T. ELIAV, A. TUVAL, C. COHEN, L. LAS, N. ULANOVSKY;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Animal brains and their behaviours have evolved in the natural world - to enable them to meet the challenges they face during their daily lives. However, our understanding of how the brain represents the world, stems from experiments performed in constrained and impoverished

laboratory settings. While laboratory-based experiments reveal what neurons can encode, they leave the fundamental question unexplored - how does the brain actually represent the real world? To tackle this challenge, we performed the first single-unit neural recordings from freely flying bats as they navigated outdoors on a remote oceanic island. To achieve this, we developed a wireless neural-logger with an integrated high-precision GPS and altimeter - allowing us to record electrophysiological activity from hundreds of single neurons, together with the bat's position and altitude in the real world. We recorded dorsal CA1 hippocampal place cells - neurons that are known to represent an animal's location in small environments and in enclosed large one-dimensional (1D) tunnels. We found that as the bats navigated the island, hippocampal neurons exhibited place fields in three-dimensions (3D), with different neurons representing different locations - as expected from place cells. Single place cells had multiple place-fields in 3D. The unconstrained bats flew at different altitudes over the island, and preliminary results indicate that different place-fields of single neurons encode distinct altitudes. Here we will describe these and other findings from a large dataset that we recently recorded from bats flying outdoors - providing the first view on spatial coding in the mammalian hippocampus during real-world navigation.

Disclosures: S. Ray: None. **S. Palgi:** None. **S.R. Maimon:** None. **T. Eliav:** None. **A. Tuval:** None. **C. Cohen:** None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

PSTR191: Navigation Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.04/V37

Topic: H.09. Spatial Navigation

Title: Action-specific spatial coding in the medial entorhinal cortex of flying bats

Authors: *G. GINOSAR[1], D. MCNAMEE[2], L. LAS[1], N. ULANOVSKY[1];
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Abstract: Medial entorhinal cortex (MEC) contains a diversity of spatially-tuned cells. These cells are typically studied in animals randomly foraging for food. However, real-world behaviors go beyond random-foraging, and it remains unclear how the animal's actions affect spatial coding. Here we recorded from MEC of flying bats as they either randomly foraged for food, or engaged in two distinct actions: takeoff and landing. Bats flew in a large flight-room containing 6-11 identical rest-platforms at various locations. A substantial fraction of cells in deep layers of MEC fired at specific locations, near specific platforms, but only under specific actions - during landing or takeoff from the platform, but not when flying through the same location in random-foraging. Thus, these neurons exhibited action-specific spatial coding. We show that in the reinforcement-learning framework, while grid cells provide a low-dimensional basis of space under random foraging, a signal encoding position-by-action, as found here, provides a low-dimensional basis of space under directed action.

Disclosures: G. Ginosar: None. **D. McNamee:** None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

PSTR191: Navigation Circuits

Location: MCP Hall A

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Topic: H.09. Spatial Navigation

Support: NDSEG Fellowship
NSF Grant IIS1718991
NIH Grant R01-EB026955

Title: Phase precession and randomized place ensembles in hippocampus as potential signatures of variable binding

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Abstract: It is widely believed that the hippocampal formation encodes a cognitive map, affording a structured representation of the environment that supports flexible behavior. A core feature in forming these representations is variable binding, such as associations between contents and contexts. Neuroanatomical projections and experimental data support the hypothesis that the hippocampus combines inputs from lateral and medial entorhinal cortex, respectively, but it is unknown how this combination is realized at the level of neural circuits and whether it is a form of binding.

To trace potential hallmarks of variable binding in hippocampus, we propose an algebraic, full-stack framework for forming a cognitive map with neural population codes. The framework is algebraic in the sense that it is equipped with additive operations for forming sets and multiplicative operations for variable binding. It is full stack because it proposes how these operations can be realized by phase coding in a spiking neural network with oscillations. It extends prior work in cognitive science known as holographic reduced representations (HRR), which provided foundations for developing compositional representations with randomized high-dimensional vectors. However, our modeling approach goes significantly beyond HRR by proposing a realization with sparse activity patterns (spike timing codes) and locally connected circuits.

After building the cognitive map model, we then compare it to experimental data. Specifically, we perform simulations of animals moving in open fields constrained by observed statistics of rodents. We analyze the single cell receptive fields of the model, often finding spatially localized place fields. We ask to what extent individual units exhibit phase precession. We then quantitatively compare our model outputs to those of existing computational models of phase precession, as well as to datasets of neural recordings from CA1 and CA3. In addition, we evaluate the predictions of the model for remapping in the hippocampus, finding that binding operations can serve as a mechanism for randomly selecting which neural ensembles are

recruited for specific places in different environments. Finally, we discuss biophysical signatures and experimental predictions that would further validate the model.

Disclosures: C.J. Kymn: None. **E.P. Frady:** A. Employment/Salary (full or part-time):; Intel Labs. **F.T. Sommer:** A. Employment/Salary (full or part-time):; Intel Labs.

Poster

PSTR191: Navigation Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.06/W1

Topic: H.09. Spatial Navigation

Support: ERC Synergy Grant 951319
Gatsby Charitable Foundation

Title: Common mathematical principles underlie place field properties across species and geometries

Authors: *N. MAINALI[1], R. A. DA SILVEIRA[2,3,4,5], Y. BURAK[6,7];
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Abstract: According to the classical view of spatial coding in the hippocampus, place cells express unimodal firing fields with a stereotyped, bell-shaped profile. Recent recordings from area CA1, however, reveal that this picture breaks down in large environments. Place cells in large environments typically fire in multiple locations. Furthermore, the multiple firing fields of individual cells, as well as those of the whole population, vary in shape and size, often deviating substantially from the classical bell-shaped form. Here, we report that a surprisingly simple mathematical model, in which firing fields are generated by thresholding a realization of a random Gaussian process, explains in quantitative detail a wide range of statistics of the observed place fields. The model simultaneously provides excellent fits to the distribution of field sizes, the distribution of inter-field distances, and the number of fields per cell, in several data sets that differ in the species and the dimensionality of the environment: from bats and rodents, in 1d, 2d, and 3d enclosures. In addition, the model makes testable quantitative predictions on the statistics of field shapes - the distribution of the number of local maxima within a field and the joint distribution of a field's width and its peak firing rate - as well as on topological properties of the fields. These predictions are all borne out when checked against experimental data, without any refitting of the parameters inferred from fitting field sizes. The description of place fields in terms of threshold crossings of a Gaussian process is

phenomenological, yet it is suggestive of a mechanistic interpretation. Gaussian processes naturally arise when many statistically independent spatial fields are summed. Thus, the model's successful explanation of the place field statistics is consistent with a picture in which synaptic weights associated with projections into CA1 from its input regions are predominantly random.

Disclosures: N. Mainali: None. **R.A. Da Silveira:** None. **Y. Burak:** None.

Poster

PSTR191: Navigation Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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Topic: H.09. Spatial Navigation

Support: UTSA Start-Up Funds

Title: Factors favoring off-track sampling behavior by rats running on linear tracks

Authors: *P. J. DAVIS, S. T. JONES, R. J. BENAVIDEZ, E. T. LE, F. SAVELLI;
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Abstract: Place cell studies typically rely on over-trained, uninterrupted animal foraging. A remarkable case of spontaneous attentive behavior linked to place field potentiation is "head-scanning" (Monaco et al., Nat. Neurosci., 2014). This phenomenon was observed on circular/hexagonal tracks, and it was hypothesized that the ballistic character of trajectories followed by rats rewarded at the two ends of linear tracks might reduce its occurrence. We explored conditions influencing off-track scanning on a 2.5 m long linear track, using cue manipulation (CM) and room change (RC) paradigms. Eleven Long-Evans rats (5M/6F) were trained to collect food rewards either throughout the track (n=7) or only at its ends (n=4) in one of two room conditions (Room A/B: n=6/5). After 7-21 days of training, rats ran 3 standard (STD1/2/3) sessions of 30 laps each for 3 reference (REF) days before testing (TEST) for 3 CM days followed by 3 RC days, in which the middle STD2 session was replaced by a CM2 or RC2 session. We defined scanning as the rat's ears midpoint moving beyond the track boundaries by >2.5 cm for >400 ms, with scanning events within 400 ms merged into a single event. We excluded scanning events occurring at the two ends of the track. Both groups of rats, rewarded throughout the track or at its ends, scanned on the REF days, although the latter group scanned significantly less (scan count per session; mean +/- SEM: 20.29 +/- 2.03 and 6.25 +/- 0.67, respectively; $t(93)=4.85$, $p<<0.001$). Scanning decreased from the first to second session of REF days (scan count difference STD2-STD1, mean +/- SEM: -4.58 +/- 1.55). Our CM paradigm did not overcome this tendency (scan count difference CM2-STD1, mean +/- SEM: -3.96 +/- 1.49; REF vs. CM; $t(57)=-0.29$, $p=0.78$). In contrast, scanning increased during RC sessions (scan count difference RC2-STD1, mean +/- SEM: 14.96 +/- 3.03; REF vs. RC, $t(55)=-5.89$, $p<<0.001$). These findings remain significant when restricted by food reward condition (track

ends, throughout). We also directly compared the mean scan count during the second sessions of REF and TEST days. There were significantly more scans in RC2 than in STD2 or CM2 (scan count per session, mean+/- SEM; RC2: 28.42 +/- 4.16; STD2: 13.4 +/- 3.55; CM2: 12.55 +/- 2.57; paired t-test by rat, STD2 vs. RC2: t(9)=-3.14, p=0.012; CM2 vs. RC2: t(9)=-4.24, p=0.002). Our results imply that head-scanning could exert more influence on place cells in classic experimental apparatus comprising linear segments than is currently appreciated. Further, the use of a 2.5 m track could be leveraged to test non-local effects of head-scanning on place cells producing multiple firing locations.
(PJD and STJ contributed equally)

Disclosures: P.J. Davis: None. **S.T. Jones:** None. **R.J. Benavidez:** None. **E.T. Le:** None. **F. Savelli:** None.

Poster

PSTR191: Navigation Circuits

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Program #/Poster #: PSTR191.08/W3

Topic: H.09. Spatial Navigation

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Pratiksha trust grant PE/CHAIR-19-025.03
Shiv Nadar Institution of Eminence intramural funding

Title: Task demands and structure-function relationships in the hippocampal formation

Authors: *S. DESHMUKH[1,2], A. BISHNOI[3], I. R. JAKHALEKAR[4];
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Abstract: Neural function is often predicted based on structural connectivity. While such predictions have regularly been successfully tested experimentally, such tests often underplay the influence of task demands and their interaction with structure on function. We demonstrate using multiple experimental and modelling paradigms that the neural function can change drastically in response to changing task demands and relative strengths of different inputs. Proximal CA1 (pCA1) receives stronger projections from the medial entorhinal cortex, while distal CA1 (dCA1) receives stronger inputs from the lateral entorhinal cortex. This led to the hypothesis that pCA1 is more spatially selective than dCA1, which was confirmed in multiple experiments (Henriksen et al., 2010, Oliva et al., 2016, Ng et al., 2018). Theta modulation and phase precession were also shown to be stronger in pCA1 than dCA1 (Henriksen et al., 2010, Oliva et al., 2016). We demonstrate an experimental paradigm in which dCA1 is spatially as selective as pCA1 and responds with higher coherence to cue manipulations than pCA1 (Deshmukh, 2021). In this paradigm, dCA1 place cells show theta modulation and phase precession comparable to

pCA1 place cells, and dAC1 shows higher theta power in LFPs than pCA1 (Bishnoi and Deshmukh, 2023, bioRxiv). This finding raises a question of what inputs contribute to theta modulation and phase precession. We modify a model of theta phase precession by Chadwick et al. (2016) to show that increase in non-theta non-spatially modulated inputs can transform non theta phase precessing cells into precessing cells. Similarly, representation of 2D space in CA1 is altered based on the spatial scale of the arena. Typical experimental arenas for spatial navigation are limited to sizes (~ $1m^2$) substantially smaller than the sizes of the home ranges of rats (Taylor, 1978, Calhoun, 1936). There has been a recent interest in testing representations of space in larger arenas studies (Harland et al, 2021, Tanni et al, 2022). We demonstrate a systematic change in spatial representation strategies in dorsal CA1 as a function of spatial scale using arenas of varying areas (0.23 m^2, 1 m^2, 2.16 m^2, 4.7 m^2, 8.8 m^2, 17.2 m^2). Together, these findings demonstrate that task demands and corresponding changes in relative strengths of inputs can drastically alter the hippocampal functional output. Thus, while structural connectivity sets absolute boundaries on the range of possible functional outputs, actual output cannot be predicted from structure without regard to the task demands and other experimental variables.

Disclosures: S. Deshmukh: None. **A. Bishnoi:** None. **I.R. Jakhalekar:** None.

Poster

PSTR191: Navigation Circuits

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Program #/Poster #: PSTR191.09/W4

Topic: H.09. Spatial Navigation

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Shiv Nadar Institution of Eminence intramural funding

Title: Contribution of non-theta non-spatially modulated inputs to the shape of theta phase precession

Authors: *A. BISHNOI[1], S. S. DESHMUKH[2,3];
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Abstract: Theta phase precessing cells maintain a temporal code of space by firing at earlier phases of theta oscillations (5-10 Hz) as the animal traverses through the neuron's place field. Theta phase precession accelerates with position when averaged across dorsal CA1 place cells. Models of the interaction of spatial excitation and theta inhibition can successfully generate phase precession (Castro & Aguiar, 2012; Seenivasan & Narayanan, 2020; Jaramillo et al., 2014), but the simulated dynamics are typically decelerating, i.e., the rate of change of phase slows down towards the end of the place field. Decelerating phase precession has not been previously reported experimentally. In this study, we show that non-theta non-spatially (NTNS)

modulated inputs simulated as Poisson noise can contribute to theta-modulated spiking dynamics as seen in the brain. We modify the Chadwick et al. (2016) E-I circuit model of theta phase precession and demonstrate phase locking, phase precession and theta phase skipping generated by systematically varying the strength of the three input sources to the model - spatially modulated, theta oscillations, and NTNS excitation, along with the asymmetry of spatial input. Of the 13328 combinations of the four input parameters tested, 3712 combinations generated phase locking and 969 combinations generated theta phase precession (254 decelerating, 556 linear and 159 accelerating). Increasing the strength of NTNS inputs reduced the tendency of the model to generate decelerating precession and generated linear / accelerating precession. Increasing the negative skewness of the spatial input and the strength of NTNS inputs increased the tendency of the model to generate accelerating precession. For comparison, decelerating precession was observed in a small minority of 291 dorsal CA1 place cells (Deshmukh, 2021; Bishnoi and Deshmukh, 2023), with accelerating (n = 113 cells) and linear (n=110) phase precession being more prominent than decelerating (n= 36) and phase locked (n = 32) cells. This difference in the propensity of theta phase precession to decelerate between the experimental data (accelerate ≈ linear > decelerate ≈ phase locked) and the model (phase locked > linear > decelerate > accelerate) suggests that the combinations of the input parameters in the real neurons occupy a small fraction of the parameter space explored in the model. A subset of combinations with non-zero NTNS inputs (45 combinations showing theta phase precession, 214 phase locked) also exhibited theta phase skipping. Our study demonstrates the importance of NTNS inputs for generating biologically realistic theta phase precession.

Disclosures: A. Bishnoi: None. **S.S. Deshmukh:** None.

Poster

PSTR191: Navigation Circuits

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Program #/Poster #: PSTR191.10/W5

Topic: H.09. Spatial Navigation

Support: India Alliance Grant IA/S/13/2/501024
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Shiv Nadar Institution of Eminence intramural funding

Title: Spatial selectivity and multiscalarity of dorsal CA1 neurons increase with increasing spatial scale.

Authors: *I. R. JAKHALEKAR[1], S. DESHMUKH[3,2];
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Abstract: Neural correlates of space in rodents have mainly been studied in arenas that are much smaller than the typical burrow systems and home ranges of the rats in the wild (Taylor, 1978,

Calhoun, 1936) with the exception of a few studies (Harland et al, 2021, Tanni et al, 2022). Additionally, a systematic study of the effect of arena size on spatial representations is missing. We made 2 rats forage in arenas of varying sizes and shapes and recorded 115 dorsal CA1 units from 0.23 m^2 hexagon, 138 units from 1 m^2 square, 121 units from 2.16 m^2 rectangle, 220 units from 4.7 m^2 circle, 263 units from 8.8 m^2 square, 245 units from a 17.2 m^2 rectangle. We observed that the number of place fields per cell increased with arena size (linear regression, $R^2 = 0.39$, $p= 10^{-117}$, slope= 0.44). The median of place field size distribution in 2 largest arenas i.e. 8.8 m^2 square and 17.2 m^2 rectangle, was smaller than the median of place field size distributions in all arenas smaller than them. The fraction of the arena covered by all the place fields of a cell decreased with increasing arena size (linear regression, $R^2 = 0.133$, $p =10^{-35}$, slope = -0.01). Consistent with this, the spatial information scores increased with the arena scale (linear regression, $R^2 = 0.067$, $p = 10^{-18}$, slope = 0.034). Thus, the larger arenas had higher resolution of spatial representations than smaller arenas. Further, we observed that the place cells displayed multiscale representations in all the arenas of size 1 m^2 and above. Cells with more than one field showed a wide distribution of place field sizes, such that the largest place field of a cell was a number of times bigger than the smallest place field of the same cell. The ratio of maximum to minimum field size per cell increased with arena size. The median field size ratio increased from 5.75 in 1 m^2 to 16.75 in 17.2 m^2 arena. The linear regression of field size ratios with arena size was significant with $R^2 = 0.03$, $p =10^{-6}$ and slope =2.5. This increase in field ratio was accompanied by an increase in maximum field size per cell (linear regression, $R^2 = 0.02$, $p = 10^{-5}$, slope= 0.02) while the minimum field size per cell decreased with arena size (linear regression, $R^2 = 0.05$, $p = 10^{-11}$, slope = -0.002). A report in bats (Eliav et al 2021) observed multiscale representations in 200 m long tunnels but not in a 6m long tunnel. The study also demonstrated that multiscale representations are more efficient and have a higher capacity for encoding larger arenas. Thus, our results show the existence of an efficient encoding strategy in rat CA1 across 2D arenas of different scales that increases its spatial selectivity and multiscalarity with the arena size.

Disclosures: I.R. Jakhalekar: None. **S. Deshmukh:** None.

Poster

PSTR191: Navigation Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.11/W6

Topic: H.09. Spatial Navigation

Support: Deutsche Forschungsgemeinschaft projects YO177/7-1

Title: Disruption of integrative property of individual neurons selectively degrades spatial tuning without disrupting speed tuning in retrohippocampal cells: a potential cellular mechanism of path integration

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Abstract: Grid cells and other spatially selective neurons are thought to integrate running velocity signals to keep track of an animal's position. This process is called path integration. However, cellular mechanisms of path integration have not been determined empirically. Here, we tested the hypothesis that individual cells' ability to integrate input over time through persistent spiking supports spatial tuning of neurons. Using in vitro patch clamp recording in the medial entorhinal cortex (MEC), we first demonstrate that optogenetic activation of cholinergic fibers combined with speed cell's activity enables neurons to integrate input over tens of seconds. We identified that TRPC4 channels, which are membrane cationic channels activated by cholinergic neuromodulation, support this property. We then conducted in vivo electrophysiological recordings from mice in which TRPC4 channels were knocked-down (KD) using an injection of shRNA-based KD virus to the MEC. TRPC4 KD significantly reduced spatial tuning of retrohippocampal neurons without disrupting velocity coding. These results suggest that integrative properties of individual neurons through TRPC4 channels may support path integration.

Disclosures: Y. Brahimi: None. **S. Calabrese:** None. **A. Reboreda:** None. **A. Dityatev:** None. **K. Allen:** None. **M. Yoshida:** None.

Poster

PSTR191: Navigation Circuits

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR191.12/W7

Topic: H.09. Spatial Navigation

Support: ESF, 2014–2020, project number ZS/2016/08/80645
DFG, YO177/4-1
DFG, YO177/4-3
DZNE

Title: Persistent firing in individual hippocampal cells supports spatial working memory and stable coding of space

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Abstract: Since the hypothesis of Donald Hebb, it has widely been believed that persistent spiking that underlies working memory is supported by the 'reverberating' activity in a recurrent synaptic network. On the other hand, it has been repeatedly indicated that individual neurons possess an ability to persist spiking for tens of seconds after a triggering stimulus offset, potentially serving as an element for active memory maintenance. However, whether such intrinsic neuronal properties underlie in vivo neural activity and contribute to memory function remains unclear. Here, we developed an in vivo model in which this ability of individual cells to support persistent firing was disrupted, using knockdown of TRPC4 channels selectively in the hippocampus. Using in vivo recordings in behaving mice, we demonstrate that this manipulation significantly decreases persistent firing and impairs working memory performance. We further demonstrate that this lack of persistent firing in the hippocampus reflects a reduction of consistent firing of place cells selectively when the animals were confined to the same place for extended duration of time. These data suggest that the cellular mechanism of persistent firing contributes to the maintenance of spatial representations and cognitive function, supporting the view that individual neurons may serve as an element of short-term memory maintenance.

Disclosures: A. Reboreda: None. **B. Saber Marouf:** None. **F.M. Theissen:** None. **R. Kaushik:** None. **M. Sauvage:** None. **A. Dityatev:** None. **M. Yoshida:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.01/W8

Topic: H.06. Social Cognition

Support: 20GC1018
22GC1004
JSPS KAKENHI 24K02374
the General Insurance Association of Japan
Inogashira Hospital Grants for Psychiatry Research

Title: The Impact of Brain Damage on Moral Judgment: Exploring Hypermorality

Authors: *M. SUGANAMI[1,3], Y. MIMURA[4], M. YAMAMOTO[5], M. MIMURA[2], T. MURAMATSU[6];
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[5]Dept. of Neurol., Yokohama Brain and Spine Ctr., Yokohama, Japan; [6]Keio Univ. Sch. Med., Tokyo 160-8582, Japan

Abstract: Human capacities for moral judgment and altruistic punishment are fundamental to social cooperation and are governed by broadly accepted, though unwritten, norms. Brain injuries can significantly alter these moral evaluations, often leading to a condition known as "hypermorality," where individuals exhibit excessive punitive behaviors. The precise behavioral pathophysiology of hypermorality, however, remains poorly understood. This study aims to explore the effects of brain damage on moral judgment, particularly focusing on hypermorality. We employed a modified third-party punishment method to assess moral judgments in 35 individuals with brain damage and 30 age-matched healthy controls. Participants evaluated the actions of characters in 40 carefully crafted vignettes, designed to provoke consistent responses across different scenarios. Our findings indicate that individuals with brain damage are more likely to attribute blame in situations where harmful intentions are absent, compared to healthy controls. Notably, these altered moral judgments in the brain-damaged group were not attributable to changes in empathy or emotional responses. This research highlights that brain damage may lead to disproportionately punitive responses to benign actions, offering new insights into the mechanisms of hypermorality. These findings have significant implications for enhancing the social functioning of individuals with brain injuries, providing a basis for targeted interventions.

Disclosures: M. Suganami: None. **Y. Mimura:** None. **M. Yamamoto:** None. **M. Mimura:** None. **T. Muramatsu:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.02/W9

Topic: H.06. Social Cognition

Support: The JPB Foundation

Title: Variability in mother baby interactions across the first three years of life

Authors: G. YOUNG, ***B. THOMPSON**;
Michigan State Univ., Grand Rapids, MI

Abstract: Background: Adversity can broadly disrupt neurodevelopment beginning very early in development and continuing through childhood and later adulthood. Probing mother-baby interactions during a naturalistic play session may provide unique insight into the development of a child over the first years of life. We coded interactive behaviors between infants and mothers between birth and 3 years to monitor trajectories of social and emotional behavior. **Methods** This study enrolled 57 mother-baby dyads from a community clinic in Los Angeles, California, and seven different visits occurred across the first 36 months of life. Demographics

and questionnaires were obtained at each visit. Videotapes of mom-baby dyads participating in a dyadic play session with each other were gathered at each visit and then scored using the Coding Interactive Behavior Scale. **Results** The sample included 51 dyads, 88% of mothers were Latinx, and 70% spoke primarily Spanish or were bilingual. Statistical analyses examined correlations between CIB constructs and infant and maternal characteristics at each time point, and significant heterogeneity in individual scores of dyadic interactions were found with limited change over the first three years of life. Preliminary analyses reveal specific and different variables at each infant age correlating with specific CIB constructs. **Conclusion** The data indicate that observation of parent-infant interactions in a clinical setting can provide valuable insight into dyadic relationships very early in development. Using the Coding Interactive Behavior (CIB) scale provides valuable insight beyond what questionnaires and surveys reveal. Future studies will probe developmental trajectories of the CIB using longitudinal linear mixed effects modeling and linear regression models to determine the influence of the external environment on behavioral constructs.

Disclosures: G. Young: None. **B. Thompson:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.03/W10

Topic: H.06. Social Cognition

Title: Setting the Stage: Investigating Adolescent Risk Factors for Alcohol Use Disorder Utilizing Preregistered ABCD Study Data

Authors: *A. MILLER[1], S. J. DEAMICIS[2], C. MIKKELSEN[3];
[1]Sch. of Cognitive Sci., Hampshire Col., Amherst, MA; [2]Dept. of Psychological and Brain Sci., Univ. of Massachusetts, Amherst, Amherst, MA; [3]Smith Col., Northampton, MA

Abstract: Alterations in neural reward circuitry have been identified as key components of addictive disorders (Volkow et al., 2015). However, there is a dearth of scientific understanding regarding the relationship between perceptions of substance use and the characteristics of reward processing. Further investigations into adolescent alcohol attitudes, impulsivity, and neural reward networks are required to uncover early-life indicators of addictions such as alcohol use disorder (AUD). To investigate this question we utilized data from the Adolescent Brain and Cognitive Development (ABCD) study (Casey et al., 2018). We utilized regression models to quantify the associations between outcomes on the Alcohol Expectancies Questionnaire to measure attitudes towards alcohol use, the delay discounting task to measure impulsivity, and differences in neural activity in the nucleus accumbens for the large vs small reward anticipation contrast of the Monetary Incentive Delay (MID) Task. We found no significant main effects among the variables we tested. However, we found a significant moderating effect of neural activity, in which adolescents with heightened nucleus accumbens sensitivity exhibited positive

relationships between impulsivity and favorable alcohol attitudes($p<0.001$). The results obtained from this study provide insight into how increased impulsivity and reward sensitivity relates to adolescent openness to alcohol use. Further research into the brain's reward circuitry, reward processing, and alcohol exposure may improve clinical practices and outcomes for adolescents at risk for addictive disorders like AUD.

Disclosures: A. Miller: None. **S.J. DeAmicis:** None. **C. Mikkelsen:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.04/W11

Topic: H.06. Social Cognition

Support: Brain Canada

Title: Social Media Screen Time Use and Brain Connectivity in Adolescents

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Abstract: Media screen time use, including social media use (SMU), increased exponentially in adolescents during the pandemic. Some adolescents reported checking social media over 100 times per day, which has previously been linked with structural brain changes in regions involved in social cognition and the theory of mind. Less is known about the influence of social media on social brain functional connectivity (FC). The present study investigated the association of SMU with FC in the prefrontal cortex (PFC) and temporo-parietal junction (TPJ) using functional Near-Infrared Spectroscopy (fNIRS) in adolescents. Data collection for this study is ongoing. A total of 13 participants have been recruited so far (10 Boys, 3 Girls). Participants ranged from 12-18 years of age, and testing occurred at Western University in London, Ontario. SMU was assessed using a self-report questionnaire (SCREENS-Q). Participants completed a 7-minute resting-state task where they watched the Inscapes video (7-minute computer-generated animation of non-social shapes) during fNIRS recording. The fNIRS data were acquired using a NIRx NIRScout system (NIRx medical systems, Berlin, Germany) with 14 sources and 19 detectors covering cortical regions of interest (PFC & TPJ). Data were preprocessed and analyzed using the AnalyzIR toolbox and custom scripts in MATLAB (R2024a). Daily SMU ranged from 17.5 to 172.5 minutes (mean=98.91 minutes). The FC results revealed that increased SMU was associated with hyperconnectivity between the PFC and left TPJ (4 channel pairs, $p<0.05$). Excessive SMU may influence the interactions of the social brain network and have downstream effects on social cognition. Future research in this area should examine SMU, brain connectivity and the association with prosocial behaviour. Overall, findings

contribute to the understanding of how increased SMU can moderate adolescent social brain function and development and could help inform guidelines for SMU and other media screen time use.

Disclosures: B. Krivoruk: None. **H. Vahidi:** None. **M. Kent:** None. **E.G. Duerden:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.05/W12

Topic: H.06. Social Cognition

Support: KAKENHI JP21H04425

Title: Neural Substrate of Social Desirability Bias

Authors: *R. OSU, T. OKADA, R. OHKUMA, Y. KURIHARA;
Waseda Univ., Tokorozawa, Japan

Abstract: Social desirability bias (SDB) is a type of response bias occurring when respondents give answers to questions that will make them look good to others, concealing their actual opinions or experiences. SDB is a severe marketing research problem, and marketers want to detect and avoid SDB. However, there has been no neuroscience research directly focusing on SDB. Since SDB is a type of prosocial behavior, brain regions related to prosocial behavior, such as the temporoparietal junction (TPJ), may be activated in SDB. To explore the brain regions associated with SDB, we set up a realistic scenario by running the experiment in a survey room where marketing research is conducted. Participants believed that they were participating in marketing research until they were debriefed. Thirty-four participants saw images of ten products, such as dumplings, Chinese noodles, cream puffs, etc., from one of the two convenience store brands: Family Mart or LAWSON. The logo on the image allowed the participants to identify which brand the product was from. They answered the impression of the product with a nine-point Likert Scale as quickly and intuitively as possible. Participants conducted the above survey task under three conditions: control, SDB, and simple lie conditions. In the control condition, no specific instruction was given. In the SDB condition, an instruction that the study was conducted by one of the two brands, and the response was significant for the marketing staff. In addition, the experimenter, who pretended to be a survey staff member, watched the participants during the task. Half of the participants received Family Mart bias, and the others received LAWSON bias. In the simple lie condition, we gave the participants a debriefing and asked them to answer the opposite of their true feelings. We used fNIRS to measure brain activity. Probes were placed around bilateral TPJ and prefrontal cortex, including DLPFC and inferior frontal gyrus IFG. The data were analyzed with a 2-way ANOVA of three survey conditions and whether the presented image was an SDB target brand. The survey scores of the target brand in the SDB condition were higher than those of the non-target brand. The

scores for the non-target brand product were not different in all conditions. Right-TPJ and Right-DLPFC were more activated in the SDB condition than others. Right-IFG was also activated in the SDB condition compared to the simple lie condition. We were able to set up realistic SDB experimental conditions and quantify the magnitude of SDB. SDB intervention biased the response of the target brand but not that of the non-target brand. TPJ activity could be a marker of SDB.

Disclosures: R. Osu: None. **T. Okada:** None. **R. Ohkuma:** None. **Y. Kurihara:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.06/W13

Topic: H.06. Social Cognition

Support: CONAHCYT

Title: Social cognition dysfunction as a risk factor for suicide ideation

Authors: *L. GALINDO-DEY[1], B. BERNAL-MORALES[2], R. L. CASTILLO LÓPEZ[3], R. TRIANA-DEL RIO[4], T. CIBRIAN-LLANDERAL[5];
[1]Inst. de Neuroetología, Xalapa-Enríquez, Mexico; [2]Neuropharm. Lab., Inst. de Neuroetología. Univ. Veracruzana, Xalapa-Enríquez, Mexico; [3]Clínica Psicológica Bienestar, Xalapa-Enríquez, Mexico; [4]Ctr. for Neural Sci., New York Univ., New York, NY; [5]Inst. de Neuroetología. Univ. Veracruzana, Xalapa-Enríquez, Mexico

Abstract: The perception of emotionally relevant stimuli can alter emotion, cognition, and behavior. The emotional stimuli to which human beings are most exposed are facial expression and social interaction. Theory of Mind (ToM) and empathy as subprocesses of social cognition are the capabilities that allow the understanding of these social stimuli. The correct functioning of both elements is essential to establish the socio-affective bonds necessary for physical well-being and mental health; however, dysfunction may be associated with the development of avoidance or escape behaviors, such as suicidal ideation. The objective was to identify ToM and empathy capacity in a sample with suicidal ideation (SI) compared to a sample without suicidal ideation (NIS). It was an observational, descriptive, comparative, cross-sectional study, with convenience sampling. The sample consisted of 1,033 Mexicans of legal age: 77% women, 20.3% men and 2.7% did not respond. The survey included the Reading the Mind in the Eyes (RME) test based on ToM and was divided into three types of stimuli: positive, neutral and negative; Interpersonal Reactivity Index for the evaluation of cognitive (CE) and affective (AE) empathy and the Roberts suicidal ideation scale. Two groups were created: IS n=409 and NIS n=624. A significance value of $p<0.05$ was established. Statistically significant differences were identified using the Mann-Whitney U test (Table1). The results indicate that the deficit of ToM, CE and AE can contribute to the development of cognitions of suicidal ideation. This may be due

to the fact that the IS group perceives the social environment as more negative, with greater emotional discomfort and a greater ability to fantasize. It is concluded that there is alteration in ToM and empathy in the group with suicidal ideation. The research project was approved by: Research Committee with registration COFEPRIS 19CI 30 087 041 and by the Research Ethics Committee, registration CONBIOÉTICA-30-CEI-001-20180131, both from the Instituto de Ciencias de la Salud of the Universidad Veracruzana.

Table 1. Comparison of variables based on the presence/absence of suicidal ideation

Variables	NSI (n=624) Mdn(range)	SI (n=409) Mdn(range)	U	p
RME	21(21)	20(23)	112,514.00	0.001
RME positive valence	5(8)	4(8)	113,071.50	0.002
RME negative valence	7(10)	7(11)	125,852.00	0.704
RME neutral valence	10(12)	9(13)	114,134.00	0.004
Perspective-Taking	26(28)	25(26)	115,186.00	0.008
Fantasy	26(26)	27(25)	112,640.50	0.001
Empathic Concern	26(25)	26(24)	126,306.00	0.781
Personal Distress	20.5(28)	23(28)	96,980.50	0.001

Disclosures: L. Galindo-Dey: None. **B. Bernal-Morales:** None. **R.L. Castillo López:** None. **R. Triana-Del Rio:** None. **T. Cibrian-Llanderal:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.07/W14

Topic: H.06. Social Cognition

Title: Embodied cognition leading to flexibility in stereotypical thinking.

Authors: *T. TAKANO[1], K. MOGI[2];
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Abstract: According to social identity theory (Tajfel & Turner, 1979), the existence of outgroup will generate an intergroup bias. However, one's flexible attitude toward out-group members can moderate the bias. Flexibleness includes a cognitive component of social attitude like impression

formation. Participants with high Openness to Experience score, which is one of Big Five factors of personality (Goldberg, 1993), find it easy to accept the opposite opinion (McCrae, 19867) and understand people who are different (Strauss & Connerley, 2003), resulting in their prejudice reduction (Flynn, 2005). Openness as a personality trait is thought to be related to cognitive flexibility. Participants with high Openness score have not only an open-minded thinking (Rokeach, 1960) but also an open-minded attitude toward other's emotions and experiences (Rogers, 1961; Tellgan & Atkinson, 1974). Information from the body influences cognition ("embodied cognition"), often modulating one's social representation. Slepian & Ambady (2014) advocated Simulated Sensorimotor Metaphor, suggesting that conceptual processing requires both sensorimotor-based and metaphor-based processes. Ackerman et al. (2010) showed that participants sitting on a soft cushioned chair where they got the softness in a passive manner took more flexible attitude on a negotiation task, because they came to regard others as more flexible persons. Participants grabbing a soft ball tend to judge gender-ambiguous faces as females evoking kind and soft image, compared with those holding a hard ball (Numazaki et al., 2016). Taking these studies into consideration in the context of embodied cognition, it would appear that soft sensation, provided in a passive or active manner, would literally and unconsciously promote one's flexible thinking or attitude in the social context. Although one of the typical intergroup biases is racial bias, people generally hide their own undesirable attitudes, lowering the credibility of explicit racism scale where participants respond by self-report (Wittenbrink et al., 1997). Here we studied whether sitting on a soft-cushioned chair would mitigate participant's prejudice by using the Implicit Association Test (IAT), which can implicitly assess some stereotypes held by participants. Our hypothesis was that softness would make participants form greater pliability of perception, leading to the mitigation of the degree of stereotypical thinking, whereas the stereotypical thinking would be hardened by the tactile perception of hardness. The present study reports a new insight of embodied cognition of softness, especially in the social context.

Disclosures: T. Takano: None. **K. Mogi:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.08/W15

Topic: H.06. Social Cognition

Support: Tulane University Summer Award for Faculty Mentored Undergraduate Research
Tulane University Newcomb College Argote-Epple & Data Hub Grant

Title: A novel behavioral measure of long-term memory for naturalistic social content

Authors: *D. L. ROSS[1], B. M. DEEN[2];
[1]Tulane Univ., Bernardsville, NJ; [2]Tulane Univ., New Orleans, LA

Abstract: How do individuals differ in their ability to remember information about other people? Existing measures of long-term memory performance use simple stimuli such as words or images. This approach offers tight stimulus control, but doesn't capture the abstract social information that humans encode about others in naturalistic experience, including others' personality, relationships, and mental states. To address this gap, we developed and validated a novel behavioral task for measuring long-term social memory from narrative movie stimuli. Participants (N = 72) watched the pilot episodes of either *Friday Night Lights* or *Gossip Girl*, which were chosen for their rich social content and character development. Both immediately after watching the episodes and following a 3-week delay, participants answered multiple choice probe questions assessing their memory for social information. Probes were separated into six categories based on the type of information they were designed to test, including "event-based" questions about specific events in the narrative (actions, statements, and mental states), and "abstract" questions about information not tied to a specific event (personality, relationships, and mental states). Performance with no delay was high (>90%), demonstrating consensus on correct answers to potentially subjective questions. Performance dropped significantly after the delay, with an interaction between question category and delay reflecting a larger decrease in memory for event-based versus abstract information. Delay performance ranged from 50 to 95% across participants with moderate split-half reliability, indicating the utility of this measure to capture individual differences. To compare our novel social memory measure to other tests of long-term memory performance, we included several additional measures in a subset of participants: 1) the Rey Auditory Verbal Learning Task, a standard test for verbal memory; 2) a face naming task; and 3) an image recognition task. Social memory performance was strongly correlated with face naming, and weakly with the RAVLT, suggesting convergent validity with other related measures. These results provide preliminary evidence that our novel social memory task captures reliable individual variance in long-term memory ability, providing a tool for future studies to assess memory in the social domain.

Disclosures: D.L. Ross: 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.; Tulane University. **B.M. Deen:** A. Employment/Salary (full or part-time):; Tulane University.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.09/W16

Topic: H.06. Social Cognition

Title: Exploring how the brain-gut axis is affected by personality traits, eating habits, and meditation practices.

Authors: *M. ISHII[1,2], T. ISHIKAWA[3,4,2], Y. TAMORI[5], K. MOGI[6,2];
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Meguro-ku, Japan; [3]2 ADSP, R-IH, RIKEN, Yokohama city, Japan; [4]Keio Univ. Sch. of Med, Shinjuku-ku, Japan; [5]Grad. Sch. of Arts & Sci., The Univ. of Tokyo, Meguro-ku, Japan; [6]Sony Computer Sci. Labs., Shinagawa-Ku, Japan

Abstract: The brain-gut axis (Martin et al. 2018) has attracted much attention in recent years, revealing possible relations between gut microbiota, brain functions, mental states, and behavior. The notion that what happens in the gut, sometimes termed the second brain (Ridaura and Belkaid 2015), would affect brain states (Mayer 2011), mind (Neary et al. 2003), and behavior (Cryan and O'Mahony 2011), and those, in turn, for example through eating habits, would affect the makeup of microbiota in the gut, has changed our views on how the health and wellness (Greenberg 1985) might be nurtured and maintained. Gut microbiota might also affect general intelligence (Spearman 1904, Zhou et al. 2023, Yao et al. 2024) and emotional intelligence (Yip et al. 2020), with implications for the development of children (Wakefield 2002, Cowan et al. 2020).Here we report secondary data analysis as well as meta-analysis of the interaction between the brain and the gut with focus on big five personality traits (Cobb-Clark and Schurer 2012), eating habits (Ezra-Nevo et al. 2020), meditation practices (Ningthoujam et al. 2021), circadian rhythms (Teichman et al. 2020), fatigue (Lakhan, and Kirchgessner 2010), life satisfaction (Zhou and Foster 2015), and ikigai (Mogi 2017). We analyze the bidirectional nature of the interactions between the brain and gut states, and how they are affected by an interaction of personality traits, dietary habits, and behaviors such as meditation.Based on the results, we discuss study frameworks for a cohort study involving the relation between eating habits, health-related behaviors, frequency of meditation practices, and personality traits to understand the best practices of promoting pro-health nurturing of gut microbiota conditions. We explore implications for different demographics, such as vegetarians without meditation, vegetarians who practice meditation regularly (Japanese Buddhist monks in the Soutou sect, Shinfuku and Kitanishi 2010), non-vegetarians who practice meditation regularly (Japanese Buddhist monks in the Rinzai sect, Borup 2008) and the general public.

Disclosures: M. ishii: None. **T. Ishikawa:** None. **Y. Tamori:** None. **K. Mogi:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.10/W17

Topic: H.06. Social Cognition

Support: FBRI support

Title: Can proximity to future index serve as an alternate to delay discounting as a measure of temporal window?

Authors: *F. QUDDOS[1], W. K. BICKEL[2];
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Abstract: Substance use disorders (SUDs) present a significant public health concern in the United States, with 46.3 million adults meeting diagnostic criteria in 2021. The Reinforcer Pathology (RP) theoretical framework suggests that a constricted temporal window contributes to unhealthy behaviors such as excessive substance consumption. Delay discounting (DD) serves as a behavioral biomarker for such behaviors and has been suggested to serve as a proxy measure for temporal window. However, DD tasks have intertemporal choices involving commodities, often investigating discount rates by using the preference of smaller sooner or larger later rewards. In this study, we propose utilizing the Proximity to Future Index (PTFI) measure as a commodity-free measure of temporal window. We conducted two experiments to examine the relationship between PTFI and DD. First, we assessed the association between PTFI and DD, and their independent predictive power for recovery outcomes in a cross-sectional study. Next, we conducted a longitudinal study to investigate the impact of Episodic Future Thinking (EFT) on both measures. In experiment 1, results indicate that PTFI and DD are independent predictors of recovery outcomes, such as Quality of life and remission status, indicating potential utility of PTFI as an independent measure of temporal window. In experiment 2, EFT changed both DD and PTFI, in a rate-dependent manner. A simpler and less time-consuming approach, such as the PTFI measure, could be more effective in measuring an individual's temporal window. Future studies investigating addiction and recovery or other unhealthy behaviors, in terms of RP, should incorporate PTFI to better understand if it can serve as an alternative to DD.

Disclosures: F. Quddos: None. **W.K. Bickel:** 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.; HealthSim, LLC; BEAM Diagnostics Inc.; Red 5 Group, LLc. F. Consulting Fees (e.g., advisory boards); Ria Health; Lumanity.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.11/W18

Topic: H.06. Social Cognition

Support: Interdisciplinary ICT Research Center for Cyber and Real Spaces, Tohoku Univ.
KAKENHI 23K21946
NeuroGlobal Program fellowship
Pioneering Research Support Project

Title: The role of the precedence of conversational facial signals in understanding intentions: an fMRI study

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Abstract: Proper conversational facial signals help to rapidly recognize speakers' intentions underlying the speech and seem to appear earlier than the accompanying speech in face-to-face communication. However, the role of the precedence of facial cues remains unknown. To investigate the brain activity involved in perceiving the early appearance and late appearance of facial signals, an fMRI experiment was conducted. We created video clips of a person speaking one sentence while making facial expressions that involves eyebrow frown or raise. In the video clips, the onset of facial signals was edited to be 750ms earlier or later than the onset of the speech, and the speakers were either asking questions or stating facts. Participants (n = 49) watched videos inside scanner, and judge whether the speaker was speaking a question or statement. The response time was faster in early appearance conditions compared to late appearance conditions, but there was no difference in accuracy rates between conditions. We predicted that understanding intentions would be more cognitively efficient in early appearance conditions, so we also conducted ROI analysis on the temporal-parietal junction and middle prefrontal cortex regions. The ROI analysis shows no significant differences among conditions. The whole brain analysis revealed a main effect of timing in the middle temporal gyrus (MTG), with decreased activation in early appearance conditions compared to late appearance conditions. Combined with the involvement of MTG in observing rational actions, these findings suggest that accurately understanding speakers' intentions does not rely on the precedence of facial signals, but the precedence helps to efficiently predict speakers' intentions based on the perception of facial movement.

Disclosures: Y. Liu: None. **H. Jeong:** None. **A. Takemoto:** None. **M. Sugiura:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.12/W19

Topic: H.06. Social Cognition

Support: Beca CONACYT 931445

Title: Psychometric properties of the COVID-19 modified Yorkshire Rehabilitation Scale (C19-YRSm) for Long Covid in Mexican population

Authors: *E. ACOSTA-MARI[1,2,3], T. CIBRIAN-LLANDERAL[4], Y. CAMPOS-USCANGA[5], H. ACOSTA[6], R. L. CASTILLO LÓPEZ[7], R. TRIANA-DEL RIO[8];

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Abstract: Background: Long COVID or Post-COVID-19 Syndrome is a multisystem syndrome that can occur in the long term after COVID-19 infection. It is estimated that at least 100 million individuals worldwide suffer from it. More than 200 symptoms have been reported in Long Covid in different organs and systems. The most frequently reported include fatigue, cognitive alterations, pain, sleeping problems, difficulty breathing, among others. These symptoms, by lasting for long periods of time, have caused a negative effect on the quality of life of the individuals who suffer from it. The modified Yorkshire Rehabilitation Scale (C19-YRSm) is a scale that was specifically developed to assess symptom severity, functional independence, and disability in patients with Long Covid. Since it was developed and validated, it has been used in a wide variety of clinical and research contexts, including hospitals and first-level care centers, which is why it has become a useful tool to determine and evaluate the need for interventions in rehabilitation and in the epidemiological record of Long Covid symptoms**Objective:** Translate, adapt and validate the psychometric, reliability and validity properties of the English version of the modified Yorkshire Rehabilitation Scale (C19-YRSm) into Spanish to apply to the Mexican population.The research protocol and informed consent were prepared in accordance with the principles of the Declaration of Helsinki.**Results:** A total of 889 participants with a history of COVID-19 completed the C19-YRSm on one occasion. The large percentage of female participation in the sample (80%) stands out, as well as the high participation of participants with a bachelor's or postgraduate educational level (74%). Participants who had a history of having been hospitalized for COVID-19 reported higher scores in SS and FD, compared to those who had not been hospitalized (p=0.001). These differences were not observed for OH.Conclusions: The results of this study agree with previous validations in other populations, in which good internal consistency and convergent validity have been reported in the instrument, providing more evidence of the psychometric properties of the C19-YRSm with significant factors or domains such as SS, FD and OH. This supports the use of the scale as a validated assessment instrument for the specific condition of Long Covid.

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Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.13/W20

Topic: H.06. Social Cognition

Title: Telomere Length is Unrelated to Cognitive Changes in after COVID-19 Individuals at a Southeastern Mexican Hospital

Authors: G. V. JUÁREZ[1,2], A. GENIS-MENDOZA[3], ***I. JUAREZ-ROJOP**[4], H. NICOLINI[3], J. CRUZ-CASTILLO[5], M. RAMOS-MENDEZ[6], Y. HERNANDEZ-DIAZ[7], D. MAGAÑA MÁRQUEZ[8];
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Abstract: Telomere Length is Unrelated to Cognitive Changes in post-COVID-19 Individuals a Mexican HospitalGuillermo Efrén Villar-Juárez, Alma Genis-Mendoza; Isela Esther Juarez-Rojop, Humberto Nicolini,,Juan Daniel Cruz-Castillo, Miguel Angel Ramos-Méndez, Yazmín Hernández-Díaz.The evidence available to date suggest that patients who have suffered from COVID 19 disease have neurocognitive manifestations. On the other hand, some reports indicate that the length of the telomeres (TL) may be related with neurodegenerative disorders, cardiovascular disease, type II diabetes, and cancer. The aim of the study was to determine the relationship between telomere length and cognitive changes in health care workers after COVID-19 in a hospital in southeastern Mexico. Forty samples from healthcare workers (21 males and 19 females) at a hospital in southeastern Mexico who had recovered from SARS-CoV-2 infection confirmed by RT-PCR were analyzed. All subjects participating in the study underwent a survey (sociodemographic questionnaire, medical history, and the Mini-Mental State Examination and Montreal Cognitive Assessment scales) and blood collection in EDTA tubes. Telomere length determination was performed using real-time quantitative polymerase chain reaction. Statistical analysis utilized the Mann-Whitney U test. The results indicated that 15% of the participants showed cognitive impairment according to the MMSE, while 72.5% showed it according to the MOCA. Regarding telomere length, a mean of 0.076 ± 0.13 was found. Besides, there was no significant difference in telomere length between the groups with and without cognitive impairment, assessed both by the MMSE and the MOCA ($p = 0.425$ and $p = 0.835$, respectively). The findings suggest that, although TL is affected by SARS-CoV-2 infection. However, TL is not associated with cognitive changes in healthcare workers post-COVID-19 in this study.

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Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.14/W21

Topic: H.01. Attention

Title: Follow-up rs-fMRI study on Post COV19 children reveals functional implications of the Dorsal Attention Network.

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Abstract: Abstract After the COVID-19 pandemic, "long COVID" became a popular term among survivors of COVID-19, primarily adults [Asadi-Pooya A. A., 2022]. In the first half of 2022, in collaboration with *Hospital Infantil de México Federico Gómez* (HIMFG), we conducted a functional analysis using functional Magnetic Resonance Imaging (fMRI) on a group of children who had survived COVID-19 (n=14, age (11.5 [10,13])). We utilized resting-state functional MRI (rs-fMRI) to identify any functional variations compared to a control group (n=35) selected from volunteers with no history of COVID-19 at the time of functional imaging. Two years later, we conducted a follow-up study to investigate any long-term difference between the post-COV19 group and the control group.This study and its predecessor were approved by the hospital's ethics committee in accordance with international practice, including procedures from the Helsinki Declaration. The original Study Cohort was selected from the Hospital's records; their functional data was acquired a few months (7.5[4,15]) after the onset of COVID-19. The functional data was analyzed using the CONN: functional connectivity toolbox [Whitfield-Gabrieli et al., 2012]. The results showed higher temporal correlations in 3 clusters. One of these clusters included the left and right nodes of the Dorsal Attention Network (Frontal Eye Fields (DAN.FEF)) associated with visuospatial attention [Castellanos, F.X. & Aoki Y., 2016], as well as the Sensorimotor Lateral Network associated with the planning of complex movements [Podgórski P., 2021]. The new cohort from 2 years after the original study exhibited higher temporal correlations in 3 clusters of Networks. One of these clusters included again DAN.FEF, now with fronto-parietal (Posterior Parietal Cortex (PPC)), is associated with rapid coordinate behavior but also with AD/HD disorder [Marek & Dosenbach, 2018]. This suggests that even after 2 years, the post-COVID-19 children may present higher resource demands when the DAN is engaged compared to the control group. However, the symptomatology associated with these functional variations is still under observation. **References**[1] Asadi-Pooya, A. A., et al. *J. Med. Virol., 94*(3), 979-984. (2022)[2] Castellanos, F.X. & Aoki Y. *BP:CNNI*. 1(3), 253-261. (2016)[3] Marek, S., & Dosenbach, N.U.F. *DCNS*. 20(2), 133-140. (2018)[4] Podgórski, P., et. al. Front. Neurol. (2021)[5] S. Whitfield-Gabrieli, et al. *(*2012) *Conn.*

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Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.15/W22

Topic: H.06. Social Cognition

Support: Ministero Istruzione Università e Ricerca (PRIN 2022, NextGenerationEU. Project code: 2022L3AALJ)

Title: Unmasking vaccine hesitancy: a neuroscientific investigation

Authors: *A. CASULA[1,2];
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Abstract: Introduction. The COVID-19 pandemic has highlighted the urgent need for widespread vaccination to mitigate its devastating effects. However, vaccine hesitancy (VH) remains a significant barrier for achieving high vaccination coverage. While existing research primarily focuses on explicit reasons for VH, there is a notable gap in understanding the neurophysiological underpinnings of this phenomenon. Addressing this gap could offer valuable insights into developing effective strategies to promote vaccine acceptance and address VH. **Methods.** We conducted a study to investigate the neural correlates of sensorimotor mapping and associated affective-autonomic appraisals of others' vaccination experiences in 23 healthy individuals. Using Transcranial Magnetic Stimulation (TMS), we measured Motor-Evoked Potentials (MEPs) in the lateral deltoid muscle and a control muscle. Additionally, we assessed participants' disgust sensitivity and specific disgust responses to others' vaccination experiences and vaccine vials, compared to control vials, alongside their vaccine hesitancy levels. **Results.** Our findings revealed a significant reduction in MEPs specifically in the lateral deltoid muscle, but not in the control muscle, among subjects with both high VH and elevated specific disgust towards the injection of the influenza vaccine during pictures observation. This effect was observed for both the influenza vaccine vial and injection images, but not for those related to COVID-19 or control fluids. Moreover, we found that the higher the levels of VH and disgust, the greater the reduction in MEPs in the lateral deltoid muscle during observation of influenza vaccine-related images. No significant muscle-specific effects between MEPs amplitude and disgust sensitivity were found. **Conclusions.** Our study identifies a neurophysiological marker associated with the observation of others' vaccination experiences. This marker is particularly pronounced in individuals with heightened perceptions of vaccine risk and is moderated by specific disgust responses to vaccines, shedding light on underlying mechanisms of VH.

Disclosures: A. Casula: None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

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Topic: H.06. Social Cognition

Support: NIH grant MH124004
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NSF grant DRL2024462
NIH F99/K00 grant 8K00DA058542

Title: Introducing the Supramarginal gyrus, Insula, Prefrontal Network (SPIN):Discovery, Replication, and Implications for the Study of Pain Perception

Authors: *H. L. KOSAKOWSKI[1], R. L. BUCKNER[1,2,3];
[1]Psychology and Ctr. for Brain Sci., Harvard Univ., Cambridge, MA; [2]Department of Psychiatry, Massachusetts General Hospital, Charlestown, MA; [3]Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, MA

Abstract: Within-individual precision neuroimaging has yielded novel discoveries about human brain organization and function. For example, precision neuroimaging demonstrated that the so-called “default network” is composed of multiple parallel, anatomically distinct networks, each with separate functional properties (Braga et al., 2020; DiNicola et al., 2020; Du et al. 2024). Another discovery is the unexpected description of inter-effector regions along the precentral sulcus (Gordon et al., 2023) that are likely aligned to the cingulo-opercular (CG-OP) network (Du et al., 2024; Dosenbach et al., 2024). These details were difficult to appreciate in prior studies using group-based averaging methods because of spatial blurring. Here, we used precision neuroimaging techniques to identify a novel candidate network that sits near to the CG-OP and Salience (SAL) networks. Participants read stories of people experiencing physically or emotionally painful events (Bruneau et al., 2011; Jacoby et al., 2016). In each participant, the physical pain task contrast (physical pain > emotional pain) robustly activated regions in the supramarginal gyrus, insula, and prefrontal cortex. Remarkably, this distributed topography was replicated in each individual using functional connectivity analysis of resting-state data, suggesting the distributed regions form an interacting network. We refer to this candidate network as the Supramarginal gyrus, Insula, Prefrontal Network, or SPIN. In independent task data, we found SPIN had robust responses to the pain task contrast that were significantly greater than responses in the juxtaposed CG-OP and SAL networks. Further, SPIN does not overlap with well-defined motor responses in secondary somatosensory cortex (SII), representing an important divergence from the traditionally defined “pain matrix,” a group of distributed regions that respond to first-person physical pain (Ploghaus et al., 1999). The identification of SPIN as a candidate network highlights the importance of accounting for idiosyncratic anatomical differences between individuals and has implications for research on pain perception and attribution.

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Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

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Program #/Poster #: PSTR192.17/W24

Topic: H.06. Social Cognition

Support: NIH UO1NS121616

Title: Neural signatures of social personality traits and their impairments: insights from cortical network dynamics

Authors: *A. T. PHAN[1], B. MASH[1], D. J. KELLAR[1], M. L. MUSTROPH[1], P. NG[1], Y. KFIR[2], Z. WILLIAMS[1], M. JAMALI[1];
[1]Dept. of Neurosurg., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; [2]R&D, Modulight.Bio, Ramat-Gan, Israel

Abstract: Our ability to navigate social situations relies on a range of social personality traits that influence our behavior and shape our interactions with peers, family, and society at large. Impaired social functioning serves as a core hallmark of neuropsychiatric conditions such as autism spectrum disorder (ASD). While prior imaging studies have provided valuable insights into the neuroanatomical networks involved in social cognition and behavior, they are limited by their coarse spatial and/or temporal resolution. Specifically, the precise cortical network properties and dynamics underlying these traits or contributing to their dysfunction in ASD remain largely unknown. Here, we leverage rare access to participants implanted with intracranial depth electrode recordings (sEEG) for clinical epilepsy monitoring to examine the neural activities across multiple brain regions involved in social cognition during both resting state and a visual social-emotional perceptual task. Additionally, we collect self-reported behavioral surveys from participants, including the Autistic Quotient (AQ), Empathy Quotient (EQ), and Systemizing Quotient (SQ) questionnaires, which measure personality traits related to ASD symptoms. By studying local- and network-activities across brain regions and individuals, we seek to describe patterns that underlie specific traits and identify social-cognitive neurocircuitry uniquely associated with ASD-related traits. Preliminary results from power spectrum analysis reveal consistent differences in resting-state gamma frequency when compared between participants scoring high vs. low on AQ questionnaires. Additionally, initial analyses revealed a relation between ASD-like traits and local functional connectivity within the hippocampus concerning theta and high gamma frequencies. Together, this research sheds light on the neural mechanisms contributing to social cognition and sets the groundwork for building a more comprehensive network-based understanding of social behavior and its disruption. By providing valuable insights into the pathophysiology of ASD, further investigations may help refine our understanding of the underlying neurocircuitry and potentially inform targeted interventions for individuals with ASD.

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Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.18/W25

Topic: H.06. Social Cognition

Support: NIH Grant R01HD098097

Title: Lower Sensitivity to Social Pressure is Associated with Lower Engagement with Digital Media and Better Mental Well Being

Authors: *J. CHEIN, B. TANRIVERDI, H. GREEN, D. ZWEBEN, S. MARTINEZ, L. SKALABAN;
Psychology and Neurosci., Temple Univ., Philadelphia, PA

Abstract: Recent discussions around digital media engagement focus on its potentially detrimental effects on well-being, but only a limited subset of research explored the relationship between social sensitivity and digital media habits. Here we test how sensitivity to social feedback is associated with mental well-being and engagement with digital media. Data is collected as part of a larger effort to understand how digital media habits interact with cognitive development. In a cohort of young adults (N=132, Mage=19.2, SD=.826, Females=93), we assessed digital media habits using the Smartphone Addiction Scale (SAS) and Mobile Technology Engagement Scale (MTES); mental well-being using the Adolescent Wellbeing Scale; and finally, we measured sensitivity to social feedback with two behavioral assessments (Resistance to Peer Influence (RPI) and Adults Rejection Sensitivity Questionnaire (ARSQ), which we combined for a composite social sensitivity score) as well as a functional neuroimaging task (Peer Affinity Task). While we found no direct correlations between social sensitivity and digital media usage (MTES), we observed that individuals with lower social sensitivity scores reported lower addiction-like digital media behavior (as measured by SAS, $r = 0.25$, $p = .004$) and more positive well-being ($r = 0.21$, $p = .017$). This suggests that lower sensitivity to peer feedback might be a protective factor against developing unhealthy digital media habits and mental health problems, which we are probing further by testing whether socially relevant brain regions exhibit heightened reactivity to social feedback that is similarly correlated with the digital media behavior.

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Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

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Program #/Poster #: PSTR192.19/W26

Topic: H.06. Social Cognition

Support: EPSRC
Yale Kavli Neuroscience

Title: Neuro-vr: blending neuroscience and virtual reality to facilitate next-generation cognitive investigation

Authors: *C. KELLY;
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Abstract: Neuro-VR describes the pairing of mobile neuroimaging and virtual reality. As the field of cognitive neuroscience continues to strive towards creating more naturalistic environments, technology such as virtual reality presents a viable, immersive route. Here, I present an example of this pairing, investigating social cognition using EEG-VR to probe the effects of eye-gaze between a virtual human and a participant. The experiment focused on achieving joint attention between the virtual human and participant through a puzzle task. The virtual human would communicate to the participant which board a puzzle piece belonged to, using only eye-gaze. When the virtual human would engage in eye-contact, behaviorally, responses were significantly faster. In the EEG data, we observed significant differences in theta oscillations during the trials where the virtual human engaged in eye-contact when compared to trials where no eye-contact was made. In addition, I will present the combination of these techniques in a similar set-up to investigate the dyad of mother-infant interactions, specifically looking at the effects of eye-gaze in response to infant cues (happy, neutral and sad). Both examples will demonstrate the applicability of Neuro-VR to enhance experimentation in cognitive neuroscience, ultimately improving the ecological validity of experimental paradigms.

Disclosures: C. Kelly: None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

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Topic: H.06. Social Cognition

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NEA Research Lab at Rice University
The Musiqa
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NobleMotion Dance

Title: Neural dynamics of social interaction during creative movements from a state of discord to "Meeting of Minds": A longitudinal art-science performance.

Authors: *M. A. PACHECO RAMÍREZ[1], A. J. AGUILAR HERRERA[1], Y. E. LIMA CARMONA[2], B. KHALEGHIAN[3], A. BRANDT[4], A. NOBLE[5], D. NOBLE[5], J. L. CONTRERAS-VIDAL[6];
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Abstract: Dance is a multifaceted brain activity that engages emotional, social, cognitive, sensory, motor, reward, and creative neural networks. It can also provide a window to study social interaction in public settings and to engage the audience. In this research, we collaborated with NobleMotion Dance and Musiqa to create *"Meeting of Minds"* - a multidisciplinary art-science research collaboration to assay coupled brain activity, dance and music as two dancers interact during rehearsals and public performance from a state of discord to collaboration. This project implements brain-computer interface (BCI) and analysis techniques such as hyperscanning, adaptive noise canceling, and mobile brain-body imaging (MoBI), to capture and analyze the level of intra- and inter-brain synchrony throughout the dance. Multimodal data was simultaneously (28 channel scalp electroencephalography (EEG), 4 channels of electrooculography (EOG), 1 IMU for head motion and video recordings) during rehearsals and the public performances. *"Meeting of Minds"* performance is composed of 10 scenes, each representing a different type of interaction between the dancers, aiming to cause fluctuations in the level of intra and inter brain synchrony, where the first 4 scenes showcase solo performances from dancers. Scene 5 marks a significant moment where the dancers perform together for the first time, but in a state of conflict. As the choreography progresses, dancers engage through eye contact, touch, and synchronized movement, culminating in a cooperative climax that integrates all these interactions. During the recordings, data was not only collected but also filtered and processed in real time via a BCI to control visual projections that showed the level of interbrain synchrony in real time, making this dance performance one of the first ones to visualize inter-brain synchrony during a dance performance. A novel denoising pipeline to remove artifacts from the EEG will be presented together with the analysis of the shared brain networks along with the intra and Inter brain synchrony across the dancers. This ecological approach to social neuroscience facilitates the understanding of how interactions and choreographic elements influence brain functions and processes, shedding light on the complex interplay between neural activity and live artistic expression.

Disclosures: M.A. Pacheco Ramírez: None. **A.J. Aguilar Herrera:** None. **Y.E. Lima Carmona:** None. **B. Khaleghian:** None. **A. Brandt:** None. **A. Noble:** None. **D. Noble:** None. **J.L. Contreras-Vidal:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.21/W28

Topic: H.06. Social Cognition

Title: Machine Learning Classification of Affect Decoding in Human Intracranial EEG

Authors: *L. J. DUAN[1], A. R. KIMATA[2], W. F. ASAAD[3];
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Abstract: Facial affect processing is critical in human social interactions. How affect processing interacts with, and might be discriminated from, other aspects of facial recognition remain unclear. In this study, we investigated these dynamics by recording neuronal activity from epilepsy patients with implanted stereotactic electroencephalography electrodes during a modified delayed-match-to-sample paradigm. Subjects were asked to view a face, then after a brief delay, select the correct match in a 2-alternative, forced-choice paradigm. The "match" was defined as either the same face regardless of emotion or gender, the same emotion regardless of face or identity, or the same gender regardless of identity or emotion; the relevant dimension was cued prior to each trial. In this way, we could disambiguate signals related to particular aspects of facial processing. We trained support vector machine and linear discriminant analysis classifiers on event-related potentials in the high gamma (50-150 Hz) range to differentiate neural responses associated with three dimensions of facial affect: emotion (happy vs. angry), gender (female vs. male), and identity (same vs. different). Our results demonstrated that each dimension of facial affect was most accurately decoded in the amygdala, hippocampus, and orbitofrontal cortex. Decoding of identity was generally less accurate than decoding of emotion or gender. Additionally, we observed that decoding accuracy remained consistent across sample, matching, and choice phases in all tested regions except the hippocampus, which had high decoding during sample and matching only. These findings suggest that representation of dimensions of facial affect in amygdala, hippocampus, orbitofrontal cortex, middle frontal gyrus, and superior frontal gyrus was stable across different phases of affect processing and highlight a potential network of regions involved in affect decoding.

Disclosures: L.J. Duan: None. **A.R. Kimata:** None. **W.F. Asaad:** Other; Supported by U01 NS121616 (PI: Z. Williams), Norman Prince Neuroscience Institute (Providence, RI), NIH Office of Director S10OD025181.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.22/W29

Topic: H.06. Social Cognition

Support: MUR PRIN 2022 G53D23004500006

Title: Acting jointly is not acting side-by-side. A dual EEG study

Authors: M. FANGHELLA[1], G. BARCHIESI[2], A. ZAZIO[3], M. BORTOLETTO[3], A. BATTAGLIA MAYER[4], ***C. SINIGAGLIA**[5];
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Abstract: Anyone who has ever walked, cooked, or crafted with a friend knows that acting jointly is not just acting side-by-side. Unlike acting side-by-side, where agents pursue *individual goals*, acting jointly requires that a *collective goal* guide their actions. Yet previous studies have largely ignored this difference, thereby failing to isolate what is distinctive of acting jointly. Indeed, they typically contrasted solo with dyad actions, comparing intra- and inter-individual coordination. However, moving from one to two agents is not necessarily moving from individual to joint action, and walking alongside each other in a crowded alley may require more coordination than walking together. Our study used a dual EEG approach to investigate the brain markers of action planning and execution specific to joint action. We recruited twenty dyads (n=40, 19 female, mean age 24.1 yo) and had them play a joystick video game. The game involved grabbing and transporting one object, either jointly (Joint-Action Condition, JA) or in parallel but merely individually (Parallel-Action Condition, PA). We designed the tasks to ensure equal coordination demands across conditions, as measured by success rates. Our behavioral measurements included RT, velocity, and movement direction, while our EEG measurements focused on two event-related potentials—late Contingent Negative Variation (CNV) and Motor Potential (MP). Late CNV is associated with motor preparation, while MP occurs around movement initiation. Given the similar coordination demands, a collective goal is supposed to facilitate action planning and execution, thereby enhancing the predictability of each agent for the other. We should expect reduced behavioral variability and EGG negativity in JA compared to PA. This was what we found. The mean variability of RT ($F_{(1,38)} = 29.801$, $p < .001$, $p\eta^2 = .440$; JA: 0.173, ± 0.040; PA: 0.246, ± 0.089), velocity ($F_{(1,38)} = 27.407$, $p < .001$, $p\eta^2 = .419$; JA: 75.594, ± 31.130; PA: 113.742, ± 64.155), and movement direction ($F_{(1,38)} = 4.375$, $p = .043$, $p\eta^2 = .103$; JA: 0.311, ± 0.081; PA: 0.331, ± 0.080) was lower in JA than in PA. Strikingly, we also found that the CNV mean amplitude was lower in JA than in PA ($F_{(1,38)} = 8.629$, $p = .006$, $p\eta^2 = .185$; JA: -3.979, ± 1.733; PA: -4.646, ± 2.247). The MP exhibited a similar pattern, with its mean amplitude lower in JA than in PA ($F_{(1,38)} = 8.636$, $p = .006$, $p\eta^2 = .185$; JA: -5.462, ± 2.419; PA: -6.238 ± 3.049). Our findings demonstrate that actions are processed differently when planned and performed jointly compared to side-by-side. The difference arises from the collective nature of the goal rather than mere disparities in coordination.

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Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.23/W30

Topic: H.06. Social Cognition

Support: PSR2022_DIP_007_SINIGAGLIA
BIAL Foundation 2022

Title: Readiness potential as a marker of the sense of commitment in a joint action task

Authors: *M. FANGHELLA, A. KAUFMANN, C. SINIGAGLIA, J. MICHAEL;
Univ. of Milan, Milan, Italy

Abstract: Recent research underscores the role of the sense of commitment in joint actions, showing it is influenced by expectations and reliance cues from others. However, its underlying psychophysiological mechanisms remain unexplored. Our study addresses this gap by combining EEG and behavioural measures to unravel the neurophysiological correlates of the sense of commitment in joint action. Specifically, we aim to explore the executive control mechanisms underlying the capacity to remain committed in a joint action task. We use EEG measures of Readiness Potential (RP) to explore how individuals prepare for and inhibit actions that would break their commitments in a coordination game. A group of dyads of human participants (n = 40, male and female, mean age 23) engaged in a coordination game with two virtual partners. In one condition, the virtual partners adopted a cooperative strategy (signalling condition), while in the other they didn't (no signalling condition). After each round of the game, participants could choose between accepting a default even payoff (50% participant, 50% partner) or an alternative payoff of either a tempting condition (e.g., 70% participant, 30% partner) or a non-tempting condition (40% participant, 40% partner). Participants were instructed to press a button if they chose to accept the alternative payoff (defect), and otherwise to remain still to accept the default payoff (no defect). During the 2600-ms response phase, we measured RP from each participant to explore whether they were tempted by the alternative option and therefore preparing a button-press action (i.e. defection) but then inhibited this action to remain committed to their partner (gritted teeth commitment hypothesis), or whether they were simply ignoring the temptation (engaged commitment hypothesis). We then compared the amplitudes of RP in defect VS no defect, and tempting VS no tempting conditions for each condition (signalling and no signalling conditions). Our behavioural data confirmed that participants had a lower defection rate when the virtual partner acted cooperatively (signalling condition) compared to the non-cooperative (non-signalling) condition. Preliminary EEG results showed increased amplitudes of RP in the tempting compared to the no tempting options and no significant differences in RP for defect and no defect options. These results suggest that, in a cooperative joint action, participants mostly resisted the temptation by gritting their teeth, i.e., inhibiting the temptation to defect through executive control mechanisms.

Disclosures: M. Fanghella: None. **A. Kaufmann:** None. **C. Sinigaglia:** None. **J. Michael:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.24/W31

Topic: H.06. Social Cognition

Support: NSF Grant DGE 1922598.

Title: Continuous theta-burst stimulation of the posterior superior temporal sulcus and intersubject synchrony during naturalistic viewing

Authors: *J. C. THOMPSON[1], P. A. KAKALEC[2], R. ROY[2], C. MARSH[2];
[1]George Mason Univ., Fairfax, VA; [2]Psychology, George Mason Univ., Fairfax, VA

Abstract: Sharing an understanding of commonly experienced events is important for forming and maintaining social connections. Friends share similar patterns of functional MRI activity (intersubject correlation, or ISC) when viewing the same naturalistic stimuli, such as movies. Individuals central in a social network show greater synchrony with their network partners than those who are less central. The superior temporal sulcus (STS) is one region that might play an important role in forming the shared neural representations that underlie ISC. The STS tracks social interactions during task-based and naturalistic viewing, and ISC in the STS is associated with greater encoding of social information into memory. Here, we propose that shared representations rely on neural processing in the pSTS underlying the perception and understanding of social interactions. In this study, we used TMS to examine the causal contribution of the STS to ISC and encoding of social information during naturalistic viewing. In one session, participants viewed videos consisting of people, places, food, objects, and scrambled videos to localize pSTS. Resting motor TMS thresholds were also acquired. In a second scanning session, we administered inhibitory (continuous) theta burst TMS (cTBS) to functionally-localized right pSTS or vertex (sham) in a between-groups design, before participants viewed a 20min movie ("The Neighbors Window") during multiband/multiecho fMRI scanning. After scanning, participants were asked to recall details from the movie. ISC and intersubject pattern similarity (ISPS) were calculated from fMRI responses from parcels derived using the Schaeffer 200 parcel atlas. Memory accuracy for social but not non-social details was lower following cTBS to the pSTS, relative to sham. Lower ISC during movie watching following cTBS to the pSTS relative to sham TBS was observed in the oribitofrontal and anterior temporal cortex. Increased ISC following cTBS, relative to sham, was observed in the parahippocampal cortex. Decreased ISPS following cTBS, relative to sham, was observed in the right amygdala and retrosplenial cortex, while several frontoparietal regions showed increased ISPS following cTBS. More idiosyncratic neural activity was observed in limbic and cortical-limbic regions following cTBS to the pSTS, while some regions showed increased synchrony. This study provides details about the causal role of the STS to ISC and ISPS across multiple brain networks during the viewing of naturalistic social stimuli and helps identify the contribution of social perception to the formation of shared neural representations.

Disclosures: J.C. Thompson: None. **P.A. Kakalec:** None. **R. Roy:** None. **C. Marsh:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.25/W32

Topic: H.06. Social Cognition

Support: NIH Grant R01MH076136-16
NIH Grant R21MH124674-02

Title: Valence expectation effects in the human amygdala

Authors: *B. GRAUL[1], K. BUJARSKI[2], J. HONG[2], T. D. WAGER[1];
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Abstract: Amygdala activity during tasks designed to elicit emotion has been well documented as an early marker of salience, but the extent of valence processing is less clear. Does the amygdala integrate contextual information during emotional responses to stimuli, or is activity limited to salience signaling? Although animal models have enabled extensive electrophysiological mapping of circuit-level activity, human amygdala function during emotional processing remains understudied due to the practical difficulties involved in gathering data. Furthermore, prior work in animal models have required extrapolation of emotional states from observed behavior. To overcome these limitations, we gathered basolateral amygdala recordings from human participants (n = 7) with intracranial electrodes implanted during treatment for epilepsy. Subjects were shown a series of OASIS images and asked to report their anticipated and experienced valence ratings for each image. Prior to seeing each image, valence ratings of the upcoming image were shown. Subjects were informed that these ratings came from prior subjects. However, these ratings were fabricated to systematically shift the subject's anticipated valence estimate from the normative rating established by prior raters. Exploratory analysis indicates that the amygdala processes both valence and salience information during image viewing. Furthermore, activity in the low/mid gamma range (30-70 Hz) increases following large discrepancies between expectation ratings and valence ratings. This occurs at a timescale that corresponds to prediction error N400 signals in scalp EEG studies. These new data support the hypothesis that amygdala activity is multidimensional during emotional processing, clarifying that its activity is not merely an indication of emotional salience alone.

Disclosures: B. Graul: None. **K. Bujarski:** None. **J. Hong:** None. **T.D. Wager:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.26/W33

Topic: H.06. Social Cognition

Support: R01 DC 021663
R01 DC 018539

Title: Fast amygdala face processing is modulated by breathing

Authors: *G. ZHOU[1], X. MENG[2], G. DENG[3], G. LANE[1], F. CHEN[4], P. XU[3], C. ZELANO[1]; [1]Northwestern Univ., Chicago, IL; [2]Shenzhen Gen. Hosp., Shenzhen Univ., Shenzhen, China; [3]Beijing Normal Univ., Beijing, China; [4]The Univ. of Hong Kong- Shenzhen Hosp., Shenzhen, China

Abstract: Recent findings have established a fast subcortical pathway between the retina and the amygdala in humans, and have shown that it enables rapid, subconscious responses to faces (Méndez-Bértolo et al. 2016, Wang et al. 2022). Concurrently, other work has shown that respiratory phase entrains neural oscillations across a wide range of human brain areas, including the amygdala, and that an olfaction-based evolutionary process exploited nasal inhalation to facilitate and tune responses within neuronal assemblies (Perl et al. 2019, Zelano et al. 2016). Thus it is possible that breathing may entrain local field potential oscillations in the amygdala that serve to modulate input gain, opening windows of greater effective connectivity through which the fast amygdala pathway may more efficiently operate. However, whether fast responses to faces in the amygdala are modulated by respiration is not well understood. In this study, we presented fearful and neutral faces to participants at random intervals occurring equally during inhales or exhales, while intracranial electroencephalographic data (iEEG) and nasal airflow were recorded. Time-frequency analysis of the iEEG data in the amygdala revealed a significantly higher gamma response when faces were presented during inhale, compared to those presented during exhale, beginning within 50 ms after face onset. A support vector machine decoding analysis based on the gamma activity within 100 ms after face stimuli onset dissociated amygdala responses to faces by respiratory phase: Specifically, using the early face-induced gamma signal, we could predict the phase of breathing during which the stimulus was presented. These findings further support prior work showing a fast subcortical pathway to the human amygdala in rapid detection of faces, and hint that the speed of the fast amygdala pathway depends on inhalation. These findings support the notion that human cognition, including electrophysiological responses to ecologically and socially important stimuli in the human amygdala, is modulated by breathing.

Disclosures: G. Zhou: None. **X. Meng:** None. **G. Deng:** None. **G. Lane:** None. **F. Chen:** None. **P. Xu:** None. **C. Zelano:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.27/W34

Topic: H.06. Social Cognition

Support: Conacyt 736014

Title: Brain-to-brain coupling during a social cooperation - competition activity

Authors: *L. QUESADA OLGUÍN[1], Y. DEL RÍO-PORTILLA[2];
[1]Univ. Nacional Autonoma de Mexico, Coyoacán, Mexico; [2]UNAM, México City, Mexico

Abstract: In this study, we conducted EEG hyperscannings to measure the neuroelectric activity in 24 couples of subjects while they engaged in the "Chicken's game," a variation of the Prisoner's Dilemma derived from game theory. This game involves two players facing choices of cooperation or defection, where the outcome depends on mutual decisions. The simultaneous EEG recording in interacting subject pairs allowed us to directly observe and model the neural signature of human interactions, shedding light on the cerebral processes involved in social cooperation or competition.We integrated measures of closeness between participating subjects using the Inclusion of Other in the Self (IOS) scale to contrast with hyperscanning results. Our findings indicated that couples reporting higher levels of closeness tended to achieve higher scores in the game, indicating greater cooperation. This prompted further analyses with EEG signals obtained from pairs during the game, aiming to create functional network matrices and measure the level of coupling between them through correlations.Preliminary results from this ongoing analysis have shown correlations of approximately 0.5, consistent with existing literature. These correlations exhibited positive trends in homologous electrodes and negative trends in cross electrodes, reflecting the intricate neural dynamics underlying social interactions. These results underscore the importance of simultaneous EEG acquisition in understanding brain activities during social interactions.

Disclosures: L. Quesada Olguín: None. **Y. del Río-Portilla:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.28/W35

Topic: H.06. Social Cognition

Support: JST, Moonshot R&D JPMJMS2013, Japan

Title: Nirs hyperscanning study of social interactin using vr avatars with enhanced facial expressions

Authors: *H. KODAMA, K. HIGO, S. SHIMADA;
Meiji Univ., Kawasaki, Japan

Abstract: Recent technology allows users to communicate with each other by using virtual reality (VR) avatars reflecting their facial expressions. Oh et al. (2016) found that avatars with an enhanced smile of the user made the partner feel a higher sense of social presence than avatars with the same smile as the user. In this study, we examined the effects of the avatar's enhanced facial expression on social interaction between two participants using functional near-infrared spectroscopy (NIRS) Hyperscanning. A previous study found that cooperation between pairs increased inter-brain synchronization (IBS) in the right temporo-parietal junction (r-TPJ) (Lu et al., 2018).
In this experiment, we prepared three types of avatars, to which different facial expression weighting coefficients were applied. The coefficients were 0 (None-expression condition), 1 (Normal condition), and 2 (Enhanced condition). The facial data were multiplied by the coefficient to enhance or reduce the facial expressions. In this experiment, the pair of participants performed an alternative uses task. At the end of each task, the participants were asked to complete a questionnaire about the sense of ownership of the avatar, social presence of the partner and interpersonal attraction towards the partner. NIRS Hyperscanning (24 channels each over the right temporo-parietal area) was also conducted to examine IBS, especially in the r-TPJ region, during the task.
The questionnaire results showed that body ownership was significantly greater in the Enhanced condition than that in the None condition ($p = 0.033$). Additionally, the social presence score was significantly greater in the Enhanced condition than in the Normal ($p = 0.005$) and None conditions ($p = 0.001$). The interpersonal attraction score was significantly higher in the Enhanced ($p = 0.034$) and Normal ($p = 0.034$) conditions than that in the None condition. Furthermore, NIRS data analysis showed significantly greater IBS in the right angular gyrus in the Enhanced ($p = 0.004$) and Normal ($p < 0.001$) conditions than in the None condition.
The results of the questionnaire indicate that enhancing the facial expressions of avatars increases the sense of ownership of the avatar and elicits greater social presence and interpersonal attraction toward the partner. In addition, we confirmed that the r-TPJ showed significant IBS in conditions where the participant's facial expressions were reflected on the avatars (Enhanced and Normal conditions). These results suggest that enhancing avatar's facial expressions facilitates social interaction with the partner in VR environment.

Disclosures: H. Kodama: None. **K. Higo:** None. **S. Shimada:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR192.29/W36

Topic: I.04. Physiological Methods

Support: NIH-1RF1MH117155-01

Title: Assessing the hierarchy of numerical cognition using stereoelectroencephalography

Authors: *A. ROCKHILL[1], A. RASLAN[2], K. COLLINS[3], B. SHAFIE[4], A. FECKER[5], M. SHAHIN[6], C. LOPEZ RAMOS[6], Z. A. ZOOK[7], M. ISMAIL[6], D. R. CLEARY[8];
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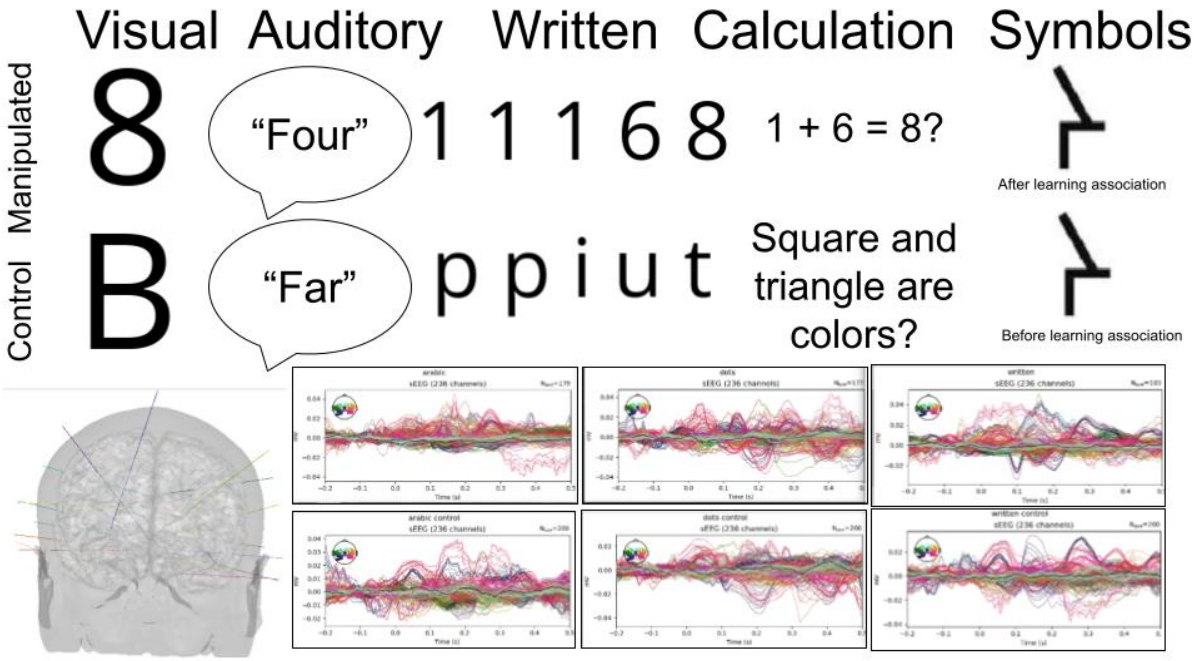
Abstract: Previous studies have used electrocorticograpgy (EcoG), functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG) to study numerical cognition in humans but, to our knowledge, brain activity related to numerical cognition in the deeper structures recorded by stereoelectroencephalography (sEEG) have not been reported. These findings implicate a triple code model (TCM) where symbolic visual number processing occurs in the inferior temporal gyrus (ITG), symbolic auditory number processing occurs in the superior temporal gyrus (STG) and non-symbolic number processing occurs in the intraparietal sulcus (IPS), and that a symbol learning network specific to humans that coordinates between IPS and regions of the frontal lobe (Dehaene & Cohen 1995; Amalric & Dehaene 2019).
A 22 year old male underwent an sEEG study to determine the origin of their seizures. He also participated in five tasks assessing numerical cognition: 1) visual cues of numbers, 2) auditory cues of numbers, 3) writing numbers, 4) calculating equations and 5) symbol learning. Time-frequency spectrograms around the time of stimulus presentation were computed and used to classify stimuli from controls using a linear discriminant analysis (LDA). Areas activated by the task were largely confirmatory of the triple code model and frontal network while adding timing information and implicating subcortical areas in symbol binding.
Dehaene, S., & Cohen, L. (1995). Towards an anatomical and functional model of number processing. Mathematical cognition, 1(1), 83-120.
Amalric, M., & Dehaene, S. (2019). A distinct cortical network for mathematical knowledge in the human brain. NeuroImage, 189, 19-31. https://doi.org/10.1016/j.neuroimage.2019.01.001



Disclosures: A. Rockhill: None. **A. Raslan:** None. **K. Collins:** None. **B. Shafie:** None. **A. Fecker:** None. **M. Shahin:** None. **C. Lopez Ramos:** None. **Z.A. Zook:** None. **M. Ismail:** None. **D.R. Cleary:** None.

Poster

PSTR192: Human Cognition: Behavioral and Neural Processes

Location: MCP Hall A

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Program #/Poster #: PSTR192.30/W37

Topic: H.10. Human Learning and Cognition

Support: Postdoctoral Research Abroad Program of Ministry of Science and Technology, Taiwan [111-2917-I-564-007]

Title: A 7T fMRI Study on Cortical Layer-Specific Repetition Suppression to Faces in the Fusiform Face Area

Authors: *S.-M. LEE[1], D. APŠVALKA[2], M. CORREIA[2], R. HENSON[2];
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Abstract: Repeated face images cause reduced neural activity, i.e., repetition suppression (RS), in brain regions such as fusiform face area (FFA). A predictive coding framework suggests that repetition increases the precision of "top-down" predictions from higher to lower visual regions, reducing prediction errors. Within this framework, the FFA, as a higher visual processing region, is thought to show reduced prediction error in its middle layers with face repetition, leading to RS. To investigate this, we leveraged high-resolution laminar BOLD-fMRI. In a face repetition paradigm, participants made left-right symmetry judgments to novel and repeated face images for 9 sessions. Imaging data were acquired on a 7T scanner. Structural images were acquired using a T1-weighted sequence and segmented. Functional images were acquired using a two-band GE sequence, and then denoised, timing-corrected, motion-corrected, and coregistered to the structural images. The voxels were assigned to three layers (superficial, middle, deep) using the equi-volume approach. Our current findings reveal a pronounced RS effect primarily in the middle cortical layers of the FFA, supporting the role of predictive coding in efficient visual processing through top-down feedback. Additionally, we propose exploring RS effects in lower visual areas, such as occipital face area, expecting greater RS effects in superficial and deep layers compared to middle layers. Our results align with the hypothesis that during the repetition of faces, prediction error signals in the middle layers of the FFA are reduced. We also demonstrate the potential of laminar BOLD-fMRI in elucidating the complexities of cortical processing.

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Poster

PSTR193: Language and Communication: Neural Circuits and Mechanisms

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR193.01/W38

Topic: H.11. Language

Title: Symmetry and asymmetry in human-AI cooperation.

Authors: *S. YOSHIZAWA[1,2], K. MOGI[3,4];
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Abstract: ChatGPT (OpenAI 2023), one of the state-of-the-art Large Language Models (LLMs), showed unprecedented performance in reasoning and inference capability (Bubeck et al. 2023). In a false-belief task (Baron-Cohen 1992) to evaluate the capability of the “theory of mind” (ToM), ChatGPT showed performance comparable to humans (Kosinski 2023, Moghaddam 2023). Metacognition is a type of social cognition that carries a role in monitoring and controlling information in cognitive processes (Nelson and Narens 1990) and is related to decision-making (Yeung and Summerfield 2012). Confidence Judgements have been considered a typical example of metacognition (Kepecs and Mainen 2012). Mentalizing (Frith C and Frith U 1999) is a significant factor for cooperation and joint action between self and others (Frith 2012). Here we examined what asymmetries exist in the cooperative decision-making between LLMs and humans from empirical and theoretical points of view. Comparison was made with human metacognition subserved by neural networks including the lateral and medial prefrontal cortex (Frith 2012, Fleming and Dolan 2012). We used the Stag Hunt (Skyrms 2004) game to analyze confidence level differences in the game theoretical settings. The Stag Hunt game is one of the coordinated games designed to increase reward when players coordinate. We used this game to estimate which the opponent (Human/LLM) chose when a subject (Human/LLM) chose either stag or rabbit. After that subject answered the confidence level of the opponent (Human/LLM) based on the task. Subject (Human/LLM) assumed that the opponent (Human/LLM) had two choices of Pure Nash equilibrium between (Stag, Stag) and (Rabbit, Rabbit). The uncertainty as to whether they chose Stag or Rabbit was represented as a confidence level. The task was conducted under four conditions (Human-Human, Human-LLM, LLM-Human and LLM-LLM). We also compared the subject (Human/LLM) to evaluate whether the strategy taken was changed when the opponent player is a human or an LLM. We analyze appropriate cooperative strategies between humans and AI. Finally, based on the results of this experiment, we discuss human trust in AI (AI-trust) in the context of AI Safety.

Disclosures: S. Yoshizawa: None. **K. Mogi:** None.

Poster

PSTR193: Language and Communication: Neural Circuits and Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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Topic: H.11. Language

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Title: Comparing dorsal and ventral auditory pathways in chimpanzees and humans: A tractography study of individual variation and evolutionary implications

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Abstract: The capacity for language is a distinctive characteristic of the human species. Understanding how language evolved from animal communication remains a challenging question for evolutionary biology. Comparative research provides crucial insights into the evolution of language by examining the neural pathways underlying communicative capacities in non-human primates. Our language abilities rely on a network of frontal and temporal brain areas. The arcuate fasciculus (AF) is a white matter tract that connects frontal and temporal regions through a dorsal route, but these regions are also connected by other white matter connections that run ventrally between the frontal and temporal lobes. Comparative connectivity research between humans, great apes, and macaques indicates human-unique features of the AF, including expansions into the temporal lobe and leftward asymmetry. However, intraspecies variation within these auditory pathways in great apes remains largely unexplored, including in apes who have been trained to use communicative signals such as lexigrams and hand signs. To address this gap, this study investigated the dorsal and ventral pathways in a large sample of captive chimpanzees (N=67) using ROI-based probabilistic tractography. The ROIs included the homologs of Broca's and Wernicke's areas, which were delineated using sulcal boundaries. Individual variation in the volume and fractional anisotropy of the dorsal and ventral streams, as well as the effects of sex, age, and rearing history, were examined. The same tractography analysis was conducted on a human dataset to identify species-specific differences in the organization and variability of the dorsal and ventral auditory pathways. The two language-trained chimpanzees exhibited overall AF and ventral tract volume, and fractional anisotropy (FA), within the typical intraspecies variation, although one chimpanzee showed pronounced leftward asymmetry in these measures. Age and rearing history correlated with AF tract characteristics in chimpanzees, with variations observed in the associations between these factors and arcuate volume and asymmetry across different rearing environments. These results provide insights into the evolution of auditory pathways in primate brains and highlight the importance of considering individual variation in large samples of chimpanzees to better understand the unique

aspects of human language abilities. These findings contribute to our understanding of the neural basis of language and the evolutionary changes that may have occurred in the human lineage to support complex linguistic abilities.

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Poster

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Topic: H.11. Language

Support: NIH T32MH065214
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Google PhD Fellowship
NDSEG Fellowship
CV Starr Fellowship

Title: Shared functional specialization in transformer-based language models and the human brain

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Abstract: When processing language, the brain is thought to deploy specialized computations to construct meaning from complex linguistic structures. Recently, artificial neural networks based on the Transformer architecture have revolutionized the field of natural language processing. Transformers integrate contextual information across words via structured circuit computations. Prior work has focused on the internal representations ("embeddings") generated by these circuits. In this paper, we instead analyze the circuit computations directly: we deconstruct these computations into the functionally-specialized "transformations" that integrate contextual information across words. Using functional MRI data acquired while participants listened to naturalistic stories, we first verify that the transformations account for considerable variance in brain activity across the cortical language network. We then demonstrate that the emergent computations performed by individual, functionally-specialized "attention heads" differentially predict brain activity in specific cortical regions. These heads fall along gradients corresponding to different layers and context lengths in a low-dimensional cortical space.

Disclosures: S. Kumar: None.

Poster

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Program #/Poster #: PSTR193.04/X3

Topic: H.11. Language

Support: NIH Grant MH119099

Title: Intracranial Signatures of Temporal Integration and Separation During Narrative Comprehension

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Abstract: MOTIVATION:
Our brains must flexibly integrate related information into meaningful units while separating unrelated information into distinct units. What are the neural signatures of these integration and separation processes? In the context of language processing, temporal integration has been linked to ramping of neuronal activity over the course of visually presented sentences. Temporal separation processes are less well understood. We hypothesized that, as participants listen to a naturalistic spoken narrative, we would observe ramping in broadband high-frequency (65-200 Hz) power over the course of each sentence in language-responsive electrodes (integration), and transient increases in broadband power at event boundaries in the discourse (separation).
METHODS:
Intracranial stereo electroencephalography (sEEG) signals were recorded from 7 participants who listened to a 14-minute auditory narrative. Broadband high-frequency (65-200 Hz) power time courses were extracted in each channel. Multiple linear regression was used to model the power time courses as a function of (i) word onsets; (ii) sentence position; (iii) GPT-2 contextual embedding vectors. We also collected human-rated event boundaries, in order to characterize boundary-related and segment onset/offset responses.
PRELIMINARY RESULTS:
In 4 of 6 participants, we identified electrodes, including in temporal and frontal cortices, exhibiting reliable word-locked high-frequency broadband power responses. However, in the same word-responsive and sentence-responsive electrodes, we did not identify reliable ramping signatures from the start to end of sentences.
CONCLUSIONS and NEXT STEPS:
Continuous naturalistic auditory narratives did not engender overt ramping responses from the start to end of sentences, even though temporal integration was engaged. Both the presentation modality (auditory vs. visual) and the stimulus type (isolated sentences vs. natural discourse) could explain this result's divergence from prior reports. The variability of natural speech (e.g. prosody and sentence structure) may also reduce our power to detect ramping. Our ongoing analyses will tease apart these possibilities, while also the transient boundary-locked responses, and extending the analysis to 4-14 Hz power modulations.

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Poster

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Topic: H.11. Language

Support: R01 DC019354
R01 DC015260
Simons Collaboration on the Global Brain

Title: Distinct neural networks and response motifs support interactive speech

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Abstract: During spoken interaction, humans must comprehend an incoming message, plan a reply, and articulate that response at the appropriate time. This behavioral cascade requires the brain to execute multiple sensorimotor and cognitive operations sequentially and in parallel. However, little is known about how the circuits underlying these functions are organized and interact during naturalistic language use because previous work has largely focused on dissecting specific aspects of speech perception or production in isolation. To address this issue, we recorded high-resolution intracranial signals from over 1,700 electrocorticography electrodes spread across the left and right lateral cortices of 19 neurosurgical patients. We observed that ~70% of electrodes displayed significant modulation during a question-answer task, indicating a broad cortical network is recruited during interactive speech production. Despite this widespread engagement and a high degree of functional heterogeneity across electrodes, we found that neural responses were organized into four clusters related to sensory processing (20%), motor function (25%), planning (35%), and suppression during the task (20%). These categories of neural activity were furthermore localized to separate neural substrates - with sensory, planning, and motor responses significantly overrepresented in bilateral temporal cortex, left frontotemporal lobe, and bilateral sensorimotor structures, respectively - which indicates that a small number of discrete subnetworks underlie language generation. Finally, we use a dimensionality reduction analysis to demonstrate that each subnetwork possesses multiple response profiles; for example, planning responses contain signatures of three preparatory functions while articulation-related dynamics are composed of premotor, motor, and movement-offset activity motifs. In sum, this study identifies the core systems underlying spoken communication in the human brain and provides neurobiological evidence for distinct sensorimotor and cognitive processes relevant for interactive speech production.

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Poster

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Topic: H.11. Language

Title: Evaluating the Temporal Order of Motor and Auditory Systems in Speech Production using Intracranial EEG

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Abstract: Theories propose that speech production can be viewed as a temporal reversal of speech perception. For example, phonological encoding in the auditory system is assumed to precede phonetic encoding in the motor system during speech production. However, empirical evidence supporting such temporal order of motor and auditory systems in speech production is rare. In this study, we investigated the neural dynamics of speech production using stereotactic electroencephalography (sEEG) with both high temporal and spatial resolution while participants spoke single-character Chinese words. The latency analysis revealed that the activation in the inferior frontal gyrus (IFG) preceded the posterior superior temporal gyrus (pSTG). Moreover, representational similarity analysis (RSA) revealed that in the gamma band (30-70 Hz) IFG and pSTG encoded similar representations at least in parallel. These results suggest that the auditory-phonological system is not necessarily activated before the motor-phonetic system during speech production. Additionally, in the high-gamma band (70-140 Hz), a similar representation was observed again in the IFG at the time of articulation, suggesting that IFG may mediate both encoding and execution of speech production. The findings, combining both temporal and spatial precision in human electrophysiological recordings, prompt the reevaluation of the classical theories and inspire the reconsideration of the temporal relations between language and motor systems in speech production.

Disclosures: S. Li: None. **X. Luo:** None. **Z. Chen:** None. **X. Tian:** None.

Poster

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Topic: H.11. Language

Support: NIH Grant R01EY026025

Title: Syntactic-feature encoding of language in the human brain

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Abstract: Understanding the internal representation of language within the human brain remains a challenge with significant implications for neuroscience, artificial intelligence, and clinical medicine. The localization and functional structure of higher-order language features, such as grammatical and semantic processing for sentences, requires further study to better understand human language processing. We recorded neurophysiologic responses from 1593 electrodes in 17 patients with medically refractory epilepsy during depth electrode monitoring, while they were presented with a four-word sentence [e.g. “the girls ate cakes” (correct grammar + syntax), “the cakes ate girls” (correct grammar + incorrect syntax), or “the ate girls cakes” (incorrect grammar + syntax)] followed by a picture. The sentences were presented either auditorily or visually, and the subjects had to indicate whether the sentence was grammatically and syntactically correct, as well as correctly described the image. High spatiotemporal resolution recordings enabled us to identify selective signals within frontal, temporal, and parietal regions that encoded semantic and syntactic differences between these sentences. This representation of grammar and syntactic features also showed invariance across both auditory and visual presentation modalities. These findings highlight the domain-general (i.e. audiovisual) processes of grammar and semantic perception and provide a novel understanding of language processing in humans.

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Poster

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Program #/Poster #: PSTR193.08/X7

Topic: H.11. Language

Support: 5R01DC004290

Title: Human neural network for vocal production and control: comparing network modes during vocalization and listening

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Abstract: Speech is a human-specific evolution adaptation for communication and relies on a highly adaptable neural system to facilitate vocalization. This network engages several cortical regions—including the prefrontal, auditory, and motor cortices—which function across the sensory-motor hierarchy to integrate auditory and motor processes. Dysfunctions within this network, including hearing loss or developmental communication impairment, can significantly impair vocal control and communication. There is substantial interest in understanding how the vocal production network changes between listening and vocal production; however, outside of intracranial recordings with nonhuman animals, the human vocal production network may have specialized for speech and language, requiring direct study in humans. To address this gap, we studied local-field potentials (LFPs) recorded intracranially from neurosurgical patients undergoing stereo-electroencephalography and electrocorticography for clinical purposes. The recordings were obtained while participants were performing a vocal production task, learning how to control their voice to create sustained or varying pitch vocalizations. The participants' real-time vocal pitch was extracted and displayed on the screen; they were instructed to modify their vowel vocalizations by following a visual target to match their vocal pitch. The target's starting point was calibrated to each subject's baseline pitch, and a subsequent increase of 300 cents per second above this baseline was introduced after approximately 1 second. Each vocalization trial was recorded and immediately played back to the participant through insert earmolds to obtain listening only trials. We analyzed the LFP event-related broadband (1-150 Hz) signals from the dorsolateral prefrontal cortex, inferior frontal gyrus, superior temporal gyrus, and precentral gyrus during vocalization and passive listening trials Our findings revealed a low-frequency (3-8 Hz) suppression pattern during vocalization in the inferior frontal gyrus, whereas an inverse pattern emerged during passive listening. A similar pattern of suppressed low-frequency activity during vocalization was also observed in the premotor cortex. In contrast, the ventral regions of the precentral gyrus exhibited enhanced high-frequency activity (30-70 Hz) during vocalization and enhanced low-frequency activity during listening. These preliminary findings suggest that regions involved in vocal motor control are differentially active in passive listening. The results identify how the vocal production network switches between listening and vocal production.

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Poster

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Topic: H.11. Language

Support: NIH K12 Neurosurgeons Research Career Development Program

Title: Network interactions mediate one-shot learning in the human brain

Authors: *M. GHOSH[1], S. LOWE-HINES[1], A. CRANDALL[1], Q. CHENG[2], K. E. WEAVER[3], A. KO[1], J. OJEMANN[1], B. L. GRANNAN[1];
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Abstract: Network interactions mediate one-shot learning in the human brain*Megha Ghosh[1], Sophia Lowe-Hines[1], Adam Crandall[1], Qi Cheng[2], Andrew L. Ko[1], Jeffrey G. Ojemann[1], Benjamin L. Grannan[1*]*
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Humans have the remarkable ability to learn by making context-based, relational inferences. This often needs to occur “on-the-fly” after a single exposure to a novel stimulus, a behavior referred to as one-shot learning. This behavior is essential for effective communication and linguistic learning. Prior clinical and electrophysiological studies suggest an important role of the hippocampus in verbal learning and context-based prediction. Here, we study hippocampal single neuron activity and brain-wide neural responses and oscillatory interactions during a one-shot language learning task.
We performed intracranial recordings in 15 human participants undergoing intracranial-EEG monitoring as they read sentences of a varying contextual constraint such that the last word of the sentence was either highly predictable or not predictable. The last word was substituted with a pseudoword in 60% of sentences. Sentences with high context and pseudoword insertion provided epochs of one-shot learning and were compared to non-learning trials: low context with pseudoword, low context with real word, or high context with real word. Behnke-Fried micro-macro electrodes were used to isolate single neurons from the hippocampus.
We found significant differences in theta and gamma power and in local theta-gamma phase-amplitude coupling (PAC) between the learning and non-learning trials in the hippocampus and dominant lateral temporal regions. To assess network interactions, we performed PAC and phase-locking analysis of hippocampal units to rhythms across multiple regions. Single hippocampal units showed higher phase locking to theta rhythms during learning trials compared to non-learning, accompanied with differences in PAC between trial types. In addition, there were significant increases in the hippocampal neuronal coupling during correct vs incorrect trials.
Together, these results support a network level response spanning cortical and neocortical structures, that may mediate rapid linguistic learning. Further understanding of this learning mechanism can inform therapeutics for individuals with verbal learning deficits and provide keen insights into development of artificial language models being engineered to achieve “one-shot” word learning.

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Poster

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Topic: H.11. Language

Support: 1RF1NS125026-01A1

Title: Network properties distinguish cortical sites critical to speech and language function

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Abstract: Prior to epilepsy or tumor surgery, it is important to identify focal brain areas that are “critical” for speech and language. The current standard of care is to identify these critical nodes using electrocortical stimulation (ECS), which disrupts the function. ECS is also used to modulate neural activity, e.g., in directly treating epilepsy or pain. However, despite its long history of clinical use, the precise mechanisms of ECS are poorly understood. For example, it is unclear whether ECS’ effects on behavior are due to affecting only the local cortex, other cortical areas, and/or underlying white matter. To investigate these questions in the brain's language network, we recorded electrocorticography (ECoG) from sixteen participants while they performed a word-reading task. We extracted high-gamma activity from ECoG recordings and computed the pairwise connectivity between electrodes. We then calculated graph theory metrics from the functional connectivity to examine the network properties of critical sites for speech and language (those producing speech arrests and language errors, respectively) and compared to other (non-critical) cortical sites. Critical sites could be distinguished by their profile of network properties. This profile included global and local connectivity (strength, clustering coefficient, and local efficiency) that was lower in both speech arrest and language error sites than in non-critical sites. Notably, language error sites had higher participation coefficients than all other sites, indicating that they served as connectors between modules in the language network. We used the network metrics to classify critical vs. non-critical sites with relatively high accuracy, including across participants. This suggests that a site’s pattern of connections within the language network helps determine its importance to language function.

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Poster

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Support: Alchemist Project (20012355, Fully implantable closed loop Brain to X for voice communication) funded by the Ministry of Trade, Industry & Energy (MOTIE, Korea).

Title: Analyzing Spatiotemporal Dynamics of Semantic and Syntactic Processing: Intracranial Neural Decoding Before Speech Production

Authors: *Y. PARK[1], J. KWON[2], C. CHUNG[3];
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Abstract: Sounds and sound patterns are just one aspect of language; meanings are conveyed through semantics and syntax. Semantics involves the rules for meaning, while syntax governs the combination of words into phrases and sentences. Current research on speech Brain-Computer Interfaces (BCIs) lacks detailed understanding of the neural mechanisms behind semantic and syntactic processing. Our study aims to fill this gap by exploring the spatiotemporal features of these processes using intracranial recordings before speech occurs. This study involved 20 intractable epilepsy patients who underwent surgical placement of electrocorticography electrodes. Data were recorded from these patients during a task that required reading words, which were grouped either semantically—as body parts or non-body parts—or syntactically—as subjects or predicates. We extracted averaged amplitudes of high-gamma (70-170 Hz) using continuous wavelet transform data within a 150ms time window. Non-parametric t-tests were applied to identify crucial spatiotemporal neural features that distinguish between these semantic and syntactic groups. For the decoding task, a random forest classifier was employed.
In this study, notable differences in brain activity between semantic and syntactic categories were observed. Spatially, brain activity initially involved the frontal areas, particularly the left inferior frontal gyrus (IFG), and progressed to include the frontal, parietal, and temporal areas—highlighted by activity in the left IFG, angular gyrus, and medial temporal gyrus (MTG) as speech onset approached. The highest accuracy for distinguishing between body parts and non-body parts was 81% ±14, occurring during the middle phase (300-450 ms) after word presentation. In terms of syntax, the IFG showed dominant activity during both the initial (0-150 ms) and the last phase (600-750 ms). The highest accuracy in distinguishing between subjects and predicates was 75.5% ±8, occurring during the last phase.

Our findings have elucidated the spatiotemporal processes engaged in pre-speech language processing through successful decoding. Specifically, our results align temporally with previous research, including N400, ELAN and P600 in speech processing. Also, we could successfully align previously identified speech-related areas spatially, such as the IFG, superior temporal gyrus (STG), and MTG. By investigating these spatiotemporal contributions, we have decoded semantic and syntactic processing, potentially extending beyond the current limitations of speech BCI technologies.

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Poster

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Title: Neural entanglement of working memory and language: insights from human laminar fMRI and array recordings

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Abstract: The extent to which human language functions interact with those for domain-general cognition across the cortical layers is unknown. Studies of dorso-lateral prefrontal cortex (DLPFC) in nonhuman primates have implicated layer 3 neurons in a recurrent circuit for working memory (WM), and a canonical microcircuits model describes the superficial and deeper layer involvement in feedforward and feedback interactions between brain areas. Here, we focus on DLPFC, often implicated in WM but not language function, to test the hypothesis of a neural entanglement between WM and language combinatorial semantics across layers. In our task, participants heard two or three words, and they either alphabetized or maintained the word order during the delay, after which they verbally reported their mental order. On a trial-by-trial

basis, participants would either manipulate the words into or out of a grammatical order. We analyzed high-density laminar array recordings obtained during deep-brain stimulation procedures in neurosurgery patients at the University of Iowa. Extracellular recordings were compared with laminar fMRI (0.8mm)-capable of resolving sets of layers and broader system interactions-in healthy participants and patients scanned preoperatively. Laminar array recordings from DLPFC showed single neurons, including those in the approximate location of layer 3, modulated by all task components. WM manipulation-specific effects were strongest in layers 3 and 6. The language grammatical effects, although expectedly weaker than those for WM, engaged both superficial and deeper layers in DLPFC. The laminar fMRI results recapitulated many of the laminar array recording effects, and they showed overlapping clusters for grammatical and WM effects in a network of regions, including DLPFC. Patient clinical tissue samples taken from the recorded area after task performance are being analyzed for single-nuclear multiomics (RNA+ATAC sequencing) and compared to control samples taken prior to task performance. The results provide initial insights into the interplay of working memory and language across cortical layers from an area in the human brain often identified for its cognitive domain-general function but not language.

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Poster

PSTR193: Language and Communication: Neural Circuits and Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR193.13/X12

Topic: H.11. Language

Support: University of Sussex start-up funds

Title: Electrophysiological evidence for prediction errors during perception of degraded spoken sentences

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Abstract: Prediction facilitates language comprehension but how are predictions combined with sensory input during perception? Previous work suggests that cortical speech representations are best explained by prediction error computations rather than the alternative ‘sharpened signal’ account. The key signature of prediction error is an interaction between bottom-up signal quality and top-down predictions. When predictions are uninformative, increasing bottom-up signal

quality results in enhanced neural representations. However, the opposite occurs when predictions are informative (suppressed neural representations with increasing signal quality). Here we explore a listening situation more naturalistic than in previous work in which listeners heard sentences and predictions obtained directly from the speech signal i.e. from sentence context. In Experiment 1, listeners (N=30) heard degraded (16-channel noise-vocoded) sentences in which the context was strongly or weakly predictive of the last word, based on cloze probability. All sentences were semantically coherent. We also manipulated signal quality of the final word (2/4/8 channels). Using Temporal Response Function (TRF) analysis of EEG responses to the final word, we measured cortical representations of speech acoustic features (spectral and temporal modulations). We observed a significant interaction between context predictiveness and signal quality (F-test, $p = .04$). However, follow-up tests showed that there was only a marginal effect of signal quality on TRF model accuracies within the weakly predictive condition. In follow-up Experiment 2, listeners (N=31) listeners heard final words varying more strongly in signal quality (4/8/16 channels). We also included a control condition in which sentence context was unintelligible (1-channel noise-vocoded) and therefore completely uninformative about the final word. Here we observed a more robust interaction between context predictiveness and signal quality (F-test, $p < .001$). For the unintelligible context, increasing signal quality led to increased TRF model accuracies (F-test, $p < .001$) while for the strongly predictive context, increasing signal quality led to reduced model accuracies (F-test, $p = .008$). These findings are more consistent with the prediction error account and show that previous findings extend to more naturalistic listening situations.

Disclosures: J. Webb: None. **E. Sohoglu:** None.

Poster

PSTR193: Language and Communication: Neural Circuits and Mechanisms

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Topic: H.11. Language

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Title: Variability of neural effective connectivity caused by self-image in a mirror in linguistic judgments

Authors: *S. TOKIMOTO[1], N. TOKIMOTO[2];
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Abstract: The self-awareness of one's behavior can change the behavior itself, and the self-image in a mirror can increase the self-awareness. In this study, we examine the neural mechanisms for the variability of linguistic judgment by manipulating the self-image in a mirror. 27 native speakers of Japanese listened to 180 Japanese simple sentences in two experimental blocks that described categorical judgments, in which their naturalness was manipulated in three

ways: typical, less typical, and anomalous, with 60 sentences for each (e.g., A sparrow is a bird, A chicken is a bird, and Gas is a bird, respectively). They were asked to press a button when they judged a sentence unnatural, with their EEG recorded using a 64-channel EEG amplifier. A whole-length mirror was placed in front of them in one of the two blocks. As a behavioral result, the less typical sentences were judged unnatural more often for With-Mirror condition than No-Mirror condition in the first block and more often for No-Mirror condition than With-Mirror condition in the second block. The unnatural judgment rates for less typical sentences in With-Mirror condition were significantly correlated with the participants' scores of Imagination in Autism Spectrum Quotient (AQ) and those of Empathy Concern (EC) in Interpersonal Reactivity Index (IRI). To examine the possible change of effective connectivity caused by the self-image, we placed 27 regions of interest (ROIs) in the brain, referring to the recent fMRI study on the self-recognition, in which the first- and the third-person perspectives were manipulated by video clips. We also localized the source of the N400 elicited by the anomalous sentences as the 28th ROI at the left posterior cingulate cortex (PCC) through the dipole fitting. We observed a significant enhancement of event-related spectral power on the scalp in gamma band (30-50 Hz) from 100 to 300 ms after the onset of a critical word for With-Mirror against No-Mirror conditions. We thus calculated partial directed coherence (PDC) between the 28 ROIs for the gamma bands in 100 to 300 ms latency. We observed significant decreases of PDC for the connectivity to the left PCC from the left precuneus and from the right inferior frontal gyrus. The former decrease was significantly correlated with the participants' age and the score of EC in IRI, and the latter was correlated with their sex, age, the scores of Social Skills and Imagination in AQ. Our analysis suggests that the self-image affected interaction between the default mode network and language network. This study is the first to show the neural mechanisms of linguistic judgments and its possible change due to the speakers' individual differences of sociality.

Disclosures: S. Tokimoto: None. **N. Tokimoto:** None.

Poster

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Topic: H.11. Language

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Title: Exploring the interplay between auditory-motor synchronization and cognitive skills

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Abstract: Auditory-motor synchronization is the inherent human ability to align motor gestures with a rhythmic auditory stimulus. This ability has gained attention over the past 20 years, with research indicating a correlation between auditory-motor synchronization and language related abilities1,2. Our main goal is to determine whether this relationship is restricted to linguistic abilities or if it extends to other more general cognitive domains. In this direction, we evaluated a large cohort of participants using: (i) an extensive cognitive battery that included six language related tasks and eight non-related ones; and (ii) a simple behavioral protocol2,3, that binarily categorizes subjects as good or bad auditory-motor synchronizers. Subsequently, we conducted a PCA analysis of the cognitive scores. We discovered that the component explaining the most variance, which also has a similar weight across almost all scores, significantly distinguished between bad and good synchronizers. Our results evidence an interplay between general cognition and auditory-motor synchronization which is more extensive and complex than previously assumed.

Disclosures: F. Lizcano Cortés: None. **F.A. Barrios:** None. **M.F. Assaneo:** None.

Poster

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Topic: H.11. Language

Title: How bringing EEG-Steady State Visual Evoked Potentials (EEG-SSVEP) reading studies into schools is actively reshaping our lab-based experimental paradigms

Authors: *F. WANG;
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Abstract: How bringing EEG-Steady State Visual Evoked Potentials (EEG-SSVEP) reading studies into schools is actively reshaping our lab-based experimental paradigms.
Fang Wang[1], Elizabeth Y. Toomarian[1,2], Radhika S. Gosavi[1,2], Lindsey R. Hasak[1],Vani Dewan[1], Stephen Gonzalez[1], Bruce D. McCandliss[1]
[1]Graduate School of Education, Stanford University, Stanford, CA, USA[2]Synapse School, Menlo Park, CA, USAEEG research on reading development is typically a lab-bound enterprise that relies heavily on paradigms optimized for adults. The causal forces that drive reading development, however, take place within the minds of children, interacting with teachers, situated within school environments. In this talk, we review how conducting research in the context of a partnership with a local elementary school, by bringing EEG into schools, has changed the way we carry out research, especially in understanding the central role of orthographic coding in English learning. We began by adapting EEG-SSVEP paradigms proven to index visual word hierarchy to a deliberately slower frequency (e.g., 3Hz), closer to an optimal pace for children learning to read. We distinguished unique cortical sources for whole word lexical representation and visual word form structure (VWFS) in early readers.

Longitudinal investigations revealed remarkable changes in brain responses to VWFS which were linked to reading growth, supporting the functional role of VWFS in early reading ability. Next, in collaboration with teachers, we counterbalanced instructional materials to examine the relationships between different spelling activities (focused on lexical and sublexical units) and changes in brain sensitivity to VWFS. This project will provide insights for teachers on how to effectively teach children to flexibly utilize different aspects of orthography (e.g., onsets, rime units). Our EEG work in a school context helps bridge the gap between laboratory research and classroom dynamics, which allows for a better understanding of the complexities involved in orthographic coding and early reading acquisition.

Disclosures: F. Wang: None.

Poster

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Support: This study has been supported by a General Research Fund (GRF) from the Research Grants Council of the Hong Kong Special Administrative Region, China [Project No 17608922].

Title: Neural Correlates of Orthographic, Phonological, and Semantic Processing in Biscriptal Readers of Chinese and English

Authors: T. JIANG[1,2], W. DENG[2], N. WANG[1], *W. SIOK[1];
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Abstract: Reading involves the integration of existing phonological and semantic knowledge with later acquired orthographic knowledge to interpret visual symbols. While previous studies have examined the neural mechanisms involved in reading for monolinguals across various languages, it remains unclear whether reading processes are universally similar or differ across writing systems, such as Chinese and English. To address this gap, this study aims to precisely identify the neural representations associated with orthographic, phonological, and semantic processing in Chinese and English among biscriptal readers.
Thirty-one college students (26 females) aged between 18 and 25, who were native Mandarin (L1) speakers with over 10 years of English (L2) learning experience, participated in the study. Functional magnetic resonance imaging (fMRI) was used to measure brain activity during the experiment. Participants were presented with pairs of Chinese or English words/pseudowords, as well as line/figure patterns in blocks. They performed tasks involving component/letter search, rhyme judgment, and synonym judgment. Baseline tasks included font-size comparison, line-pattern judgment, and figure judgment. Univariate activation analysis and representational

similarity analysis (RSA), were conducted to explore the similarity of neural representations for orthographic, phonological, and semantic tasks in Chinese and English.
Compared to the font-size baseline task, the univariate activation analysis revealed both overlapping and distinct neural activation patterns in orthographic, phonological, and semantic processing across both scripts. The overlapping activation was primarily observed in the left mid-inferior frontal region. Script-specific differences were more prominent in orthographic and phonological processing, while semantic processing showed a higher degree of cross-script similarity. Specifically, orthographic tasks elicited activations in the inferior parietal lobule and bilateral occipital regions in both scripts. However, the Chinese orthographic task recruited a more extensive visual network, including bilateral fusiform areas. The whole-brain RSA analysis indicated categorically distinct neural representations for orthographic processing compared to phonological and semantic tasks in Chinese, whereas the differences were less pronounced in English. Overall, these findings suggest that reading involves a combination of universal and script-specific processing across different scripts.

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Poster

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Program #/Poster #: PSTR193.18/X17

Topic: H.11. Language

Title: Neural Pathways of Vocal Pitch Modulation: An fMRI Study

Authors: *M. KIM, G. S. HICKOK;
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Abstract: Human communication heavily relies on the modulation of vocal pitch, yet the neural mechanisms underlying both the control and perception of pitch remain largely underexplored. This research investigates these mechanisms using functional magnetic resonance imaging (fMRI) to enhance our understanding of vocal pitch coordination and its evolution in human communication. To accomplish this, we have developed two fMRI experimental approaches. The first experiment seeks to identify the neural circuits involved in pitch modulation within the speech production network, where we compare neural activations during speech repetition tasks (Hickok *et al.* 2009, *J. Neurophysiol.*; Rong *et al.* 2018, *PLoS ONE*) under two scenarios: one involving complete rehearsal and the other limited to humming the prosodic patterns of the auditory stimulus. The second experiment aims to separate the pitch-specific pathways from general speech coordination networks by analyzing neural responses to speech repetition tasks under conditions of Altered Auditory Feedback (AAF; Tourville *et al.* 2008, *Neuroimage*; Niziolek & Guenther 2013, *J. Neurosci.*). In both experiments, we aim to delineate the specific neural circuits that are activated by changes in pitch. Early findings from these studies suggest a consistent neural link between pitch variations and specific areas of the premotor cortex,

particularly area 55b (Glasser *et al.* 2016, *Nature*). These observations support hypothetical pitch-related functions of this region independently proposed by Silva et al. (2022, *J. Neurosci.*) and Hickok et al. (2022, *Brain*). This work not only deepens the understanding of the neural basis of vocal pitch and human language but also holds potential implications for clinical interventions in speech and language disorders.

Disclosures: M. Kim: None. **G.S. Hickok:** None.

Poster

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Topic: H.11. Language

Support: NBRC Core Fund

Title: Reorganization of structural and functional connectivity in the language network across lifespan ageing trajectories

Authors: *P. JAIN[1], A. AKHTER[1], A. BANERJEE[2];
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Abstract: Age-related decline is recurrently seen in various cognitive functions like attention, executive functions, memory, and language production, while language comprehension tends to remain intact throughout the lifespan. This phenomenon may reflect the preservation of functional or anatomical connections between the key regions (Pars-T, Pars-O, MTG, STG, and STS) of the Language Network (LAN). To investigate this hypothesis, we analysed the relationship among Functional Connectivity (FC), Structural Connectivity (SC) and Language behaviour (Comprehension and Production) across the cross-sectional adult lifespan of a large healthy cohort (n =652, age range = 18:88, CamCAN). Partial correlation and essential non-parametric statistics were employed to control for confounding factors (handedness, age, gender) and non-linearity, respectively. Our analysis revealed a general decline in FC with age, particularly among inter-hemispheric connections of the LAN, while some functional connections were preserved within the right hemisphere. Although the inter-hemispheric SC decreased, SC among MTG, STG, and STS was found to be significantly increasing with age, accompanied by decreased connectivity involving the Pars-T and Pars-O regions. Furthermore, SC among MTG, Pars-T, and Pars-O was significantly associated with language production tasks (verbal fluency and Tip of Tongue), while SC between STS and MTG correlated significantly with language comprehension tasks (vocabulary). Our findings suggest that FC reflects the SC to a greater extent, but there is not always a straightforward one-to-one mapping. Language behaviour appears to be more closely related to variations in SC than FC within the LAN, emphasising the importance of preserving certain connections within the LAN for maintaining language comprehension across the lifespan. This study highlights the significance of the

conservation of brain function and structure to sustain healthy cognitive abilities throughout one's life trajectory.

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Poster

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Topic: H.11. Language

Support: Veteran's Affairs RR&D (USA) grant 1IK2RX002934

Title: Resting-state functional connectivity underlying semantic fluency task difficulty

Authors: *R. PANG[1], L. KRISHNAMURTHY[2], E. SMITH[3], V. KRISHNAMURTHY[2]; [1]Biol., Emory Univ., Atlanta, GA; [2]Atlanta VA Healthcare Syst., Atlanta, GA; [3]Georgia State Univ., Atlanta, GA

Abstract: Exploring mechanisms underlying cognitive processes is essential to advance our understanding of human behavior. Resting-state functional connectivity (rsFC) estimates the correlated signal between functionally related brain regions in the absence of any stimulus or task. Currently, there is a knowledge gap in our understanding of underlying rsFC supporting semantic fluency (SF) that is modulated for task difficulty.35 participants, 18 young and 17 old, underwent a resting-state functional magnetic resonance imaging (fMRI) scan along with the SF task fMRI in the same scan session. Task stimuli were titrated for difficulty (e.g. easier categories such as "flowers" versus harder categories such as "types of fabric") and were assessed for accuracy (i.e. % correct response) from 144 stimuli averaged together. rsfMRI scans were processed using the standard procedures. rsFC maps were generated using seed-based correlation analysis for 9 ROIs identified from the task fMRI. 3dTtest++ was used to determine the rsFC differences between age groups that were assessed at a voxel-wise $p \leq 0.01$. False positives (FP) were controlled for using 3dClustsim. Multivariate analysis (LESYMAP) was used to estimate the brain-behavior relationships.We observed a stronger rsFC for the young group seeded from the posterior cingulate cortex (PCC), anterior preSMA, and posterior preSMA (FP rate <8%). LESYMAP showed PCC to right angular gyrus (R-AG) connectivity that had an inverse relationship with accuracy ($p \leq 0.01$ for young and old groups). No significant brain-behavior relationships were observed for anterior or posterior preSMA.As a function of aging, we observed a strong decline in functional brain connectivity (i.e., seeded from PCC and preSMA), consistent with the aging literature. Irrespective of age group, the inverse relationship between PCC-to-R-AG rsFC and task accuracy suggests that stronger rsFC from PCC to R-AG impedes task accuracy. Considering the R-AG involvement in processing increased SF difficulty, which is accompanied by the need to access semantic memory (governed by PCC), our results show the underlying brain connectivity supporting a naturalistic task such as semantic fluency.

Thus, while we did not investigate difficulty as a variable, previous literature is consistent with the observed accuracy drop as reflected by the inverse relationship. Since preSMA is involved in the executive functioning aspects of SF, it is not surprising that we did not observe its rsFC contributing directly to task accuracy. Our future work will systematically investigate the rsFC profile specific to the degree of SF difficulty as a function of aging.

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Poster

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Title: Simultaneous suppression as a measure of processing capacity in the reading circuitry

Authors: *V. S. CHAUHAN[1], K. MCCOOK[2], A. WHITE[1];
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Abstract: Our ability to recognize written words depends on a brain region in the occipito-temporal sulcus known as the visual word form area (VWFA). Nearly all neuroimaging studies of this region have presented just one word at a time, which clearly differs from typical reading conditions. It is therefore unknown how the response of the VWFA varies with the number of words being read. We present a human fMRI study that fills that gap by leveraging the simultaneous suppression paradigm, which has demonstrated processing capacity limits in other parts of visual cortex. On each trial, participants viewed a sequence of three pairs of character strings in which we embedded 0, 1 or 2 English words. Most of the character strings were “false fonts” with visual features matched to the real letters. Thus, visual stimulation was kept constant across conditions. Crucially, there were two types of two-word trials: simultaneous presentation, in which the two words appeared in the same frame, and sequential presentation, in which one word appeared before the other. We found two main results: first, a linear increase in the VWFA’s response magnitude from the zero, one and two-word sequential conditions. This demonstrates that each word recognition process adds to the overall BOLD response. Second, we found evidence for simultaneous suppression: a lower response to the simultaneous presentation of two words than the sequential presentation. This demonstrates a strong limit to the VWFA’s capacity to process two words in parallel, consistent with a series of behavioral studies.

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Poster

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Title: Investigating sulcal correlates of language processing

Authors: *A. L. PRACAR[1], S. A. MABOUDIAN[2], N. BIONDO[3], N. F. DRONKERS[4], K. S. WEINER[5];
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Abstract: Structural distinctions of the human brain from non-human primates, crucial for language, extend from microscopic cellular arrangements to macroscopic anatomical differences. These include a marked leftward asymmetry in Broca's area (BA44/45) and pronounced structural characteristics of the left Sylvian fissure and the left planum temporale. These adaptations suggest a specialized neural architecture in humans for processing language. Here we aim to delineate structural features supporting language by analyzing sulcal variations in language-associated regions across hemispheres in healthy adults.
Using T1 MRI scans processed with FreeSurfer, we manually defined 19 sulci in the lateral prefrontal cortex (lPFC) and 13 sulci in lateral parietal/lateral parieto-occipital junction (LPC/LPOJ) in 66 adults (age 18-35; 50% male) from the Human Connectome Project (HCP). We applied LASSO regression with nested cross-validation to identify sulci whose depths were most predictive of performance on a language task (Oral Reading Recognition). We then assessed the relationship between these LASSO-selected sulci and other measures of language, cognition, and emotion [Picture Vocab (language), Flanker (executive function, EF), and Penn Emotion Recognition tasks] using linear regression models, to compare specificity across cognitive domains.
Our analysis revealed an optimal model relating reading performance to the depth of 6 specific left hemisphere LPC/LPOJ sulci: the superior temporal sulcus (STS) and two caudal rami of the STS, inferior temporal occipital sulcus, anterior intraparietal sulcus of Jensen, and superior parietal sulcus were selected by the LASSO as being most associated with reading. The depths of these LASSO-selected sulci significantly predicted performance on another language task (Picture Vocab; r=0.26, p=0.03) and with trending significance in the other cognitive (EF: r=0.23, p=0.06) but not emotion (r=0.071, p=0.57) tasks. In the LPFC, the LASSO-selected model for reading selected the following sulci in the right hemisphere only: the ascending ramus of the lateral fissure (aalf); the ventral component of the intermediate frontal sulcus (infs-v);

anterior, inferior, and posterior components of the posterior middle frontal sulci (pmfs-a, pmfs-i, pmfs-p); and the pretriangular sulcus in Broca's area (prts).
These structural features provide a more nuanced understanding of the neuroanatomical basis for language capabilities, supporting findings from earlier studies (Palomero-Gallagher & Zilles 2019, Segal & Petrides 2012) that have emphasized hemispheric and regional variations in brain structure related to language.

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Poster

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Topic: H.11. Language

Support: NIH Intramural Research Support
NIDCD Intramural Research Support

Title: A functional connectivity approach to understanding large scale language related network dynamics during task-based fMRI

Authors: A. KUROSU[1], J. LIANG[1], N. SINAII[2], K. WINGERT[1], *N. BIASSOU[3,1];
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Abstract: INTRODUCTION: Recent studies have begun to apply machine learning models with patterns of functional connectivity (FC) in functional MRI (fMRI) to develop neuro-computational models for language processing. Previous studies suggested static resting-state FC in fMRI (rs-fMRI) and task-based fMRI (tb-fMRI) region-of-interest in language mapping are interchangeable. However, it is unknown whether dynamic FC (dFC) is interchangeable for the two modalities. Thus, network correlation variability overtime during rs- and tb-fMRI was analyzed in healthy participants.METHODS: We analyzed 3T fMRI data from 172 healthy participants (86 females, 27.6 ±3.8 years) from the Human Connectome Project. Participants lied down still during rs-fMRI and they completed an auditory comprehension task (listening to short stories) and a math task (addition/subtraction) as the baseline control during tb-fMRI. Sliding-scale time series correlation was employed to analyze dynamic FC time series data with a sliding window of 42 and stride of 3. Spearman correlation with Bonferroni correction was used to identify common network correlations over time. Linear mixed models with error and intercept random effects were used for statistical analyses. RESULTS: Distinct correlation variability pattern differences were found between rs- and tb-fMRI. Overall, correlation strengths during the resting state were spread across a wide range with no significant variability over time in any of

the networks ($p>0.12$). Correlation strengths during task-based condition demonstrated an increase in correlation strength in the left and right hemispheric connections, and decreased strength in inter-hemispheric connections ($p<0.001$). There was a tightening of correlations over time (as measured by SD reduction) around the new mean in left, right and inter-hemispheric connections compared to resting state correlations ($p<0.001$). In particular, mean correlation strengths in some task-based networks, left > right hemispheric networks, showed a robust oscillatory pattern, similar to auditory evoked potential N1-P2 oscillatory waveforms. Other left hemispheric networks demonstrate patterns that parallel resting state networks.
CONCLUSIONS: fMRI is thought to have good spatial but poor temporal resolution. Our findings suggest that some temporal patterns may indeed be reflected in dFC BOLD signal correlations and may be exploited to develop computational models that incorporate large scale tasked-based network dynamics to more accurately predict cerebral dominance, and/or to better understand a patient's complete neurolinguistic profile following CNS injury.

Disclosures: A. Kurosu: None. **J. Liang:** None. **N. Sinaii:** None. **K. Wingert:** None. **N. Biassou:** None.

Poster

PSTR193: Language and Communication: Neural Circuits and Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR193.24/X24

Topic: H.11. Language

Support: UT Southwestern OBI Sprouts Grant
Simons Foundation Bridge to Independence Award
NIH Award R00-HD057522
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MIT Simons Center for the Social Brain

Title: The cerebellum maintains a typical language profile following damage to the left temporal lobe in the cerebral cortex

Authors: *B. WANG[1,2], G. TUCKUTE[3,4], H. KEAN[3,4], A. M. PAUNOV[3,4], I. A. BLANK[3,4], E. FEDORENKO[3,4], A. M. D'MELLO[5,6,2];
[1]The Univ. of Texas at Dallas, Richardson, TX; [2]Department of Psychology, The University of Texas at Dallas, Richardson, TX; [3]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA; [4]McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA; [5]Dept. of Psychiatry, UT Southwestern Med. Ctr., Dallas, TX; [6]Peter O'Donnell Jr. Brain Institute, UT Southwestern Medical Center, Dallas, TX

Abstract: Language comprehension is supported by a distributed network of predominantly left-lateralized frontotemporal neocortical regions. Although rarely considered part of the canonical

language network, the cerebellum is also recruited for language comprehension. The cerebellum is connected to the neocortex via a series of closed-loop reciprocal circuits, and it is thought that cognitive functions in the cerebellum arise as a result of inputs from these contralateral neocortical regions.
An important unanswered question is how cerebellar language regions emerge and develop sensitivity to language. A powerful approach to answer this question is to examine unique case studies who are missing critical language regions. In one such case study, participant EG, perinatal damage to the left temporal lobe resulted in plasticity in the neocortical language network including a shift of language sensitive regions to right-hemisphere homologues. Notably, EG's left inferior frontal gyrus, including Broca's area, remained insensitive to language, indicating that left temporal regions are critical for the development of language sensitivity in the adjacent frontal cortex. Using data collected from EG, we asked whether and how the loss of these neocortical language regions and subsequent reorganization for language would affect the topography and magnitude of cerebellar language responses.
We compared functional MRI (fMRI) data acquired from EG to that from a comparison group of n=116 typically-developing (TD) adults. All participants completed a sentence reading task to localize language responsive brain regions. In TD participants, cerebellar language activation was predominantly right-lateralized to lobules VI, Crus I/II, VIIB, VIII, and IX, mirroring left-lateralization of language in the cerebral cortex, although also largely bilateral (p_{unc}<0.001). In EG, as expected, cerebellar language areas were primarily left-lateralized, mirroring the right-lateralization of language in the neocortex. The topography of EG's left cerebellar clusters mirrored right-lateralized cerebellar language areas identified in TD adults. Remarkably, EG also exhibited bilateral cerebellar activation for language, despite the putative absence of inputs from the contralateral left frontotemporal neocortex. These findings suggest that cerebellar reorganization patterns differ from those in the cerebral cortex and that cerebellar language regions maintain a more typical topography, even without input from contralaterally connected language regions.

Disclosures: B. Wang: None. **G. Tuckute:** None. **H. Kean:** None. **A.M. Paunov:** None. **I.A. Blank:** None. **E. Fedorenko:** None. **A.M. D'Mello:** None.

Poster

PSTR193: Language and Communication: Neural Circuits and Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR193.25/X25

Topic: H.11. Language

Support: NIH Grant NS116883

Title: Role of the human cerebellum in content prediction: distortions of cortical responses to violation of semantic predictions in cerebellar degeneration

Authors: M. J. ROTH[1], K. D. ANDRADE[2], R. B. IVRY[3], *A. BRESKA[1];
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Abstract: Adaptive interaction with our rapidly and continuously evolving environment relies on predictions of future events, based on embedded statistical regularities. The cerebellum has been associated with prediction, traditionally in spatiotemporal motor control, but recently also in sensory prediction and attention, particularly in the temporal domain. However, in addition to predicting location and time, the brain also predicts content, or the "what" of future events. A well-established example of this is semantic prediction in language, where semantic incongruency between a word and its preceding context leads to degraded performance and elicits prediction error neural responses. However, the role of the cerebellum in semantic prediction has been the subject of considerable debate, with fMRI studies finding cerebellar activations, yet cerebellar degeneration (CD) patients showing intact ability to detect violations. Here, we addressed this issue by investigating the neurophysiological signatures of semantic prediction in CD patients compared to healthy controls using EEG recording. Participants viewed sentences presented visually, word by word, with the last word either congruent or incongruent with the preceding sentence. The sentences had high cloze probability, to maximize predictability of the sentence context. In this context, an incongruent sentence-ending word is known to elicit a neural violation response, the N400 EEG potential. Impaired feature-based predictions in cerebellar patients should be reflected in an alteration or even abolition of this EEG response. Our behavioral findings indicate that cerebellar patients showed high accuracy in detecting semantic violations, though lower than controls. Nevertheless, there were discernible discrepancies between the neurophysiological patterns of patients and controls, underscoring the pivotal role of the cerebellum in these predictions. Most importantly, the N400 response was not abolished in cerebellar patients, but rather its latency was delayed. Moreover, inter-trial phase consistency (in delta frequency band) of the violation response to incongruent target words was modulated in patients, pointing to a role of the cerebellum in temporal consistency of semantic predictions. These findings reconcile previous conflicting results and reveal a novel neurofunctional role of the cerebellum in feature prediction in the language domain.

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Poster

PSTR193: Language and Communication: Neural Circuits and Mechanisms

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Program #/Poster #: PSTR193.26/X26

Topic: H.11. Language

Support: MH094258
Kiwanis Neuroscience Research Foundation

Title: Neuroanatomical correlates of response patterns in a measure of phonemic verbal fluency by participants with focal brain injury

Authors: *C. DEIFELT STREESE[1], K. OGDEN[2], J. E. BRUSS[3], J. D. SKYE[4], D. TRANEL[2,4];
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Abstract: The Controlled Oral Word Association Test (COWAT), a longstanding measure of phonemic verbal fluency, is traditionally scored by counting the number of valid responses given. However, this approach does not capture the richness and complexity of responses generated by individuals. Additional response characteristics, such as semantic or lexical categorization, can help elucidate other important features of COWAT performance. We aggregated 56,689 COWAT responses from a sample of 925 individuals (47.9% female) with focal, stable brain lesions covering the cortex, and we used these data to understand the relationship between focal brain damage and altered response patterns. A total of 5,804 unique responses were identified, and these were tagged as belonging to the categories of (1) animals or (2) verbs. These initial categories were chosen as they are known to be selectively impaired in tasks of cued word retrieval following focal brain injury, thus leading the researchers to hypothesize that altered COWAT response patterns in these categories would be associated with damage to the (1) left anterior frontal lobe and (2) left inferior frontal gyrus. Overall COWAT performance was regressed out, and participants whose responses in these categories were 1.5 standard deviations below the sample mean were considered selectively impaired. These participants (n= 36, 37) were retained for lesion overlap analyses. Lesion masks for each participant were generated via manually-selected voxels from magnetic resonance imaging and transformed into a standard template space. Lesion overlap maps indicated that fewer category-specific words generated than expected was associated with damage to the (1) left inferior frontal gyrus and (2) left inferior temporal lobe. Our results align with our hypotheses and demonstrate that established patterns of deficit in cued language generation are also present in phonologically-constrained free association. These data also support that item-level responses by individuals with focal brain lesions are sufficiently sensitive to broader patterns of word generation for use in lesion analyses and can be used to query neural correlates of these processes. These findings give confidence that item analyses of words generated in the COWAT by participants with focal brain damage can yield further insights into the underlying neuroanatomical correlates of word generation.

Disclosures: C. Deifelt Streese: None. **K. Ogden:** None. **J.E. Bruss:** None. **J.D. Skye:** None. **D. Tranel:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

Location: MCP Hall A

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Program #/Poster #: PSTR194.01/X27

Topic: H.13. Schizophrenia

Support: K23MH06683
R01MH118484
R01MH102951
R01MH113800

Title: Cortical myelin mapping in neurobiological subgroups of patients with psychosis-spectrum disorders

Authors: *V. L. KING[1], A. C. LAHTI[2], M. DEFENDERFER[3], N. V. KRAGULJAC[4]; [1]Univ. of Alabama, Birmingham, Birmingham, AL; [2]Dept. of Psychiatry and Behavioral Neurobio., The Univ. of Alabama at Birmingham, Birmingham, AL; [3]Res. Computing, The Univ. of Alabama at Birmingham, Birmingham, AL; [4]Psychiatry and Behavioral Hlth., The Ohio State Univ., Columbus, OH

Abstract: Background: Converging evidence from postmortem, genetic, and neuroimaging studies have implicated myelin abnormalities in psychosis-spectrum pathophysiology. Normative modeling was developed as a way to examine interindividual differences based on the lifespan trajectory of brain development when considering age and sex. The T1w/T2w ratio was developed as an indirect, in vivo proxy for myelin content based on the physiological properties of myelin. Here, we produced cortical myelin maps to assess potential neurobiological mechanisms of psychosis-spectrum disorders based on individual abnormalities. **Methods:** We registered the normative model developed for cortical thickness to our study site using 130 healthy controls. We then plotted our patients along the average trajectory for each region of the Destrieux atlas. We used a cutoff of 2 standard deviations in each region to determine extreme deviations. Using a cutoff of 4 extreme deviations per subject, we classified our patients into 4 groups: those with mostly positive deviations, those with mostly negative deviations, those with under 4 positive and negative deviations, and those with over 4 positive and negative deviations. We also generated cortical myelin maps in 91 antipsychotic medication-naïve first-episode psychosis patients and 107 healthy controls and calculated the average myelin content for each subject. We then compared the myelin content between patients and controls using an ANCOVA with SES as a covariate to determine group differences in gray matter myelin content. **Results:** Group membership was significantly associated with T1w/T2w ratio values (Wilks Lambda = 0.09, $p < .01$). Using a stepwise algorithm to determine the most parsimonious subset of the data that contains this signal yielded a subset of 16 regions which is sufficient to describe this relationship. The correlation of the canonical variate based on all ROIs and the canonical variate based on only these 16 ROIs is $r = .74$. **Discussion:** Our data demonstrate increased myelination in antipsychotic-naïve, first-episode patients with psychosis-spectrum disorders, suggesting aberrant myelin-related activity in the early stages of the illness. Additionally, we underscore the importance of considering age- and sex-related factors when interpreting studies of myelin. These findings highlight a potential underlying pathophysiological feature and suggest that aberrant functioning of myelin, via oligodendrocytes and/or myelin-related genes, during critical periods of development may lead to toxic overexpression of cortical myelin in psychosis-spectrum disorders.

Disclosures: V.L. King: None. **A.C. Lahti:** None. **M. Defenderfer:** None. **N.V. Kraguljac:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

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Topic: H.13. Schizophrenia

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Hungarian National Research, Development and Innovation Office (NKFIH 2017-1.2.1-NKP-2017-00002, National Brain Research Programs NAP 2.0, NAP 3.0 and NAP2022-I-4/2022).

Title: Protein aggregation of DISC1, as assayed by insolubility, varies across the brain of an individual with schizophrenia and Alzheimer's disease

Authors: *B. SAMARDZIJA[1], E. RENNER[2], M. PALKOVITS[2], N. J. BRADSHAW[1];
[1]Univ. of Rijeka, Rijeka, Croatia; [2]Semmelweis Univ., Budapest, Hungary

Abstract: Schizophrenia, along with many other major mental illnesses, is a chronic condition that has a severely debilitating effect on the life of a patient. The biology of this condition is poorly understood, as a result of significant genetic heterogeneity. Recently, we and others have therefore been studying schizophrenia as a proteinopathy, with specific proteins aggregating in the brains of patients. Several such proteins have been discovered based on insolubility assays, however most such studies only look at one brain region, which may not be representative of the whole brain. It is therefore critical to determine if aggregation of schizophrenia-related proteins is consistent across the brain or if it varies. We therefore assessed 20 post-mortem brain tissue samples from across the brain of a single patient diagnosed with schizophrenia and Alzheimer's disease. Samples were anonymized before the insoluble protein fraction was isolated from tissue samples. We determined that this individual did have insoluble (presumably aggregating) Disrupted in Schizophrenia 1 (DISC1) in his brain, however the level of insolubility varied dramatically across the brain, and even between corresponding regions in opposing hemispheres. Similar results were seen in a smaller number of samples from two other individuals. While caution must be taken in generalizing this, it nevertheless provides first evidence that protein aggregation in schizophrenia is heterogenous across the brain, and may develop over time, as is the case for neurodegenerative disorders. This has major consequences for future study of these protein aggregates in mental illness, and may mean that studies are underestimating the prevalence of such aggregates.

Disclosures: B. Samardzija: None. **E. Renner:** None. **M. Palkovits:** None. **N.J. Bradshaw:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

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Topic: H.13. Schizophrenia

Support: NSFC Grant 82090030
NSFC Grant 82090031
NSFC Grant 82288101

Title: Multi-omics dissection pinpoints causal variants and vital regulatory dynamics in human schizophrenia

Authors: *X. CHEN[1,2], B. YU[1,2], X.-M. LI[1,2];
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Abstract: Schizophrenia (SCZ) is a complex and severe psychiatric disorder characterized by high heritability. However, the molecular and cellular mechanisms underlying SCZ remain elusive, especially the regulatory dynamics of genetic risk variants. Here, we present a single cell multi-omics study of the human dorsolateral prefrontal cortex (DLPFC) in SCZ, revealing dysregulated gene expression and epigenetic regulatory dynamics. With heritability enrichment and regulatory network inference, we identified disease-associated cis-regulatory elements and their target genes in relevant cell types, highlighting functional TF modules driven by THRB in SST neurons, as well as GLI3 in astrocytes. Notably, spatial transcriptomics revealed alterations in astrocyte-neuron interactions in SCZ. Overall, our high-resolution multi-omic single cell atlas provides valuable resources for understanding the molecular and cellular dysfunction associated with SCZ, and links cell type-specific transcriptomic and epigenetic changes to etiological genetic risk factors, which could ultimately lead to the development of new therapeutic approaches.

Disclosures: X. Chen: None. **B. Yu:** None. **X. Li:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.04/X30

Topic: H.13. Schizophrenia

Support: Cleveland VA Medical Research and Education Foundation

Title: Targeted and non-targeted metabolomic evaluation of cerebrospinal fluid in early phase schizophrenia: a pilot study from the Hopkins First Episode Psychosis project

Authors: *G. E. JASKIW[1,3], M. E. OBRENOVICH[4], C. J. DONSKEY[2], F. B. BRIGGS[5], L. N. HAYES[6], K. YANG[7], R. H. YOLKEN[8], A. SAWA[9];
[2]Infectious Dis. Service, [1]VANEOHS, Cleveland, OH; [3]Sch. of Med., Case Western Reserve Univ., Cleveland, OH; [4]Pathology and Lab. Med., Cincinnati VAMC, Cincinnati, OH; [5]Publ. Hlth. Sci., Univ. of Miami, Miller Sch. of Med., Miami, FL; [6]Cell Biol., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK; [7]Dept. of Physiol., Univ. of Maryland, Baltimore, Baltimore, MD; [8]Stanley Div. of Developmental Neurovirology, Johns Hopkins Sch. of Med., Baltimore, MD; [9]Psychiatry, Johns Hopkins Univ., Baltimore, MD

Abstract: Background: Biomarker development of biological markers (biomarkers) has been identified as the critical unmet need in schizophrenia research. We posited that metabolomic evaluation of cerebrospinal fluid (CSF) from patients with First Episode Schizophrenia (FES) v. healthy controls would delineate small molecules that distinguished the two groups. Methods: Patients with FES (n = 15) and age- and gender-matched controls (n =14), were recruited by the Johns Hopkins First Episode Psychosis Center. For non-targeted analysis, we used a C18 column (Thermo Fisher CA), Vanquish UHPLC and an Orbitrap Q Exactive HF (positive and negative ionization modes). Data were processed using MSDIAL (v4.92), annotation done using MoNA and MSDIAL Metabolomics MSP Spectral Kit (80% ID cut-off). For targeted analysis, we used authentic standards with a focus on high-value phenolic molecules generated in whole or in part by the gut microbiota (doi: 10.1007/s00213-019-05267-3). (TSQ Quantiva, Thermo Fisher /Vanquish, Thermos Fisher UPHLC, F5 column (Phenomenex). Features with > 50% missing values were removed, and zero/missing values were replaced by 1/5 of the minimum peak height for each feature, which was then normalized and scaled. Statistical comparison were conducted. Random forest, a machine learning approach, was used to identify features most predictive of FES. Results: In the non-targeted analysis, n = 23 features showed a log2 Fold Change x > 1 or x < -1 (uncorrected p-value < 0.05). The FES group had lowered levels of N-acetylneuraminate, and N-acetylaspartic acid (NAAA) but elevated levels of uric acid. In the targeted analysis, inter-group analyses by the Wilcoxon test of n = 34 features detected higher levels of 3-hydroxybenzoic acid and lower levels of phenylalanine in the FES group. Random forest suggested that 8 small molecules were informative for predicting group status (out-of-bag error <0.38), with the most informative being 3-hydroxybenzoic acid, which was elevated in FES. Discussion: Our non-targeted data are consistent with other studies associating FES with lower levels of N-acetylneuraminate and NAAA, markers of neuronal integrity. Lower levels of uric acid reflect a mechanism to counteract oxidative stress. The targeted analysis implicates elevated levels of 3-hydroxybenzoic acid, a gut-microbiota derived metabolite of L-tyrosine metabolism and lower levels of L-phenylalanine. Both aromatic amino acids are precursors of catecholamines. These preliminary data suggest targets for replication in larger samples.

Disclosures: G.E. Jaskiw: Other; Member of the Editorial Board for the journal Metabolites. **M.E. Obrenovich:** None. **C.J. Donskey:** 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.; contracts with Pfizer Inc, Clorox Inc.. Other; Associate Editor - Pathogens and Immunity. **F.B. Briggs:** None. **L.N. Hayes:** None. **K. Yang:** None. **R.H. Yolken:** None. **A. Sawa:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; grant from Sumitomo

Pharmaceuticals. F. Consulting Fees (e.g., advisory boards); Sumitomo Pharmaceuticals. Other; Editor, Science magazines.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.05/X31

Topic: H.13. Schizophrenia

Support: NIDA U01DA048279

Title: Cell specific transcriptome & 3D genome profiling in ventral midbrain in subjects with Schizophrenia and Bipolar Disorder

Authors: S. SINGH[1], ***V. EVANS**[2], S. MARENCO[3], P. AULUCK[4], P. ROUSSOS[2], S. AKBARIAN[2];
[2]Psychiatry, [1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Human Brain Collection Core, [3]Natl. Institue of Mental Hlth., Bethesda, MD

Abstract: Dopamine neurons have been implicated in the dysregulation of neurotransmitter systems in individuals with schizophrenia (SCZ) and Bipolar Disorder (BD) via dopamine level mediation. While dopamine is primarily produced in the midbrain regions of the substantia nigra and ventral tegmental area (VTA), there is a dearth of research investigating changes in the genetic and epigenetic landscapes of these regions in SCZ and BD patients. To address this knowledge gap, we have developed a resource comprising cell-specific 111 RNA-seq and 34 HiC libraries sourced from midbrain tissue of SCZ, BD, and control patients. Libraries were prepared using dopaminergic and non-neuronal nuclei populations isolated through fluorescence-activated nuclei sorting (FANS) techniques using Nurr1+/NeuN+ immuno-tagging. Interestingly, the analysis of differential transcriptomic expression in dopaminergic neurons revealed 340 differentially regulated genes between SCZ and control patients, while no significant gene changes were observed in non-neuronal cells. These dysregulated genes showed a notable enrichment of risk variants associated with SCZ, BD, and other psychiatric symptoms. Furthermore, a functional pathway analysis of these genes indicates disruptions in synaptic plasticity and neuronal channels, shedding light on the underlying mechanisms of SCZ. We conducted a distinct analysis utilizing one of the software tools developed in our laboratory to identify genes with transcripts that displayed discordant effect sizes, which were significantly associated with the disease, even after considering the correlation in their effect sizes. To understand the regulation of these genes, we utilized our cell-specific Hi-C data from dopaminergic neurons to identify chromatin loops where the disease-associated genes were co-localized and tested for coregulation. Future work will include an additional 247 single nucleus RNA-seq libraries derived from midbrain tissue to augment our current findings. This approach provides further insights into the complex molecular landscape of SCZ. Overall, our research emphasizes the importance of midbrain dopaminergic transcriptome changes in psychiatric

disease, highlighting specific genetic and functional pathways implicated in SCZ. Funding: NIDA U01DA048279

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Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.06/X32

Topic: H.13. Schizophrenia

Title: Classification of cell types in the human medial pulvinar (PM) and its link to schizophrenia risk-genes

Authors: *A. J. ROMANOWSKI, J. A. BOURNE;
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Abstract: The medial pulvinar (PM), the largest primate-specific thalamic nucleus, is thought to play a key role in sensory processing and attention. This region has been closely linked to neurodevelopmental disorders such as schizophrenia, where patients show decreased cellular density in the PM and reduced PM-temporal lobe connectivity. Additionally, both sensory processing and attention deficits have been seen in schizophrenia patients, further linking the PM to this disorder. However, not much is known about the cellular makeup of this nucleus. The goal of this study is to identify the cell types found in the human PM and to link this brain region to schizophrenia risk genes. To achieve this goal, we looked at both protein and RNA expression in human PM tissue (N = 4 healthy controls) to characterize cells based on common cellular markers. With this approach, we have identified populations of both excitatory (VGLUT2+) and inhibitory neurons (GABA+), showing a local inhibitory network within the medial pulvinar. Next, using data from schizophrenia GWAS, we investigated the expression of several schizophrenia risk-genes in human PM. Genes of interest that have neuron-specific function were chosen from the Schizophrenia Working Group of the Psychiatric Genomes Consortium GWAS and then cross-referenced to be expressed in the non-human primate PM. This list included notable genes such as *Drd2*, *Opcml*, *Ptprd*, and *Rere* that have strong association with schizophrenia. We also compared these data to another thalamic nucleus, the mediodorsal nucleus (MD), which is present in both primates and rodents with high connectivity to the cortex. This allows us to determine key differences between these two nuclei that may give reason to the purpose of the evolution of the PM and its potential function in schizophrenia etiology.

Disclosures: A.J. Romanowski: None. **J.A. Bourne:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.07/X33

Topic: H.13. Schizophrenia

Support: Stanley Center for Psychiatric Research

Title: Altered acute stress response in a knockout model of schizophrenia risk gene Xpo7

Authors: *A. J. LAWLER[1,2], L. BARROS[1,2], J. XIONG[1,2], R. SZETO[1,2], D. J. KWON[1,2], M. KIM[1,2], E. MACOSKO[1,2,3];
[1]Broad Inst., Cambridge, MA; [2]Neurobio. Dept., Harvard Univ., Cambridge, MA; [3]Psychiatry, Massachusetts Gen. Hosp., Boston, MA

Abstract: Symptom burden due to major psychiatric illnesses like schizophrenia fluctuates widely over a person's life and is often exacerbated by psychological stress. We leveraged parallel acute stress paradigms in vivo (N = 34 adult male and female mice) and in cultured astrocytes to initiate disease-relevant cell states within schizophrenia genetic models and reveal elusive neurobiological roles of risk genes. In knockout models of schizophrenia risk gene *Exportin 7* (*Xpo7*), stress potentiated extensive behavioral and transcriptional phenotypes indicative of an overall dampened ability to mount an appropriate stress response. Underlying this gene-environment interaction, we identified multiple cell autonomous abnormalities around metabolic homeostasis in astrocytes. We suggest a role for hypofunction of the mineralocorticoid receptor (MR) in these deficits, a key cortisol-responsive transcription factor which has decreased expression in *Xpo7* mutants and is thought to regulate the affected metabolic pathways. A similar stress response defect was also observed in a separate schizophrenia mutant with *Rb1cc1* knockout. Altered stress response provides a valuable guiding hypothesis for diverse schizophrenia studies, nominating key behaviors, cell types, and pathways toward a unified interpretation of schizophrenia risk genetics.

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Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

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Topic: H.13. Schizophrenia

Support: NIH Grant 1R01H129343-01

Title: Exploring SETD1A Gene Regulation Using a Heterozygous Knockout Neural Stem Cell Model

Authors: *S. P. ARJONA[1], R. LEASE[2], M. E. CORTES-GUTIERREZ[3], S. A. AMENT[4];
[1]Inst. for Genome Sci., Univ. of Maryland Sch. of Med., Baltimore, MD; [2]IGS, Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Univ. of Maryland, Baltimore, MD; [4]Dept. of Psychiatry and Inst. for Genome Sci., U Maryland Sch. of Med., Baltimore, MD

Abstract: Exome sequencing studies have identified the histone H3 lysine 4 (H3K4) methyltransferase, SET domain containing 1A (SETD1A), as a risk gene for schizophrenia. Rare loss-of-function variants in SETD1A confer ~20-fold increased risk for schizophrenia and have been associated with developmental disorders. Previous studies in our lab using patient-derived induced pluripotent stem cells (iPSCs) with SETD1A variants have demonstrated effects on neural development and differentiation. Here, we describe molecular mechanisms associated with these neurodevelopmental effects using genome-edited iPSCs heterozygous for a SETD1A protein-truncating variant and isogeneic controls. We hypothesized that SETD1A loss-of-function variants influence neural induction via H3K4me3-dependent inactivation of promoters involved in neurodevelopment and cell cycle regulation. We differentiated iPSCs into cortical neurons using an established protocol via forced expression of the neurodevelopmental transcription factor neurogenin 2 (NGN2). We quantified SETD1A's genomic binding sites with CUT&RUN (native ChIP-seq) and the relationship of SETD1A occupancy with H3K4 methylation and gene expression during neural induction. Our results provide insight into the genes and gene networks that are regulated by SETD1A and the effects of loss-of-function variants.

Disclosures: S.P. Arjona: None. **R. Lease:** None. **M.E. Cortes-Gutierrez:** None. **S.A. Ament:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

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Program #/Poster #: PSTR194.09/X35

Topic: H.13. Schizophrenia

Support: Stanley Center Foundation

Title: Scalable and quantitative electrophysiological profiling of SCHEMA gene functions in human neurons

Authors: *Y. WANG[1], L. CUI[2], E. KURGANOV[3], J. Q. PAN[4];
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Abstract: Understanding the functional implications of genetic perturbations at the cellular level is a significant challenge in neurological and biomedical research. While genetics has identified numerous risk genes associated with various brain disorders, such as schizophrenia, translating these findings into mechanistic insights remains hindered by a lack of robust functional interpretation. To address this gap, we propose a methodological approach centered on scalable and quantitative measurement of neuronal functions using advanced electrophysiological techniques.We begin our methodology by generating isogenic knockout of human excitatory neuronal models targeting specific genetic loci of interest. To characterize the functional properties of these neurons, we utilize an automated and high-throughput electrophysiological platform (SyncroPatch, Nanion). This platform allows for comprehensive assessments of voltage-gated ion channel currents, crucial determinants of neuronal excitability and communication. By integrating advanced data analysis algorithms, we extract nuanced information regarding channel kinetics and conductance, enabling precise characterization of changes induced by both genotypical alterations and pharmacological interventions.In addition to electrophysiological measurements, we employ high-density multielectrode array technology to capture neuronal activity at single-cell resolution. This pipeline enables scalable and quantitative analysis of morphological and electrophysiological parameters, facilitating precise characterization of cellular function. Furthermore, our approach offers a range of techniques to extract features from spike-sorted data, allowing for the examination of functional phenotypes at both the individual cell and network levels, as well as across development. Moreover, this pipeline incorporates the capability to integrate novel features and employ machine-learning-assisted approaches, facilitating comprehensive evaluations of pharmacological interventions. To illustrate the practical application of our methodology, we apply it to human induced pluripotent stem cell derived excitatory neurons, showcasing how our pipeline enables phenotypic screenings.In summary, our methodology harnesses the power of iPSC-based models alongside advanced electrophysiological techniques to elucidate the functional consequences of genetic perturbations. By providing a robust framework for functional characterization, our approach offers valuable insights into the pathophysiology of psychiatric diseases.

Disclosures: Y. Wang: None. **L. Cui:** None. **E. Kurganov:** None. **J.Q. Pan:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.10/Y1

Topic: H.13. Schizophrenia

Support: R01MH131719-02
R01MH118298

Title: Sleep/wake eeg neurophysiology in mice with cacna1g loss of function

Authors: *A. ASAN[1], N. GOBLE[2], Y. WANG[3], E. YU[4], S. CHOI[5], J. Q. PAN[6];
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Abstract: Schizophrenia is a complex psychiatric disorder; however, its etiology remains elusive. The C*acna1g* has been identified as one of the genes that contribute to the genetic risk of schizophrenia (SCZ). By analyzing the loss of function of this gene can help us to probe the underlying mechanism and identify new neurophysiological biomarkers for the disease. In this study, we sought to characterize the neurophysiological phenotypes of mice lacking one or both copies of *Cacna1g* gene which encodes the Cav3.1 T-type voltage-sensitive calcium channels. Here, our analysis covers the macro-structure of sleep, including sleep duration and fragmentation, along with four domains of parameters derived from EEG recordings: power, connectivity, spindle/SO analysis, and their coupling. We observed marked changes in EEG power across various frequency bands, with a significant increase in gamma power in both heterozygous (Het) and homozygous knockout (KO) groups compared to the wild-type littermates (WT). Given schizophrenia is also characterized as a “failure of cortical integration”, we examined the connectivity between the frontal and parietal regions. Our results showed a sharp reduction in connectivity between cortical regions in the KO group, with a similar, but nonsignificant decrease in the Het group, indicating a dose-dependent decrease in connectivity. Additionally, sleep spindle parameters (spindle density, duration, amplitude and integrated spindle activity) significantly decreased in both Het and KO groups. In contrast, slow oscillation density and duration exhibited an increasing trend. Finally, we checked the coupling between SOs and spindles which is critical for sleep dependent memory consolidation. Our results indicated a distinct shift in coupling angles in KO animals compared to WT. Sleep macro-structure also showed mild changes in KO animals. Most of our EEG metrics demonstrated significant alterations, with spindle activity demonstrating the most drastic changes, suggesting its potential as a biomarker for SCZ. This study lays the groundwork for developing and analytic pipeline and experimental protocol to identify potential neurophysiological biomarkers relevant to psychiatric diseases associated with multiple risk genes in mouse models.

Disclosures: A. Asan: None. **N. Goble:** None. **Y. Wang:** None. **E. Yu:** None. **S. Choi:** None. **J.Q. Pan:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

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Topic: H.13. Schizophrenia

Support: Stanley Center Foundation
R01MH131719-02
R01MH118298

Title: Converging and diverging EEG measures across 6 different mouse models of schizophrenia genetic risk

Authors: *N. GOBLE[1], J. Q. PAN[2], S. P. MORAN[3], E. YU[4];
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Abstract: Electroencephalography provides non-invasive measurements of cortical activity that can be used to study changes in patient populations. Recently, exome sequencing has identified several genes whose rare loss of function mutation confers substantial risk for developing schizophrenia. Here we characterize spontaneous sleep and wake neurophysiology of six of these mouse models derived from the Schizophrenia Exome Sequencing Meta-Analysis consortium (SCHEMA) using in vivo EEG recordings. The models tested include loss of function Grin2a, Akap11, Srrm2, Cacna1g, Zmym2 and Xpo7. Overall, we find that these animal models display both converging and diverging pathophysiological metrics in our EEG recordings. Specifically, we analyzed coherence, power spectral density, and NREM oscillations such as sleep spindles and slow oscillations and provide here evidence that sleep spindle impairments are present in 50% of the models studied including decreased number of sleep spindles per minute, lower spindle amplitude, and shorter spindle duration. Slow oscillation deficits are also apparent in half of the mouse models tested, but sometimes diverge from the models which show deficits in other EEG parameters like sleep spindles. For instance, the heterozygous knock-out model for the SCHEMA gene Srrm2 (encoding for a nuclear speckle scaffold protein) exhibits both decreased sleep spindle density as well as fewer slow oscillations per minute. However, the heterozygous knock-out model for Cacna1g (encoding the alpha-1 subunit of T-type voltage gated calcium channels) exhibits decreased sleep spindle density but increased slow oscillations per minute. Overall, when we analyze these strain-specific changes across all EEG metrics, we find both converging and diverging neurophysiological signatures common to different schizophrenia risk genes. In conclusion, by mapping these mutation-specific changes in brain function we hope to provide a framework for identifying neurophysiological phenotypes in risk genes for schizophrenia.

Disclosures: N. Goble: A. Employment/Salary (full or part-time):; Broad Institute of MIT and Harvard. **J.Q. Pan:** A. Employment/Salary (full or part-time):; Broad Institute of MIT and Harvard. **S.P. Moran:** A. Employment/Salary (full or part-time):; Broad Institute of MIT and Harvard. **E. Yu:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.12/Y3

Topic: H.13. Schizophrenia

Support: R01MH131719-02
R01MH118298

Title: Methods for analyzing auditory steady state response (ASSR) EEG data

Authors: *A. MADDIRALA[1], N. GOBLE[2], E. YU[4], A. LAWLER[5], S. ARYAL[5], W.-C. HUANG[6], E. MACOSKO[7], M. SHENG[3], J. Q. PAN[8];
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Abstract: Schizophrenia is a complex psychiatric disorder affecting 0.5% of the global population and characterized by hallucinations, delusions, disorganized thought, sensory and cognitive deficits. The deficit of auditory sensory perception is identified as one of the most common features in people with schizophrenia. The auditory steady state response (ASSR) measures the evoked response potential (ERP) of the brain to auditory stimuli and has been shown to be impaired in people of schizophrenia. Even though the etiology of schizophrenia is not understood well, recently, genetic analyses has identified genes whose loss of function confer significance risk for developing schizophrenia. In this study, we utilized mouse models that lack one or both copies of six genes that have been identified in SCHEMA analyses. We recorded auditory evoked response in frontal and parietal electrodes and developed a frequency-based signal processing technique to analyze the ASSR data from these recordings. The strength of the new method is evaluated using the two common performance measures, the power ratio and the inter-trial coherence (ITC). The proposed methodology showed improved power ratio and ITC measures compared with the existing non-threshold and threshold-based methods. We found Srrm2 and Akap11 showed impaired gamma band power ratio at 40Hz. Our proposed method is an optimized computational tool to quantify the evoked response between mice when they respond to the auditory stimuli.

Disclosures: A. Maddirala: None. **N. Goble:** None. **E. Yu:** None. **A. Lawler:** None. **S. Aryal:** None. **W. Huang:** None. **E. Macosko:** None. **M. Sheng:** None. **J.Q. Pan:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.13/Web Only

Topic: H.13. Schizophrenia

Title: Effect of SNP rs2501432 on CB2 Receptor Function and Signaling, and Its Role in Schizophrenia

Authors: *A. MACÍAS GAMEZ[1], J. MORENO-ROCHA[2], E. ROBLES[2], R. SEPULVEDA SAA[2], A. GONZÁLEZ-HORTA[2], D. MONTIEL-CONDADO[2], B. GONZALEZ[3];
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Abstract: The cannabinoid CB2 receptor is a member of the G-protein-coupled receptor family (GPCR) and has a physiological role in neuroprotection and regulation of neuroinflammation. Polymorphism (SNP) rs2501432 (Q63R) of the receptor has been highlighted in genetic association studies in schizophrenia; however, it has not been clearly described what is the direct impact on the structure of the receptor, in cell signaling or affected molecular pathways that may explain its role in disease pathology. The objective of this work is to study how the SNP of the receptor impacts the structural and molecular stability of the intracellular loop 1 (ICL1) and its possible protein-protein CB2 interaction necessary for correct cellular signaling. Sequenced and structured computational tools were used to observe and measure the impact of the SNP. The molecular dynamics simulation test showed that ICL1 undergoes an increased positive ionic charge due to polymorphism, resulting in an increase in the recruitment of beta-arrestin proteins, affecting the proper functioning of CB2. With these results we suggest that these molecular changes in the receptor may contribute to the pathogenesis of schizophrenia and provide valuable information for future research in the field.

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Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.14/Y4

Topic: H.13. Schizophrenia

Support: NU22-J-04-00061

Title: The impact of neurodevelopmental perturbations on parvalbumin interneurons in schizophrenia: Insights from a rat model

Authors: *D. CERNOTOVÁ[1,2], D. RADOSTOVA[1], M. CHVOJKOVA[3], L. KLETECKOVA[3], H. BROZKA[1];
[1]Inst. of Physiol. CAS, Prague, Czech Republic; [2]Third Faculty of Medicine, Charles University, Prague, Czech Republic; [3]Natl. Inst. of Mental Hlth., Klecany, Czech Republic

Abstract: Perinatal ischemia constitutes one of the risk factors for schizophrenia. One of the most replicated findings is the loss of parvalbumin immunoreactivity in post-mortem brains of schizophrenia patients. Parvalbumin interneurons (PVIs) are susceptible to metabolic stressors such as excessive release of excitotoxic glutamate, increased reactive oxygen species, or inflammation, all of which are events linked to perinatal ischemia. Therefore, our study aims to elucidate the effects of chronic perinatal intermittent hypoxia alone and in combination with the application of NMDA receptor antagonist, MK-801, during early adulthood on PVI integrity. Furthermore, we explore the potential neuroprotective effects of citalopram, risperidone, or their combination in preserving PVI numbers in key brain regions affected by schizophrenia - the prefrontal cortex, striatum, and hippocampus. Our findings indicate that perinatal hypoxia, only when combined with MK-801 application, leads to a reduction in PVI immunopositivity, specifically in the hippocampus. Perinatal ischemia alone or repeated exposure to MK-801 during adolescence alone did not affect PVIs. Moreover, citalopram was ineffective in preventing the loss of PVI immunopositivity. Our results support the idea that the loss of PVI immunopositivity, observed in schizophrenia, is associated with perinatal ischemia and additional perturbation during puberty, as often depicted by two-hit models of schizophrenia.
The project was supported by the Ministry of Health of the Czech Republic, grant nr. NU22J-04-00061.

Disclosures: D. Cernotová: None. **D. Radostova:** None. **M. Chvojkova:** None. **L. Kleteckova:** None. **H. Brozka:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.15/Y5

Topic: H.13. Schizophrenia

Support: Czech Science Foundation (GACR) grant 22-15096S

Title: In vivo imaging of interneuronal metabolic activity in a mice model of repeated psychosis

Authors: *D. RADOSTOVA, H. BROZKA;
Inst. of Physiol. CAS, Prague, Czech Republic

Abstract: Schizophrenia (SCZ) is a severe psychiatric disorder, whose origin and causes are still unknown. Evidence suggests that with each psychotic episode, progressive deterioration of the brain occurs, pathophysiological changes in the central nervous system (CNS) responsible for the deterioration of the overall clinical condition. Several postmortem observations from brain samples of SCZ patients suggest that mitochondrial function might be compromised in SCZ. Perturbation of mitochondrial function leads to increased reliance on glycolysis, resulting in increased production of reactive oxygen species. The fast-spiking parvalbumin-positive interneurons (PVIs) are the subgroup of interneurons that were shown to be very sensitive to

oxidative stress. The application of N-methyl-D-aspartate receptor (NMDAr) antagonists, such as MK-801, is a widely used animal model of psychosis-like behavior highly relevant to SCZ. These substances trigger a hyperglutamatergic state leading to excitotoxicity and an increased ROS production.We hypothesized that a repeated psychosis-like state induced by MK-801 (0,2 mg/kg) might lead to redox and cell metabolic changes that take their toll on PVIs. We assessed a metabolic state of living transgenic mice C57BL/6-Tg(Pvalb-tdTomato)15Gfng/J (Jax, Stock No: 027395), expressing tdTomato fluorescent protein under parvalbumin promoter, by measuring the ratio of free/bound NADH before, during and after chronic application of MK-801 under the cranial window using fluorescence lifetime imaging (FLIM) using 2-photon microscope Bruker Ultima. As we hypothesized, repeated but not acute MK-801 treatment shifted the free/bound NADH ratio towards the free form, indicating increased glycolysis and decreased oxidative phosphorylation in mice treated repeatedly with MK-801 .

Disclosures: D. Radostova: None. **H. Brozka:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

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Program #/Poster #: PSTR194.16/Y6

Topic: H.13. Schizophrenia

Support: AASM Bridge to Success Grant #301-BS-23

Title: Pre-pubertal sex-specific changes to kynurenine pathway metabolism and inflammation in rat offspring exposed to embryonic kynurenic acid elevation

Authors: *C. WRIGHT, S. C. WALTHER, M. V. PIROLI, A. POCIVAVSEK; Pharmacology, Physiology, and Neurosci., Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Prenatal insults epidemiologically linked to neurodevelopmental disorders (NDDs) elevate tryptophan degradation via the kynurenine pathway (KP) and increase levels of kynurenic acid (KYNA), a neuroactive KP metabolite. Elevations in KYNA are observed in adult patients with NDDs, including schizophrenia and bipolar disorder. As an endogenous antagonist of NMDA and α7nACh receptors, elevations in KYNA may be causally linked to the cognitive deficits and sleep disturbances experienced by patients with NDDs. To model gestational elevations in KYNA that occur from prenatal insults, we employ the embryonic kynurenine (EKyn) paradigm, wherein pregnant dams are fed a control diet (ECon) or a diet laced with kynurenine (100 mg/day), the direct KYNA bio-precursor from embryonic day (ED) 15 to ED 22 (Pocivavsek et al. 2014. *Psychopharm*). We have previously determined sex-specific alterations in brain KYNA, cognition, and sleep in adult, postnatal day (PD) 56+, male and female offspring. To elucidate if sex-specific changes occur before adulthood, we currently assessed in both sexes of ECon and EKyn offspring (i) KP metabolites in fetal brains before birth (ED 21) and (ii) plasma cytokines and KP metabolites following an acute 6-hour sleep

deprivation (SleepDep) challenge before puberty (PD 28). In the fetal brain, EKyn diet elevated kynurenine and KYNA in males and females ($P<0.001$), yet the ratio of KYNA to kynurenine conversion was elevated only in EKyn compared to ECon male fetal brain ($P<0.05$). Given this early change in KP metabolism and our published findings that brain KYNA levels are not basally elevated until adulthood (PD 56+) in EKyn offspring, we challenged pre-pubertal (PD 28) male and female ECon and EKyn offspring with SleepDep, a homeostatic challenge known to induce inflammation and stimulate KYNA neosynthesis. In PD 28 offspring, SleepDep significantly increased cytokine levels (IL-10, IL-18) in EKyn females ($P<0.01$) compared to controls. Plasma kynurenine levels were also higher in PD 28 offspring ($P<0.05$) after SleepDep. Of note, the pro-inflammatory cytokines IL-17A and IL-18 were elevated basally in EKyn offspring of both sexes at PD 28 ($P<0.05$). Future studies will evaluate brain-specific changes to KYNA following PD 28 SleepDep and consider the contribution of sex and prenatal treatment. Taken together, we demonstrate early sex-dependent changes to KP metabolism and inflammation in EKyn offspring, a model translationally relevant to NDDs.

Disclosures: C. Wright: None. **S.C. Walther:** None. **M.V. Piroli:** None. **A. Pocivavsek:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

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Program #/Poster #: PSTR194.17/Y7

Topic: H.13. Schizophrenia

Support: Czech Science Foundation (GAČR), project No. 23-06546S

Title: Effect of maternal immune activation on rat pup development

Authors: *K. TUCKOVÁ;
The Natl. Inst. of Mental Hlth., Klecany, Czech Republic

Abstract: Infection of the mother during pregnancy is one of recognized risk factors for the emergence of schizophrenia in the offspring. In animal models, maternal immune system activation (MIA) leads to schizophrenia-like alterations. Our earlier work has shown that a MIA model induced by injecting bacterial lipopolysaccharide to pregnant rat dams exhibits behavioral changes in adults rats. In the planned project, we will provide a detailed description of the ontogeny of the schizophrenia-like changes seen in the model. At this conference, we will present pilot data from first cohorts of rat pups, adolescents and adults that were affected by prenatal exposure to bacterial lipopolysacharide. Pilot data will include results from behavioral experiments, such as sensorimotor abilities and neonatal ultrasonic vocalization of pups, playful behavior of juvenile rats and tests of emotionality of young adult rats. We also show pilot result from histological study on brain tissue. This project is supported by by the Czech Science Foundation (GAČR), project No. 23-06546S.

Disclosures: K. Tucková: A. Employment/Salary (full or part-time):; Czech Science Foundation (GAČR), project No. 23-06546S.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.18/Y8

Topic: H.13. Schizophrenia

Support: Donation by Isaac Larian and Family

Title: Src tyrosine kinase alters development of GluN2 containing receptors

Authors: *M. F. KIM[1], K. PAREKH[1], M. W. SALTER[2], C.-G. HAHN[3], R. E. FEATHERSTONE[1], S. J. SIEGEL[1];
[1]Psychiatry and Behavioral Sci., USC, Los Angeles, CA; [2]Neurosciences & Mental Hlth. Program, Hosp. For Sick Children, Toronto, ON, Canada; [3]Thomas Jefferson Univ., Bryn Mawr, PA

Abstract: Sarcoma tyrosine kinase (Src) is a key component in regulating N-methyl-D-aspartate receptor (NMDAR) activation and channel current via phosphorylation. Src kinase acts as a focal point where multiple schizophrenia susceptibility pathways such as dysbindin and neuregulin-1 converge on. Previous work generated by our lab has shown that Src (+/-) heterozygous mice display impaired working memory (WM) on a trace fear conditioning task (TFC) but not in standard cued fear conditioning (sCFC). Deficits in TFC were subsequently rescued by synapse specific Src-activating peptide (SAPIP). TFC is highly dependent on the prefrontal cortex (PFC) and the hippocampus, both of which do not become fully developed until adolescence, suggesting that deficits in TFC allow for understanding various late-emerging disease mechanisms related to NMDAR dysfunction and memory deficits. Despite its importance, there are few studies that have been done to assess the role of Src in development. As such, we sought to assess how Src impacts development of NMDARs. Western blotting was carried out on synaptosomal preparations to assess the phosphorylated and non-phosphorylated protein concentrations of GluN2A, GluN2B, and Src kinase in the hippocampal and PFC tissue of Src heterozygous (+/-) mice and their wild-type (WT) littermates. Tissue was collected from mice at P10, 15, 28, as well as adult mice (aged 14-17 weeks). Src het mice showed reduced expression and phosphorylation of GluN2A at P10 and reduced GluN2B at all times tested. Deficits in GluN2B closely matched altered Src expression and phosphorylation in het mice. These results show an important role for Src in regulating GluN2 receptor expression. Implications for cognition and schizophrenia will be discussed.

Disclosures: M.F. Kim: None. **K. Parekh:** None. **M.W. Salter:** None. **C. Hahn:** None. **R.E. Featherstone:** None. **S.J. Siegel:** None.

Poster

PSTR194: Schizophrenia Pathophysiology and Mechanisms

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR194.19/Y9

Topic: H.13. Schizophrenia

Support: R01MH107730

Title: Nuclear GAPDH affects stress responses in brain resulting in impaired cognitive flexibility and reveals its association with augmented cellular autofluorescence

Authors: *A. HAYASHIDA[1], A. RAMOS[2], A. SAWA[3], K. ISHIZUKA[4];
[1]Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Sanofi-Aventis Pharmaceuticals, Cambridge, MA; [3]Psychiatry, [4]Dept Psychiatry, Johns Hopkins Univ., Baltimore, MD

Abstract: Recent evidence has strongly suggested that lysosome, a small intracellular organelle, may be a central intersection for neurological and psychiatric disorders at the mechanistic level. The evidence includes: (1) neurons derived from a subset of Parkinson's disease (PD) showed a deficit at the mitochondria-lysosome contact[1]; (2) blood cells from a subset of patients with schizophrenia (SZ) showed higher autofluorescence (AF) and lysosomal deficits[2]; and (3) the levels of AF elicited from blood cells are correlated with cognitive inflexibility at least in both healthy subjects and SZ patients, and this neuropsychological construct is also impaired in patients with neurodegenerative disorders. In patients with SZ, higher cellular AF was associated with worse cognitive inflexibility[2], and (4) the genes in 22q11.2 deletion that leads to frequent occurrence of SZ and early Parkinsonism are implicated in lysosomal function[3].
Batten disease caused by genetic mutations of CLN3 is a representative juvenile type of neuronal ceroid lipofuscinoses and is featured by pathologically elevated AF[4]. CLN3 protein interacts with Rab7A, a crucial molecule for the mitochondria-lysosome contact[5]. Meanwhile, a multifunctional protein GAPDH modulates lysosomal functions under a stressed condition[6] and interacts with Rab family proteins[7].
Taken together, we hypothesize that lysosomal deficits seen in SZ and PD may share a mechanism with those in Batten disease, and their pathological conditions may be exacerbated at least in part by GAPDH under stressed conditions.
As the first step in addressing this working hypothesis, we have further characterized the lysosomal deficits and AF in SZ patients, by comparing them with clinical features, together with further consideration of potential confounding factors. Second, we have examined the protein interactions of CLN3, Rab7A, and GAPDH. Their disease implications have been tested mainly in blood cells by combining biochemical and pharmacological assays. Third, the impact of GAPDH on lysosomal functions have also been addressed whether and how nuclear translocated GAPDH under stressed conditions may mediate transactivation of lysosomal proteins using the ChIP sequencing.
References 1) Kim et al, Nat Commun, 2021. 2) Patent #: WO2023141606A2. 3) Sumitomo et al, Sci Adv, 2018. 4) Mole et al., Neurogenetics, 2005. 5) Oetjen et al, J Neurochem, 2016. 6) Ramos et al, Mol Psychiatry, 2024. 7) Tisdale et al, J Biol Chem, 2004.

Disclosures: A. Hayashida: None. **A. Ramos:** None. **A. Sawa:** None. **K. Ishizuka:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.01/Y10

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

Support: Kavli Foundation Exploration Award
NSF MCB 1818140

Title: Establishing CRISPR/Cas9-mediated gene knockout lines for crustacean neurobiology

Authors: *M. SEYMOUR[1], A. G. VIDAL-GADEA[2], W. STEIN[2];
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Abstract: Transgenic animal lines are invaluable tools for studying nervous system function. Creating stable transgenic lines typically requires microinjection of genetic constructs into embryos and subsequent outcrossing of at least one filial generation. For many species that are amenable to neurophysiological activity measurements, including most traditional arthropod model systems for cellular electrophysiology, no such lines exist. We are addressing this issue by expanding genetic tools for use in decapod crustacean neurobiology. Decapod crustaceans (crayfish, lobsters, crabs) possess large, well-characterized neurons that allow access for real time membrane physiology measurements in individually identifiable neurons and circuits, but very few genetic tools for manipulating gene expression exist. We developed a CRISPR/Cas9-based approach to create stable knockout lines of the marbled crayfish, *Procambarus virginalis*. This species has a well-sequenced genome and transcriptome and produces genetically identical offspring through apomictic parthenogenesis. We delivered a CRIPSR-Cas9 construct to internally developing oocytes via ReMOT Control (Receptor-mediated Ovary Transduction of Cargo). ReMOT Control molecularly delivers constructs to oocytes of injected vitellogenic marbled crayfish, targeting many oocytes at once. The CRISPR-Cas9 construct features a vitellogenic ligand called P2C derived from *Drosophila melanogaster*, allowing oocyte internalization. Using a P2C-GFP construct, we confirmed that the *Drosophila*-derived P2C enabled internalization into crayfish oocytes. Additionally, we confirmed the functional editing capabilities of the CRISPR construct in an *in vitro* cleavage assay. As a proof of concept for testing the CRISPR-mediated gene knockout *in vivo*, we targeted *eyeless* (E-value of 2e-68 to *Drosophila melanogaster*), a gene encoding a transcription factor involved in eye structure development, and *scarlet* (E-value of 5e-44 to *Daphnia magna*), a gene encoding a protein involved in eye pigment transportation. Sequencing data revealed that both *eyeless* and *scarlet* had been successfully edited. However, we also observed increased offspring lethality (~46% for *eyeless*; ~23% for *scarlet*). For *eyeless*, we did not observe any obvious changes in eye phenotypes, suggesting that these mutations were either lethal or not sufficient to alter eye development. In contrast, we observed a clear lack of eye pigment for the *scarlet* knockouts,

albeit only in <1% of all offspring. Our data thus indicate that genetic editing was successful, making marbled crayfish the first crustacean to have CRISPR/ReMOT Control established.

Disclosures: M. Seymour: None. **A.G. Vidal-Gadea:** None. **W. Stein:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.02/Y11

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

Title: A novel fluorescent reporter for the sequential control of the combinatorial action of Cre and Flp recombinases

Authors: *G. PESSINA[1], M. CAMERA[2], F. LOIACONO[3], A. UCCELLI[3], F. BENFENATI[4], P. MEDINI[5], F. TROVATO[6], S. SULIS SATO[3];
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Abstract: The advent of conditional site-specific recombination strategies has opened the way for precise gene expression control and development of transgenic animal models targeting specific cell types. Drug-inducible recombinases further expanded this field by allowing a temporal control of recombination events. However, when multiple recombinases are used, the combinatorial gene expression readout is not easy to detect. This scenario is further complicated by the absence of reporter systems capable of unraveling combinatorial maps and their temporal patterns. Here we present Rubik, a fluorescent genetic reporter designed to visualize the combined action of Cre and Flp recombination. Rubik has four alternative fluorescent configurations: Blue for Cre recombination, and Green for Flp recombination; while for the two different intersections of Cre and Flp, Yellow corresponds to Cre followed by Flp recombination, and Red to Flp followed by Cre recombination. Moreover, we introduced an extra level of control on the intersectional maps by co-expressing inhibitory or excitatory optogenetic proteins, depending on the sequence of recombinase activity. This setup allows to choose between photo-stimulation or photo-inhibition of cells expressing the combination of the two recombinases, by controlling the temporal sequence of Cre and Flp events. In order to achieve such a temporal control, we implemented a double inducible recombination system based on the tamoxifen-inducible Cre (ERT2CreERT2) recombinase and a novel trimethoprim-inducible FlpO (FlpO-DD) recombinase. Thus, when Rubik is expressed together with ERT2CreERT2 and FlpO-DD, recombination can be induced upon the addition of tamoxifen or trimethoprim. To validate the functionality of our reporter, we established a stable knock-in HeLa cell line utilizing CRISPR-Cas9 technology, and we performed single-cell sorting to obtain single clones of HeLa-Rubik cells. Our results show the efficacy of the system upon the recombination with either Cre or Flp

recombinases individually, as well as in combination. We also tested Rubik together with the ERT2CreERT2 and FlpO-DD recombinases, showing that, by adding tamoxifen or trimethoprim, we are able to selectively induce Cre or Flp recombination. This system could be exploited for the generation of knock-in mice expressing Rubik, which could be resourceful in studying and precisely defining the anatomical and functional roles of specific neuronal populations expressing Cre and Flp recombinases.

Disclosures: G. Pessina: None. **M. Camera:** None. **F. Loiacono:** None. **A. Uccelli:** None. **F. Benfenati:** None. **P. Medini:** None. **F. Trovato:** None. **S. Sulis Sato:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.03/Y12

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

Support: Intramural Research Program, NIDA

Title: Targeting transgene expression to rat microglia using adeno associated viral vectors

Authors: E. FIELDING, R. SVARCBAHS, E. J. GLOTFELTY, C. RICHIE, *B. HARVEY; NIDA - NIH, Baltimore, MD

Abstract: Selective expression of transgenes in rodent microglia has historically been performed only in mice and required the production of transgenic animals using an endogenous microglial locus (e.g. Cx3cr1, Tmem119, P2Ry12, and Csf1r). These microglial-specific transgenes can be in the form of Cre-drivers that can be crossed with mice that contain a Cre-dependent transgene, thus producing cell type specific expression. Rats are a preferred model for certain cognitive and behavioral experiments but there are limited transgenic rats available to perform experiments comparable to those in mice. To address the dearth of microglial specific transgenic rats, we developed a Cx3cr1-CreERT2 rat to facilitate expression of transgenes in rat microglia, though this animal does not address the limited availability of rats with Cre-dependent reporters or modulators of cellular activity. An alternative approach to express transgenes in the rodent brain is through adeno-associated viral (AAV) vectors. While AAVs readily expresses transgenes in neurons of the mouse and rat central nervous systems, expression in microglia can be achieved using a truncated Aif1/Iba1 promoter along with a micro-RNA sequence, previously demonstrated in mice by the Hirai lab in 2022. Here, we found that the same AAV expressing GFP from the Aif1/Iba1 promoter can be used to successfully express GFP in rat microglia from several brain regions, including the prefrontal cortex, striatum, and substantia nigra. Minimal expression of GFP was detected in non-Iba1-positive cells. To further restrict expression of transgenes to only microglia after AAV delivery, we will develop Cre-dependent AAV to be injected in the brains of our Cx3cr1-CrERT2 rat.

Disclosures: E. Fielding: None. **R. Svarcbahs:** None. **E.J. Glotfelty:** None. **C. Richie:** None. **B. Harvey:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.04/Y13

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

Title: Applications of human spinal motor neurons that stably express genetically encoded calcium indicators, GCaMP and RCaMP

Authors: *G. SAHIN[1], J. LAWSON[2], W. LI[1], K. REMONDINI[1], H. RUETH[1], N. ETCHIN[1], M. L. HENDRICKSON[1];
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Abstract: Genetically encoded calcium indicators (GECI) are powerful tools to track neural activity in neuronal networks by measuring Ca^{2+}-dependent fluorescence. One main advantage of GECIs over other calcium indicators is the ability to have cell-type specific expression enabling study of neural activity in a subset of cells together in a more complex and physiologically relevant culture systems such as co-cultures. In this study, we generated human spinal motor neurons by differentiating induced pluripotent stem cells (iPSCs) that stably express GCaMP or RCaMP. We detected spontaneous changes in the fluorescence intensity indicating spontaneous Ca^{2+} flux and confirmed neural activity with extracellular recordings of spontaneous and evoked action potentials of mono- and co-cultures on multi-electrode arrays (MEAs). The mono- and co-cultures were responsive to known Ca^{2+} signal agonists and antagonists. To demonstrate the applicability of these cells in a disease model system, we co-cultured the human spinal motor neurons expressing GECIs with spinal astrocytes that arbor mutations in either SOD1 or TDP43 genes, well-known amyotrophic lateral sclerosis (ALS)-associated mutations. Our results demonstrate that GECI-expressing human neurons provide a valuable platform for novel drug discovery and development as well as studying underlying mechanisms of neurodegenerative diseases such as ALS.

Disclosures: G. Sahin: A. Employment/Salary (full or part-time):; BrainXell, Inc. **J. lawson:** A. Employment/Salary (full or part-time):; BrainXell, Inc. **W. Li:** A. Employment/Salary (full or part-time):; BrainXell, Inc. **K. Remondini:** A. Employment/Salary (full or part-time):; BrainXell, Inc. **H. Rueth:** A. Employment/Salary (full or part-time):; BrainXell, Inc. **N. Etchin:** A. Employment/Salary (full or part-time):; BrainXell, Inc. **M.L. Hendrickson:** A. Employment/Salary (full or part-time):; BrainXell, Inc..

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.05/Y14

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

Title: Harnessing CRISPR-Ready ioGlutamatergic Neurons and ioMicroglia for drug discovery in neurodegenerative diseases

Authors: L. GRABNER[1], C. SCHMIDT[1], M. GAMPERL[1], H. CEYLAN[1], B. KÖNYE[1], K. ARAT[1], N. PAPAI[1], T. PERLOVA[1], B. KLAPHOLZ[2], G. SHIPLEY[2], ***A. BYRNE**[3], M. RAMAN SRIVASTAVA[3], E. METZAKOPIAN[2], T. BUERCKSTUEMMER[1], S. SALIC-HAINZL[1];
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Abstract: Navigating drug discovery in neurodegenerative diseases requires models that reflect human physiology, enabling a deeper understanding of disease mechanisms and potential therapeutic interventions. Animal models or immortalised cell lines often fall short in replicating the intricacies of disease-affected human cells. Induced pluripotent stem cells (iPSCs) offer a solution, allowing for the recreation of disease-specific cell types, but they come with differentiation challenges, including variability and scalability issues. bit.bio addresses these with their innovative opti-oxTM technology, enabling rapid and deterministic programming of cell types at scale. With the emergence of CRISPR-Cas9 technology, a new horizon in functional genomics has been unveiled, reshaping our approach to understanding the ties between genes and diseases. Herein, we spotlight the potential of bit.bio's ioCRISPR-Ready Cells™, with an emphasis on ioGlutamatergic Neurons™ and ioMicroglia™, as pivotal cell types for neurodegenerative disease studies. These ioCRISPR-Ready Cells pave the way for advanced functional genetics studies, fostering deeper insights into neurodegenerative pathologies. Our ioCRISPR-Ready Cells are compatible with scCRISPR screening workflows, as demonstrated in our proof-of-concept screens where we targeted genes essential to neurodegenerative processes. Our findings underscored the efficacy of functional genomic screening in both cell types. Single-cell transcriptomic analyses revealed disease-associated genes whose knockout caused notable transcriptomic signatures. Specifically, within the domain of ioMicroglia, our CRISPR knockout screens highlighted genes that influenced the response to LPS stimulation, shedding light on microglial activation dynamics. Combining bit.bio's opti-ox technology with bit.bio discovery's scCRISPR screening platform, we present a ground-breaking approach to drug discovery in neurodegenerative diseases. The CRISPR-Ready ioGlutamatergic Neurons and ioMicroglia form a unique platform for comprehensive exploration of disease-relevant cell states, laying the foundation for advanced disease modelling, target discovery, and validation.

Disclosures: L. Grabner: None. **C. Schmidt:** None. **M. Gamperl:** None. **H. Ceylan:** None. **B. Könye:** None. **K. Arat:** None. **N. Papai:** None. **T. Perlova:** None. **B. Klapholz:** None. **G. Shipley:** None. **A. Byrne:** None. **M. Raman Srivastava:** None. **E. Metzakopian:** None. **T. Buerckstuemmer:** None. **S. Salic-Hainzl:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

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Program #/Poster #: PSTR195.06/Y15

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

Support: R01MH113215
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T32GM007814-40
CIHRDFD-181599

Title: A functional Schizophrenia-associated genetic variant near the TSNARE1 and ADGRB1 genes.

Authors: *R. J. BOYD, M. H. WAHBEH, C. YOVO, D. AVRAMOPOULOS, A. S. MCCALLION;
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Abstract: Recent collaborative genome wide association studies (GWAS) have identified over 200 independent loci contributing to schizophrenia (SCZ) risk. The genes closest to these loci have diverse functions, supporting the potential involvement of multiple relevant biological processes; yet there is no direct evidence that individual variants are functional or directly linked to specific genes. Nevertheless, overlap with certain epigenetic marks suggest that most GWAS-implicated variants are regulatory. Based on the strength of association with SCZ and the presence of regulatory epigenetic marks, we chose one such variant near *TSNARE1* and *ADGRB1*, rs4129585, to test for functional potential and assay differences that may drive the pathogenicity of the risk allele. We observed that the variant-containing sequence drives reporter expression in relevant neuronal populations in zebrafish. Next, we introduced each allele into human induced pluripotent cells and differentiated 4 isogenic clones homozygous for the risk allele and 5 clones homozygous for the non-risk allele into neural precursor cells. Employing RNA-seq, we found that the two alleles yield significant transcriptional differences in the expression of 109 genes at FDR <0.05 and 259 genes at FDR <0.1. We demonstrate that these genes are highly interconnected in pathways enriched for synaptic proteins, axon guidance, and regulation of synapse assembly. Exploration of genes near rs4129585 suggests that this variant does not regulate *TSNARE1* transcripts, as previously thought, but may regulate the neighboring *ADGRB1*, a regulator of synaptogenesis. Our results suggest that rs4129585 is a functional common variant that functions in specific pathways likely involved in SCZ risk.

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Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

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

Support: NIH Grant Brain Armamentarium UF1MH128336
CZI Neurodegeneration Challenge Netowrk
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Aligning Science Across Parkinson's (ASAP-020495 to V.G.) through the Michael J. Fox Foundation for Parkinson's Research (MJFF)

Title: Transcriptional crosstalk between AAV genomes enables targeted delivery of large gene editing machinery for brain cell type-specific gene function interrogation

Authors: *B. H. BARCELONA, G. M. COUGHLIN, M. BORSOS, N. APPLING, A. MAYFIELD, E. MACKEY, R. ESER, C. JACKSON, X. CHEN, S. RAVINDRA KUMAR, V. GRADINARU;
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Abstract: Efficient cell type-specific manipulation is crucial for dissecting complex cell and circuit function in neuroscience research. Engineering of adeno-associated viruses (AAVs) has yielded a toolset of AAV vectors that can cross the blood-brain barrier (BBB) and enable genetic access with minimally invasive systemic delivery throughout the brain of multiple rodent models and non-human primates. Incorporation of regulatory elements (e.g. enhancers) in the AAV genome provides the opportunity to target specific cell types. However, AAV's small packaging capacity (4.4 kb, not including ITRs) restricts the use of cell type-specific enhancers to drive expression of large cargo such as Cas9 for genetic modification. This limitation can be addressed using a phenomenon termed "transcriptional crosstalk," in which an enhancer on one AAV genome can boost expression from the promoter of another, in a cell type-specific manner. By separating enhancers and coding sequences across two AAV vectors, this strategy can help to circumvent the packaging constraints. Here, with BBB-penetrant CNS AAV capsids, we describe transcriptional crosstalk using multiple cell type-specific enhancers with activity throughout the cerebellum and cortex. Using PNS-targeting AAV capsids, we also demonstrate transcriptional crosstalk occurring in peripheral structures including the enteric nervous system and dorsal root ganglia. We then test the feasibility of cell type-specific gene editing using this approach with a commonly used reporter assay and demonstrate that crosstalk is able to restrict efficient gene editing to targeted cell types. Finally, we leverage transcriptional crosstalk to facilitate cell type-specific gene disruption via CRISPR, following systemic administration of AAV-PHP.eB in wildtype animals. For this experiment, we targeted the voltage-gated calcium channel subunit alpha 1A gene (*Cacna1a*) in Purkinje cells (PCs). Loss-of-function in this gene in PCs leads to ataxia. Two sequence-independent sgRNAs were used and compared to a no-guide condition. Immunohistochemistry showed reduced *Cacna1a* staining in the cerebellar molecular layer for both sgRNAs. Furthermore, we were able to recapitulate known behavioral phenotypes of *Cacna1a* knockout mice. In sgRNA-treated animals, we observed reductions in total locomotion, skilled motor behavior, and forelimb strength. Similarly, detailed gait analysis revealed gait

abnormalities in sgRNA-treated animals. This innovative method allows for targeted gene disruption without the need for transgenic animals, offering a versatile and powerful tool for gene manipulation in a variety of model organisms.

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Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.08/Y17

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

Support: Deutsche Forschungsgemeinschaft, SFB1280 Project-ID 316803389 subproject A01

Title: Achieving cell-type specific transduction with adeno-associated viral vectors in pigeon

Authors: K. HASELHUHN, J. M. TUFF, M. ZIEGLER, O. GÜNTÜRKÜN, ***N. ROOK**; Biopsychology, Ruhr Univ. Bochum, Bochum, Germany

Abstract: Birds are excellent models to study learning, complex cognition, song and vision. However, to understand how these behaviors are realized within the brain, methods that are able to record and control cellular activity on a millisecond timescale are essential. Recent advancements in novel methods, such as optogenetics, calcium imaging or DREADDs, have revolutionized rodent research, but are not yet widely used in avian research. This is in part due to the fact that these methods rely on the introduction of genetic information for artificial ion channels or sensors. In the absence of transgenic animals, adeno-associated viruses (AAVs) are often used for viral gene transfer. AAVs can be flexibly implemented to transfer different genetic sequences as well as to achieve cell-type specificity or conditional gene expression through distinct promoter systems. While AAVs have been used in pigeons before, this study set out to verify further tools to gain specific gene expression. We provide a proof of concept that GCaMP7 and NpHR can successfully be expressed in pigeon neurons. Furthermore, we are able to show that CaMKII and mDLX promoters lead to cell-type specific expression. Likewise, this study offers a proof of concept for the functionality of the Cre/loxP and a Tet-On/Tet-Off system. While Cre/loxP expands the possibility to transfer genes that are too large for typical AAVs, the Tet-On/Tet-Off system offers conditional gene expression. These novel tools open new avenues for behavioral research in pigeons to understand the avian brain.

Disclosures: K. Haselhuhn: None. **J.M. Tuff:** None. **M. Ziegler:** None. **O. Güntürkün:** None. **N. Rook:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.09/Y18

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

Support: NIMH grants U24 MH133236

Title: A recently developed "microglia-targeting" AAV capsid enables specific genetic access to hippocampal and neocortical excitatory neurons

Authors: *W. CAO[1], X. XU[2];
[1]Univ. of California, Irvine, Irvine, CA; [2]Anat. and Neurobio., Univ. California, Irvine, Irvine, CA

Abstract: It has been recently reported that adeno-associated virus (AAV) capsid variants (AAV-MG1.1 and AAV-MG1.2) produced by directed evolution capsid engineering (Lin et al., 2022), can mediate efficient in vitro and in vivo microglial transduction, capable of delivering various genetic payloads into microglia with high efficiency. In this study we report that AAV-MG1.2 actually enables specific genetic access to hippocampal and neocortical excitatory neurons in vivo, but does not infect non-neuronal cells including microglia in vivo. We packaged CAG-EGFP and CAG-tdTomato into the AAV capsid MG1.2, respectively, and stereotaxically injected the virus into hippocampal CA1, subiculum and visual cortical regions of adult wild type mice. Through immunostaining of inhibitory neuronal marker GABA and quantification of tdTomato+/EGFP+ and GABA- cells, we identify over 95% of MG1.2 labeled cells to be excitatory cells in CA1, CA2 and CA3, and subiculum, and identify over 99 % of MG1.2 labeled cells to be excitatory cells in visual cortex. Thus, the MG1.2 capsid primarily labels excitatory neurons in the hippocampus and the visual cortex. In addition, we find that the MG1.2 capsid specifically labels the deep layer of the CA1 pyramidal layer in a titer-dependent manner. Lower virus titers ($5.15x10^{12}$ GC/ml compared to $5.15x10^{13}$ GC/ml for MG1.2-CAG-eGFP) result in more precise labeling specifically within the deep layer. This specificity for the deep layer is more pronounced in the ventral CA1. Given the cell type heterogeneity among CA1 pyramidal cells exists along the superficial-deep axis, AAV-MG1.2 can be used to genetically target the deep sublayer of CA1 stratum pyramidale for structural and functional analysis. We also incorporate cre-recombinase with MG1.2 capsid to create MG1.2-Cre in order to manipulate excitatory neurons for functional studies. By injecting MG1.2-Cre into the Ai9 reporter mouse line, we find that MG1.2-Cre specifically targets excitatory neurons with a specificity of 95.72% at CA1 and a specificity of 96.91% at visual cortex. In addition, rabies monosynaptic tracing for excitatory neurons using MG1.2-Cre at dorsal CA1 and visual cortex reveals major inputs consistent with what have been reported before. We also injected MG1.2-CAG-tdTomato into adult rat brains, and we discovered that the MG1.2 AAV capsid maintains specificity for the excitatory neurons in rats with a specificity over 85%. Taken together our new discovery regarding the AAV capsid MG1.2 expands our genetic toolset to target overall and specific excitatory cell types in the brain across different species.

Disclosures: W. Cao: None. **X. Xu:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.10/Y19

Topic: I.08. Methods to Modulate Neural Activity

Support: ASAP-020625

Title: Olfactory Sensory Neuron Environment-to-CNS Connection as a model for Alpha-Synuclein Seeding

Authors: *A. GARZA[1], B. R. ARENKIEL[2];
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Abstract: Olfactory system impairment is a common early symptom in multiple neurodegenerative disorders, including Parkinson's disease (PD). In PD, hyposmia is one of the earliest indicators of disease, occurring long before the onset of other motor symptoms. Moreover, the olfactory system is one of the first locations for the appearance of α-synuclein aggregates. Olfactory sensory neurons (OSNs) are a strong candidate for the initial aggregation and spread of pathologic α-synuclein, as they provide a direct environment-to-CNS conduit, and may serve as an origin towards the pathology of PD. To investigate the link between the olfactory system, α-synuclein pathology, and PD, we are currently investigating how misexpression of human α-synuclein variants at initial stages of the mouse olfactory system impacts aggregate formation and PD-related pathology. Towards this, we have performed an extensive screen of multiple AAV serotypes to determine the transduction efficiency of OSNs using a nasal lavage technique. For this, we lavaged mice with 11 different serotypes of AAVs engineered to express TdTomato (n=3 per serotype, total n=33). 2 weeks post lavage-mediated infection, whole brains and nasal epithelium were harvested, cryosectioned, and analyzed for transduction efficiency. We found that AAV1, AAV-DJ8, and AAV-Rh10 show robust labeling of OSNs. Moreover, single-cell AAV sequence analysis is being used to confirm both transduction and expression efficiency of the delivered serotypes. Overall, AAV administration via nasal lavage is a practical approach to OSN transduction, and useful as a new approach towards investigating α-synuclein propagation within olfactory circuitry, and throughout the CNS.

Disclosures: A. Garza: None. **B.R. Arenkiel:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

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Program #/Poster #: PSTR195.11/Y20

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

Support: HIH Grant RF1MH121274

Title: A suite of enhancer AAV vectors targeting diverse cortical populations

Authors: *Y. BEN-SIMON, J. T. TING, M. HOOPER, D. DWIVEDI, S. NARAYAN, T. L. DAIGLE, S. WAY, A. OSTER, B. THYAGARAJAN, J. K. MICH, M. TAORMINA, T. EGDORF, X. OPITZ-ARAYA, J. ROTH, N. DEE, K. RONELLENFITCH, K. SMITH, J. WATERS, S. YAO, E. LEIN, B. P. LEVI, H. ZENG, B. TASIC;
Allen Inst., Seattle, WA

Abstract: The mammalian cortex consists of a heterogeneous population of cells, each differing in morphological, physiological, and molecular properties. Understanding these differences is essential for comprehending their diverse functions, both in health and disease. Traditionally, researchers have genetically accessed and manipulated different cell types using transgenic mice, which utilize recombinases tethered to cell type-specific marker genes. However, this approach is resource-intensive and has inherent limitations. Differential gene expression across populations primarily arises due to the presence of nearby cis regulatory elements known as enhancers. These enhancers can bind transcription factors essential for activating RNA polymerase and are characterized by higher chromatin accessibility. In this study, we established a pipeline for screening putative enhancer sequences from both the mouse and human genome, exhibiting differential chromatin accessibility across different subclasses of cortical cells, to examine whether they are capable of driving cell type-specific transgene expression when packaged into a viral vector. We cloned nearly 700 putative enhancer sequences into an AAV (adeno-associated virus) backbone upstream of a minimal promoter to drive expression of SYFP2, injected these vectors systemically, and evaluated the resulting labeling pattern in the mouse neocortex. Our screen revealed many enhancers achieving a high degree of specificity for most of the targeted cortical subclasses and types, comparable to existing transgenic lines. Furthermore, we demonstrated that these sequences can be modified to enhance transgene expression or express diverse cargo, such as recombinases. Lastly, we analyzed the properties of the screened sequences to identify elements that distinguish them based on both strength and specificity. The tools we report here, along with the scalable process used to create them, should enable diverse experimental strategies and extend our understanding of the fundamental mechanisms governing differential gene expression across cell populations.

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Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

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Program #/Poster #: PSTR195.12/Y21

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

Support: RF1MH121274
U19MH114830

Title: Comparing Transgenic Tools for Cell Type Specific Targeting in the Mouse Brain

Authors: *B. WYNALDA[1], J. ROTH[1], S. NARAYAN[1], Y. BEN-SIMON[1], T. L. DAIGLE[2], D. DWIVEDI[1], M. HOOPER[1], K. RONELLENFITCH[3], S. WAY[1], A. OSTER[1], J. T. TING[2], H. ZENG[4], B. TASIC[1];
[1]Mol. Genet., Allen Inst. for Brain Sci., Seattle, WA; [2]Human Cell Types, Allen Inst. For Brain Sci., Seattle, WA; [3]Transgenic Colony Mgmt., Allen Inst. for Brain Sci., Seattle, WA; [4]Allen Inst. for Brain Sci., Seattle, WA

Abstract: The brain is composed of a myriad of different cell types, each with its own unique properties. Using novel sequencing techniques like single-cell RNAseq and ATAC-seq (Assay for Transposase-Accessible Chromatin), we have built AAV (adeno-associated virus)-based viral genetic tools and transgenic mouse lines that can precisely target specific cortical cell types. For generating the AAV-based viral genetic tools, we use cell-type specific accessible cis-regulatory elements, called enhancers. Enhancers help bind transcription factors required for RNA polymerase activation; they are found in regions of open chromatin with high accessibility. We clone these enhancers into an artificial plasmid construct, package them into AAV-based delivery system, and screen them in a high-throughput pipeline for assessing cell-labeling in the mouse brain. For generating transgenic lines, we use cell-type specific driver mice (created using a recombinase cassette tethered to a cell type specific marker gene), crossed with fluorescent reporter mice, for screening and validation in our pipeline. Successful enhancer AAVs and transgenic mice are further tested using serial 2-photon tomography and single-cell RNAseq to assess their specificity in labeling cell types. Transgenic lines can be precise in targeting specific cell types, however they can be both expensive and time consuming to generate. Additionally, as cell types are often defined by more than one marker gene, access to distinct cell types using transgenic mouse models requires complex breeding strategies. AAV-based viral genetic tools can be relatively easy to use and can be optimized to provide the same level of cell-type specific targeting. To this end, in the current study we compare best-in-class, cell-type specific enhancer AAVs with their corresponding best-in-class transgenic driver line, using single-cell RNAseq data in tandem with serial 2-photon tomography. The goal is to provide optimal genetic tools with both specificity and completeness for targeting specific cortical cell types.

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Poster

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Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.13/Y22

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

Support: ZIAM# H002619

Title: Crispr interference against choline acetyltransferase in rhesus monkey

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Abstract: Neuromodulatory pathways contribute to neural circuit dynamics by regulating synaptic strength, synaptic plasticity, and neuronal excitability. As such, neuromodulators have become promising targets for genetic manipulation. Our work aims to interfere with endogenous pathways for neuromodulator signaling in Rhesus monkey behavior experiments. As a proof of principle, we employed RNA interference (RNAi) via Lentivirus expression to decrease Choline Acetyltransferase (ChAT) protein post-transcriptionally. This resulted in suppression of ChAT protein but not complete elimination. In the present work, we instead use CRISPR interference (CRISPRi) expressed from lentivirus to downregulate the enzyme pre-transcriptionally. Our CRISPRi systems have two components: a) a gene expressing the HyperdCas12 enzyme fused with a transcriptional repressor and b) a gene expressing small guide RNAs (gRNAs) that direct the Cas-repressor protein to the promoter region of the targeted protein. These components were either expressed from a single vector construct (SVC) or split up into a dual vector construct. Two SVCs and two DVC systems, each encoding different gRNA cassettes were injected into the striatum of a monkey. The two constructs for each combination differed in size, titer, and number of gRNAs but were replicated in both hemispheres. Immunostained tissue was evaluated for viral expression (via reporter protein) and ChAT downregulation. Combined expression of DVCs covered an average volume of 1.37mm^3, while the SVCs only resulted in an average volume of expression of 0.015mm^3. Thus, DVCs lead to greater viral expression volume, most likely because its two components each have a smaller packaging size compared to SVCs. Preliminary results from co-staining for ChAT expression indicate that, at least for one of the DVC systems, there is a marked reduction of ChAT expressing cells in areas of CRISPRi expression.

Disclosures: D. Hadj-Mabrouk: None. **L. Salhani:** None. **W. Lerchner:** None. **B. Li:** None. **B.J. Richmond:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.14/Y23

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

Support: RF1MH120017

Title: Third-generation monosynaptic tracing using a nontoxic single-deletion-mutant virus

Authors: X. SHI[1], H. A. SULLIVAN[2], M. MATSUYAMA[3], L. JIN[4], P. JORWAL[5], T. LAVIN[1], *I. WICKERSHAM[1];
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Abstract: Monosynaptic tracing, or the complementation of a deletion-mutant neurotropic virus in situ in order to label neurons in direct synaptic contact with a targeted neuronal population, has become a standard technique in neuroscience, but it has been mostly restricted to anatomical applications because of the cytotoxicity of the first-generation (ΔG) rabies viral vectors on which it is typically based. We have recently introduced a second-generation monosynaptic tracing system based on nontoxic double-deletion-mutant (ΔGL) rabies virus; however, this second-generation system usually labels fewer input neurons than the first-generation system does. Separately, we have also recently introduced third-generation (ΔL) rabies viral vectors, in which only one gene (L, encoding the viral polymerase) is deleted, and shown that they are as nontoxic as second-generation ones but grow more efficiently in cell culture, resulting in higher titers and therefore more labeled neurons when they are used for direct retrograde targeting in vivo. Here we introduce a third-generation monosynaptic tracing system based on nontoxic single-deletion-mutant ΔL rabies viral vectors. Modification of the ΔL vectors allows pseudotyping with the avian retroviral envelope protein EnvA; this in turn allows selective infection of neurons expressing TVA by means of helper AAVs, just as for first- and second-generation vectors. Complementation of the single deletion in vivo by expression of the viral polymerase in trans using a knock-in allele allows replication of the rabies virus in the complementing cells and spread to input cells both local and distant. This ΔL-based monosynaptic tracing system comprises a new state of the art in nontoxic monosynaptic tracing and should allow diverse applications such as long-term imaging, optogenetic and chemogenetic manipulation, and transcriptomic profiling of minimally-perturbed synaptically-connected networks of neurons.

Disclosures: X. Shi: None. **H.A. Sullivan:** None. **M. Matsuyama:** None. **L. Jin:** None. **P. Jorwal:** None. **T. Lavin:** None. **I. Wickersham:** F. Consulting Fees (e.g., advisory boards); Monosynaptix, LLC.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.15/Y24

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

Support: KAKENHI 22H05157 and 23K27472 to K.I
KAKENHI 24H01235 and 24K02343 to K.H
AMED JP23dm0207077 to M.T.

Title: Long-term activity imaging of a neuronal population that sends input to a specific type of neurons via a low cytotoxic G-deleted rabies virus vector

Authors: *K.-I. INOUE[1,2], K. HAMAGUCHI[3], S. NONOMURA[4,2], M. TAKADA[2];
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Abstract: Investigating the input to a specific neuronal population within a given neural circuit is critical for understanding the information-processing algorithms of the brain. The G-deleted rabies virus vector (ΔG-RV) pseudotyped with envelope protein from avian sarcoma leukosis virus (Env) can selectively infect a target neuronal population expressing its receptor via recombination. Expression of the rabies virus glycoprotein in this population leads to monosynaptic transmission of the ΔG-RV and expression of fluorescent protein or probe gene for activity imaging in neuron groups projecting to the target population. However, since the conventional ΔG-RV is highly cytotoxic because it expresses the viral gene as well as the inserted gene, neuronal activity can only be measured for a very short period, resulting in the restriction of its application to chronic functional experiments. Recently, the development of modified ΔG-RVs with defective replication ability has been reported. Yet, due to their low expression capacity of foreign genes, these vectors must be inserted with recombinase genes and be used in combination with reporter animals or other viral vectors with probe genes for neuronal activity measurement. This requirement limits the use of animal lines in which recombinase was expressed in a specific population of neurons. Their ultra-low replication capacity also causes a reduction in the efficiency of monosynaptic transmission. Here, we developed a novel low-cytotoxic ΔG-RV that maintains the ability to express a foreign gene. First, we created a modified full-length vector with a super-slow growth rate, but with a high level of foreign gene expression (ssRV) by inserting a foreign gene into the tip of the viral genome and modifying the genome sequence. Then, we confirmed that the ΔG-ssRV reduced its cytotoxicity, and that the ΔG-ssRV expressing GCaMP achieved stable measurement of cortical neuron activity for several months after its injection into the mouse striatum. Furthermore, using the ΔG-ssRV-GCaMP pseudotyped with Env, we successfully and continuously performed calcium imaging of mouse cortical neurons sending input to striatal dopamine D1 receptor-expressing neurons that constitute the direct pathway of the basal ganglia. The ΔG-ssRV we developed in the present study enables us to explore the information about the input to a specific neuronal population in relation to a behavioral task, and will greatly be useful for evaluating the mechanism underlying information processing in the brain.

Disclosures: K. Inoue: None. **K. Hamaguchi:** None. **S. Nonomura:** None. **M. Takada:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.16/Y25

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

Support: University of Chicago Neuroscience Early Stage Scientist Training Program from the National Institute of Neurological Disorders and Stroke (5R25NS117360)
Whitehall Foundation Research Grant (2022-12-017)

Title: Rectal Swab DNA Collection Protocol for PCR Genotyping in Rats

Authors: *A. E. KAYE, J. W. PROCTOR-BONBRIGHT, J. Y. YU;
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Abstract: Neuroscience research often uses genetically modified rodents to study nervous system function in health and disease. DNA collection is essential to genotype these transgenic animals and enable their use. However, common collection methods—such as ear, tail, and distal phalanx clipping—require tissue amputation, causing discomfort and injury. Rectal swabbing has been proposed as an effective minimally invasive alternative, but an evidence-backed protocol for the technique remains unavailable. In this work, we identify relevant collection parameters for rectal swab DNA collection and evaluate their effect on PCR result quality. Informed by these findings, we present a new rectal swab genotyping protocol that can genotype an average litter of laboratory rats within 3-5 hours. To do this, we conducted rectal swab genotyping on 21 heterozygous transgenic parvalbumin cre (*Pvalb*^{Cre}) rats of different ages and sexes (12 males and 9 females, 6-42 weeks). We varied parameters—such as the number of scrapes made along the rectal epithelium and the presence of fecal matter or cell debris contaminants—to test their impact on genotyping outcome. Then, we performed PCR to amplify two target genes of differing lengths (*Pvalb*^{Cre}: 1803 bp; *Sox21*: 237 bp) and quantified the brightness of the resulting bands on an agarose gel. We found that rectal swab samples with 2-20 scrapes produced enough DNA to amplify targets up to ~1800bp long using PCR. These PCR results were unaffected by residual fecal matter or cell debris generated during the collection and extraction process. Further, rectal swabs produced PCR results with similar utility as invasively-collected ear clip samples, and consistently greater utility than oral swabs. Our protocol enables fast, minimally invasive, and safely repeatable genotyping, leveraging the efficiency of commercial direct-from-tissue PCR reagents. It minimizes distress to animals during routine genotyping, enabling the non-injurious identification of transgenic animals for experimental use. It may be especially useful for studies requiring fine limb movement, laboratories with limited molecular biology equipment, and field studies where invasive collection poses greater risks of

injury. Our protocol can potentially be applied to other laboratory species such as hamsters, gerbils, and guinea pigs.

Disclosures: A.E. Kaye: None. **J.W. Proctor-Bonbright:** None. **J.Y. Yu:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.17/Y26

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

Support: MSN228402 – American Cancer Society (ACS) Institutional Research Grant (IRG)
144-AAL7122 - University of Wisconsin Carbone Cancer Center Tumor Microenvironment pilot award
WPP5986 - University of Wisconsin Partnership Program award

Title: Exploring the nexus: Sensory neurons and malignant melanoma dynamics

Authors: *D. SUR[1,2], A. BIRBRAIR[3], B. SAVITA[3], T. LEE[3], J. CUNHA JUNIOR[3]; [1]Univ. of Wisconsin, Madison, Madison, WI; [2]Dermatology, University of Wisconsin, Madison, WI; [3]Dermatol., Univ. of Wisconsin, Madison, WI

Abstract: Cancer and the nervous system bear a close, entangled relationship. Sensory neurons have recently emerged as components of the tumor microenvironment. Nevertheless, whether sensory neuronal activity is important for tumor progression remains unknown. Malignant melanoma is prevalent and the most lethal form of skin cancer due to its tendency to rapidly metastasize, and its incidence is rising. Immune-based and targeted therapies have shown clinical benefit, but frequently fail to achieve efficient durable responses. Thus, efforts are urgently needed to identify novel therapeutic targets to slow or prevent disease progression. Recent findings by our group and others have demonstrated that sensory nerves infiltrate within tumors and affect their development. Since the lungs are common sites of melanoma cell metastasis, we investigated the anatomic location of sensory neurons in metastatic lungs. Using Nav1.8-Cre$^{+/-}$; Tdtomato$^{+/-}$ mice we found that sensory innervations are both surrounding and infiltrating within pulmonary metastases. To analyze the contribution of sensory nerve fibers to melanoma metastasis, we chemically denervated sensory neurons by treating animals with three consecutive daily doses (30, 70, and 100 μg/kg) of resiniferatoxin (RTX), a capsaicin analog. Pharmacologic depletion of sensory neurons was confirmed by analyses of dorsal root ganglions (DRG) of Nav1.8-Cre/TdTomato RTX-treated animals. After treatment with RTX, B16F10 melanoma cells intravenously into RTX-denervated and controlled mice. After 2 weeks of transplantation, the number of lung metastases decreased significantly in sensory neuron-denervated mice compared with controls. Along with this we developed mice that conditionally expressing Gq protein-coupled receptor (hM3Dq) or Gi-coupled engineered human muscarinic 4 receptor (hM4Di). In

the resulting mice, sensory neuronal activity can be selectively activated or silenced, respectively, by the administration of CNO. We evaluate the formation of pulmonary metastasis after intravenous administration of melanoma cancer cells in these animals. Cumulatively, our data reveal the differential effect of sensory neurons in malignant melanoma progression.

Disclosures: D. Sur: None. **A. Birbrair:** None. **B. Savita:** None. **T. Lee:** None. **J. Cunha Junior:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.18/Y27

Topic: I.08. Methods to Modulate Neural Activity

Title: Mouse model for cell type-specific ablation of high-voltage activated Ca2+ channels

Authors: S. SAJADI[1], ***M. PAUKERT**[2];
[1]Cell. and Integrative Physiol., UT Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; [2]UT Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: We report a novel transgenic mouse line for cell type-specific ablation of high-voltage activated Ca^{2+} channels (HVACCs). HVACCs are important for a wide range of biological functions including neurotransmitter release, hormone release, regulation of cellular excitability and contractility, as well as regulation of gene expression. Understanding the contribution of individual cell types or pathways to HVACC function at the level of the local cellular network up to systems level animal behavior is hampered by the widespread expression of Ca^{2+} channels, limiting mechanistic conclusions from pharmacological approaches. Another challenge is that often more than one HVACC subtype is expressed in a cell with overlapping or redundant function, or with the ability to compensate for each other upon deletion of a single subtype. To address these limitations, we took advantage of a recently reported nanobody that binds to all HVACC ß-subunits for degradation and thereby abolishes Ca^{2+} channel function (Ca_V-aßlator). We generated a transgenic mouse line that permanently and exclusively expresses Ca_V-aßlator and equimolar amounts of a fluorescent reporter upon Cre recombination. We anticipate that this new transgenic mouse line can be used to effectively ablate HVACC function from any cell type for which a specific Cre driver line is available without the need for continuous drug application. For the characterization of this new transgenic mouse line we combined awake mouse two-photon Ca^{2+} imaging and acute cerebellar slice electrophysiology to study the effects of Ca_V-aßlator on locomotion-induced noradrenergic signaling to Bergmann glia and on granule cell-to-Purkinje cell glutamatergic signaling. We chose these experimental paradigms since the bar for successful application of this mouse line is particularly high in cells where, upon expression, Ca_V-aßlator needs to reach remote cell process terminals to target HVACCs. Therefore, with far-reaching projections originating in locus coeruleus and targeting cerebellar Bergmann glia through noradrenergic volume transmission, and with classical fast glutamatergic signaling in the

cerebellar cortex, we quantified two of the most challenging and sensitive applications for the Ca_V-aßlator approach. Accordingly, our results predict the utility of this novel transgenic mouse line for revealing signaling mechanisms that involve HVACCs in specific cell types within a broad range of organs of the body.

Disclosures: S. sajadi: None. **M. Paukert:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.19/Y28

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

Title: Determining both biodistribution and efficacy of ASO using microsampling

Authors: M. S. HEINS[1], A. GOOSSENS[1], *A. J. NURMI[2];
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Abstract: Antisense oligonucleotides (ASO)s are short single strands of synthetic nucleic acids that can modify mRNA expression by a variety of mechanisms, including steric blockage and splicing. ASOs have proven to be suited therapeutics for (CNS) genetic disorders with several of them having reached FDA or EMEA approvals already. Benefits of ASOs include their long half-life and the capability to achieve a wide tissue distribution. Delivery into the human CNS is currently achieved by intrathecal (IT) administration as ASOs cannot penetrate the blood-brain-barrier (BBB).
In preclinical CNS research initial *in vivo* evaluations are typically performed by intracerebroventricular (ICV) administration of the ASO. Relevant read-outs for these studies include tolerability, biodistribution and efficacy. These are determined by behavioural assessment followed by terminal tissue collections and ASO analysis and qPCR evaluation. Experimental study designs which take the 4Rs into consideration have allowed us to stretch our capabilities. The current optimized methods enable us to both quantify ASO levels by LC-MS and perform qPCR analysis in (micro-)dissected tissues. These combined PK/PD data show the biodistribution and efficacy of the ASO of interest and can highlight potential off-target risks. The results have a direct relevance for further predictive modeling and translatability to the clinically relevant IT dose route and higher species.
This example study design details benefits of microsample collection and techniques used to allow sensitive quantitation of ASO as well as target and household gene expression. Results illustrate the reduction that can be achieve while simultaneously information per individual greatly increases. Demonstrating that LC-MS ASO quantitation can be combined with qPCR gene expression analysis in microsamples and thus providing valuable biodistribution and efficacy data.

Disclosures: M.S. Heins: None. **A. Goossens:** None. **A.J. Nurmi:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.20/Y29

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

Support: NIDA Grant P50DA037844

Title: Rattaca: genetic predictions for selected populations and experimental design in outbred rats

Authors: *T. MISSFELDT SANCHES[1,2], M. K. LARA[3], O. POLESSKAYA[4], A. A. PALMER[5], B. JOHNSON[1], A. S. CHITRE[6], D. CHEN[7];
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Abstract: RATTACA (RAT Trait Ascertainment using Common Alleles) is a new research service conducted by the NIDA Center for GWAS in Outbred Rats. The service makes use of nearly twenty thousand N/NIH Heterogenous Stock (HS) rat genotypes and thousands of measurements across hundreds of addiction-related behavioral and physiological traits. Using more than 5 million high-quality genome-wide SNPs along with classical genetic analysis tools (e.g. G-Blup, polygenic risk scores), we make genetic predictions of the behavioral and physiological traits of animals at weaning using their measured genotypes. Examples include intravenous self-administration of cocaine, oxycodone, heroin, and nicotine; alcohol drinking following vapor exposure, delay discounting, and locomotor activity. By sampling cohorts of rats predicted to show extreme phenotypes, we provide a service that is similar to selected lines, or inbred strains with divergent traits, while maintaining a diverse genetic background that avoids the genetic confounds associated with other approaches. RATTACA allows for new approaches to studying the genetic basis of individual differences, a fundamental but often overlooked aspect of most behavioral and physiological traits. The Center is providing RATTACA rats at a subsidized cost to interested researchers.

Disclosures: T. Missfeldt Sanches: None. **M.K. Lara:** None. **O. Polesskaya:** None. **A.A. Palmer:** None. **B. Johnson:** None. **A.S. Chitre:** None. **D. Chen:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.21/Y30

Topic: I.08. Methods to Modulate Neural Activity

Support: MEXT/JSPS KAKENHI JP19K08138
MEXT/JSPS KAKENHI JP23H02405
MEXT/JSPS KAKENHI JP23K27098

Title: Longitudinal assessment of DREADD expression and efficacy in macaque monkeys

Authors: *Y. NAGAI[1], Y. HORI[1], K.-I. INOUE[3], T. HIRABAYASHI[1], K. MIMURA[1,4], K. OYAMA[1], N. MIYAKAWA[1], Y. HORI[1], H. IWAOKI[1], K. KUMATA[2], M.-R. ZHANG[2], M. TAKADA[3], M. HIGUCHI[1], T. MINAMIMOTO[1];
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Abstract: Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are a chemogenetic technology enabling reversible control of neuronal activity via systemic agonist administration. While muscarinic-based DREADDs, such as hM3Dq (excitatory) and hM4Di (inhibitory), are widely used in rodent studies, their use in primate models is less documented. Given that the chemogenetic controllability depends on the expression levels of DREADDs, understanding their temporal trends is highly valuable, especially for planning long-term experiments in monkeys. In this study, we longitudinally assessed *in vivo* DREADD expression in macaque monkeys using positron emission tomography (PET) with a DREADD-selective tracer, [^{11}C]deschloroclozapine (DCZ). A total of 17 macaque monkeys that had received injections of adeno-associated virus (AAV) vectors expressing hM3Dq or hM4Di were evaluated. DREADD expression levels were estimated based on the increase in binding potential of [^{11}C]DCZ from baseline. The expression levels of hM3Dq and hM4Di peaked at about 60 days post-injection and remained at near-peak levels (86%) for about a year but subsequently declined to 37 ± 20% (7–63%) after two or more years. Despite this decline, effective neuronal modulation and behavioral changes were observed for up to two years when DREADD expression was detectable by PET. However, one hM4Di-expressed monkey, the same monkey whose expression declined to 7% as described above, had no detectable expression on PET and demonstrated no DREADD effects, suggesting that a detectable expression level is necessary for functional outcomes. Furthermore, repetitive DREADD activation through optimum doses of agonists did not significantly affect the expression level of hM3Dq or hM4Di. These results demonstrate the viability of DREADDs for long-term experiments in monkeys, providing essential data for the designs and implementation of sustainable chemogenetic manipulations.

Disclosures: Y. Nagai: None. **Y. Hori:** None. **K. Inoue:** None. **T. Hirabayashi:** None. **K. Mimura:** None. **K. Oyama:** None. **N. Miyakawa:** None. **Y. Hori:** None. **H. Iwaoki:** None. **K. Kumata:** None. **M. Zhang:** None. **M. Takada:** None. **M. Higuchi:** None. **T. Minamimoto:** None.

Poster

PSTR195: Molecular, Genetic, and Chemiogenetic Tools for Neuronal Tagging

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR195.22/Y31

Topic: I.08. Methods to Modulate Neural Activity

Support: JSPS KAKENHI JP23K11796

Title: Multiplexed chemogenetic manipulation of the default mode network in macaque monkeys

Authors: *Y. HORI[1], Y. NAGAI[1], K. OYAMA[1], Y. HORI[1], H. IWAOKI[1], K.-I. INOUE[2], M. TAKADA[2], M. HIGUCHI[1], T. HIRABAYASHI[1], T. MINAMIMOTO[1];
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Abstract: The default mode network (DMN) is a large-scale brain network that is active at rest. It is composed of several discrete brain regions, including the posterior parietal cortex (PPC) and posterior cingulate cortex (PCC). The DMN is known to be involved in internally oriented processes and has also been implicated in some psychiatric disorders. To elucidate the causal contribution of the DMN to behavior in both normal and symptomatic conditions, we aimed to establish methods for manipulating the DMN in non-human primates. Using magnetic resonance imaging (MRI), we examined the effects of chemogenetic inhibition of two core nodes of the DMN, the PPC and PCC, on the blood oxygen level dependent (BOLD) signals and functional connectivity (FC) in macaque monkeys. Two macaques received injections of adeno-associated virus vectors to express an inhibitory chemogenetic designer receptor (hM4Di) in the PPC and a designer channel (PSAM4-GlyR) in the PCC, or vice versa. Pharmacological MRI under propofol anesthesia showed a significant reduction in BOLD signals ($p < 0.001$) in the hM4Di region and in the PSAM4-GlyR region, as compared to a vehicle control, approximately 4 min and 20 min after administration of DCZ (0.1 mg/kg) and uPSEM817 (0.1 mg/kg) respectively, with effects persisting throughout our measurement period (~50 min). While the chemogenetic inhibition of either PPC or PCC alone showed limited FC reduction within the DMN nodes, simultaneous inhibition of both nodes led to a substantial decrease in global DMN connectivity. These results suggest that both the PPC and PCC play a critical role in maintaining the DMN activity. The present study thus demonstrates the promise of our methods for understanding the functional role of the primate DMN.

Disclosures: Y. Hori: None. **Y. Nagai:** None. **K. Oyama:** None. **Y. Hori:** None. **H. Iwaoki:** None. **K. Inoue:** None. **M. Takada:** None. **M. Higuchi:** None. **T. Hirabayashi:** None. **T. Minamimoto:** None.

Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.01/Y32

Topic: I.04. Physiological Methods

Support: NIH Grant U01NS123658

Title: A combined in vitro-in vivo screening strategy to develop a high-sensitivity version of the CaMPARI ratiometric calcium integrator

Authors: *D. R. MARGEVICIUS[1], M. MUSA[2], H. OROSZ[3], J. P. ICARDI[1], P. ISAK[4], M.-E. PAQUET[3], R. CAMPBELL[2], H. DANA[1];
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Abstract: Current approaches for recording large-scale brain activity patterns at cellular resolution in freely-behaving mice present several challenges. Linear microscopy methods lack the depth penetration and optical sectioning required for deep-tissue recording, while multiphoton microscopy allows for recording neuronal activity from a large single plane or few axially-shifted planes. Also, cellular-resolution recording requires a mouse to either be head-fixed under a microscope or to have a miniaturized imaging device attached onto its skull. Both imaging modalities can impact mouse behavior and underlying brain activity. Recently, it was shown that an integrator for calcium-dependent recording of neuronal activity (CaMPARI) allows for cellular-resolution imaging of large-scale brain activity patterns in freely-behaving mice without needing to attach any devices to the animal. Also, despite the enhanced *in vitro* performance of the second generation of CaMPARI (CaMPARI2), it exhibits a reduced photoconversion rate and poorer neuronal activity recording sensitivity when compared to CaMPARI1 in mice. Therefore, there are potentially unseen features that may affect how CaMPARI2 performs *in vitro* vs. *in vivo*. To address this, we developed an *in vitro-in vivo* screening pipeline that assesses the performance of new CaMPARI variants. Our findings suggest that *in vitro* and *in vivo* photoconversion rates of the same variants do not correlate. Rather, improved peak ΔF/F *in vitro* may better predict improved *in vivo* photoconversion. We present our progress developing a more sensitive generation of CaMPARI (CaMPARI3), which exhibits enhanced photoconversion, better contrast between active and less-active neurons, and increased sensitivity for detecting ΔF/F differences *in vivo*. These properties allow new experiments that require shortening the time needed to achieve a sufficient photoconversion signal-to-noise ratio. Therefore, CaMPARI3 will expand the possible experimental applications to record single-cell resolution brain activity from freely-behaving mice.

Disclosures: D.R. Margevicius: None. **M. Musa:** None. **H. Orosz:** None. **J.P. Icardi:** None. **P. Isak:** None. **M. Paquet:** None. **R. Campbell:** None. **H. Dana:** None.

Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.02/Z1

Topic: I.04. Physiological Methods

Title: Iglusnfr4: next generation of glutamate sensors for imaging synaptic transmission

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Abstract: To understand the input-output transformations of neurons, there is a need to develop genetically encoded fluorescent reporters of synaptic inputs to individual neurons. We recently reported iGluSnFR3 (Aggarwal et al 2023), a highly sensitive glutamate indicator for imaging synaptic transmission. To build on this indicator, HHMI Janelia's GENIE Project Team performed complete combinatorial mutagenesis on 11 underexplored sites identified to impact function in the original iGluSnFR3 screen. The resulting > 9000 variants were screened in cultured neurons using action potential field stimulation, and a subset of ~70 were further screened by imaging spontaneous synaptic release ('optical minis'). In the work presented here, a small subset of novel variants were further tested in vivo using two-photon microscopy, in the visual cortex of mice, under spontaneous and visual stimulation conditions as well as using fiber photometry in the ventral tegmental area. We have identified variants with further improvements in rise kinetics, higher signal-to-noise ratios, and a variety of decay kinetics in vivo.

Disclosures: A. Negrean: None. **J.P. Hasseman:** None. **A. Aggarwal:** None. **M. Xie:** None. **L. Kinsey:** None. **A. Tsang:** None. **G. Tsegaye:** None. **D. Reep:** None. **J. Zheng:** None. **I. Kolb:** None. **B.J. MacLennan:** None. **K.M. Hagihara:** None. **G.C. Turner:** None. **K. Podgorski:** None.

Poster

PSTR196: Optical Sensors for Neuronal Probing

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Program #/Poster #: PSTR196.03/Z2

Topic: I.04. Physiological Methods

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Title: Selective targeting of plasma membrane damaged cells and axons - From biosensors to pro-drugs

Authors: *D. BECKMANN[1], P. MAUKER[2], S. WANDEROY[3], A. HARBAUER[3], T. MISGELD[4], M. KERSCHENSTEINER[1], O. THORN-SESHOLD[2];
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Abstract: Loss of plasma membrane barrier integrity is a hallmark of various cell death pathways. Furthermore, disruption of the axonal plasma membrane, which leads to calcium-influx, has been shown to drive traumatic and inflammatory axon degeneration. Interestingly, this process is initially reversible: Some axons spontaneously regain membrane integrity and homeostatic calcium levels and survive long-term. We therefore hypothesize that the initial phase after loss of membrane integrity represents a window of opportunity in which therapeutic interventions including calcium chelation can promote axonal survival. To prevent unwanted side effects of CNS calcium chelation we have been exploring ways to deliver pro-drugs selectively via damaged membranes. To this end, we have recently published a proof-of-principle study, describing the development and application of a membrane damage selective fluorogenic probe (Mauker, Beckmann et al., JACS 2024, DOI: 10.1021/jacs/3c07662). This biosensor is a disulfonated fluorescein-derivative that requires intracellular enzymatic turn-on but cannot cross healthy plasma membranes due to is polarity. It is however readily taken up by cells that were exposed to a range of different damaging agents, such as pore-forming toxins, ferroptosis inducers or necrotic damage. The cytosolic localization of the biosensor also made it useful for the application on axons, which contrasts with nuclear DNA-binding dead cell stains such as PI or Sytox. Based on our findings, we have now synthesized a disulfonated pro-drug calcium chelator, BAPTA-AM4-S2, whose calcium binding and membrane crossing abilities we are assessing with live cell calcium imaging. To find the optimal dose for *in vivo* application we are also investigating how high intracellular calcium rises when plasma membrane barrier integrity is lost. To this end, we are expressing a range of genetically encoded calcium sensors with different calcium affinities from the µM to mM range both *in vitro* and *in vivo*. Taken together we believe that our studies establish novel tools to selectively label and therapeutically target neuronal membrane damage *in vitro* and *in vivo*.

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Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.04/Z3

Topic: I.04. Physiological Methods

Support: IDG/McGovern Institute for Brain Research, Peking University, Beijing 100871, China
Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China
Chinese Institute for Brain Research, Beijing 102206, China

Title: A genetically encoded melanocortin sensor

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Abstract: The melanocortin system plays a crucial role in a diverse range of physiological functions, including the regulation of feeding behaviour, energy homeostasis and sexual behaviour. This system comprises five distinct receptor subtypes and each one exhibits tissue-specific expression patterns, among which MC3R and MC4R are particularly abundant in the neural system. Dysfunctions in melanocortin, MC3R and MC4R have been linked to obesity and hypoactive sexual desire disorder, highlighting their potential as therapeutic targets. A comprehensive understanding of the spatial and temporal dynamics of the melanocortin signal will increase our understanding of the regulation of the melanocortin system during physiological processes and allow us to identify novel therapeutic targets for various disorders. However, current methodologies for *in vivo* detection of melanocortin dynamics, such as microdialysis and Tango assay, face limitation in spatiotemporal resolution and sensitivity, presenting significant challenges to comprehensive study.
To address these limitations, we have developed a highly sensitive genetically encoded **G**-protein-coupled **R**eceptor **A**ctivation-**B**ased (**GRAB**) melanocortin sensor, **GRAB$_{MC4R}$**, using the backbone of the MC4R receptor. The GRAB$_{MC4R}$ sensor exhibits a nearly 900% fluorescence increase in response to the α-MSH, both in HEK293T cells and cultured neurons. This sensor has an affinity to α-MSH in the range of tens of nanomolar, with an apparent EC50 of approximately 35 nM. In summary, we present a robust biosensor, GRAB$_{MC4R}$, which shows high response and sensitivity to melanocortin. This sensor holds promise for facilitating the study of the melanocortin system across a wide array of biological contexts.

Disclosures: W. Teo: None. **Y. Li:** None.

Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.05/Z4

Topic: I.04. Physiological Methods

Title: Multiplex Imaging of Purinergic Dynamics in vivo with an Expanded Toolbox of GRAB Sensors

Authors: *B. LI[1], H. PEIYAO[2], Z. BAI[2], J. WANG[2], Z. WU[2], Y. LI[1];
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Abstract: Purinergic transmitters, including extracellular ATP, ADP, adenosine (Ado), play essential roles in both the peripheral and central nervous systems by interacting with specific purinergic receptors on different cell types. These extracellular purines can also undergo conversions, such as ATP degrading into adenosine, which regulates sleep, motion and neuroimmune interactions. Due to the complex nature of purinergic signaling, developing tools for concurrent monitoring with high molecular specificity and spatiotemporal resolution is essential. In this study, we present a series of optimized GPCR-Activation-Based (GRAB) sensors capable of detecting various purinergic transmitters. These sensors show good plasma membrane localizations, high sensitivity, and notably, high selectivity in distinguishing different purinergic transmitters. We showcase a novel GRAB-Ado sensor with faster kinetics, enabling detailed monitoring of Ado fluctuations during sleep-wake cycles. Furthermore, the development of red-shifted purinergic GRAB sensors enables dual-color imaging of distinct neurochemicals. We successfully applied these sensors to simultaneously record Ado and ATP dynamics in cultured neurons and mice *in vivo.* Leveraging high spatial resolution, we observed localized purinergic transmitter release in response to epileptic events using widefield and two-photon imaging *in vivo*. Taken together, the expanded toolbox offers a powerful approach for dissecting purinergic signaling in diverse biological contexts. It paves the way for a deeper understanding of the dynamic regulation of the purinergic system in health and disease.

Disclosures: B. Li: None. **H. peiyao:** None. **Z. Bai:** None. **J. Wang:** None. **Z. Wu:** None. **Y. Li:** None.

Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.06/Z5

Topic: I.04. Physiological Methods

Title: Long-term, high-resolution telemetry monitoring of circadian rhythmicity using temperature and activity in socially housed mice.

Authors: *T. VELIE, K. NICHOLS, K. HOLLIDAY-WHITE, M. HOOVER, J. VAN HEE, J. ALVAREZ;
Data Sci. Intl., St. Paul, MN

Abstract: Studying the circadian rhythms governing physiological processes is vital to understanding their impact on health and diseases. The effect of circadian rhythms on mouse thermoregulation and locomotor activity has been widely studied. However, in experimental settings employing socially housed mice, technological restrictions can impose a practical hurdle to investigate the circadian rhythmicity of body temperature and locomotor activity. Current methods used for group-housed mice may present drawbacks in combining high temporal resolution data, long-term data recording capabilities, access to the data in real-time, and device surgical implantability. To address these limitations, we developed a sophisticated telemetry platform, SoHo™, to monitor body temperature and gross locomotor activity in socially housed small animals. This study aims to validate the SoHo™ telemetry system for simultaneous measurement of core body temperature and gross locomotor activity in group-housed mice. CD1 male mice (6 weeks old; N=5) were surgically instrumented (intraperitoneally) with the SoHo™ telemetry transmitters. Following the recovery period, the animals were group-housed under a standardized 12-hour dark/light cycle. Synchronized core body temperature, gross locomotor activity, and video data were continuously collected for 12 days. Actograms and hourly temperature average box plots show that the circadian clock regulates the gross locomotor activity and core body temperature, as well as a positive correlation ($r = 0.85$) between these two parameters. Average gross locomotor activity (calculated as counts per minute; cpm) and core body temperature were significantly increased during dark periods compared to light periods (75.7 ± 10.2 cpm vs. 48.1 ± 4.7 cpm, $p < 0.05$; 37.6 ± 0.4 ^{o}C vs. 36.4 ± 0.4 ^{o}C, $p < 0.05$). The magnitude of average gross locomotor activity and core body temperature fluctuations across the circadian cycle were 94.4 ± 37.9 cpm and 3.5 ± 0.7 ^{o}C, respectively. The temporal relationship between peaks of gross locomotor activity and peaks of core body temperature yielded an average phase angle of 12.9 ± 7.7 minutes. The data demonstrates that SoHo™ telemetry is a powerful platform for evaluating temperature and activity variations in socially housed mice. Further experiments, such as photoperiod modifications and pharmacological intervention, are needed to address circadian rhythm modulation.

Disclosures: T. Velie: A. Employment/Salary (full or part-time):; Employee. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stockholder. **K. Nichols:** A. Employment/Salary (full or part-time):; Employee. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stockholder. **K. Holliday-White:** A. Employment/Salary (full or part-time):; Employee. **M. Hoover:** A. Employment/Salary (full or part-time):; Employee. **J. Van Hee:** A. Employment/Salary (full or part-time):; Employee. **J. Alvarez:** A. Employment/Salary (full or part-time):; Employee.

Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.07/Z6

Topic: I.04. Physiological Methods

Support: Howard Hughes Medical Institute

Title: Optimization of genetically encoded functional indicators for in vivo imaging: GENIE Project Team updates

Authors: *J. P. HASSEMAN[1], A. AGGARWAL[2], B. J. ARTHUR[3], D. BUSHEY[3], H. R. FARRANTS[3], V. JAYARAMAN[3], J. N. KOBERSTEIN[3], I. KOLB[3], W. KORFF[3], A. NEGREAN[4], K. PODGORSKI[4], D. REEP[3], E. R. SCHREITER[3], H. M. SHIOZAKI[3], N. P. SPRUSTON[3], A. G. TEBO[3], A. TSANG[3], G. TSEGAYE[3], G. C. TURNER[3], A. K. WARING[3], J. ZHENG[3];
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Abstract: GENIE is a Janelia Project Team that uses systematic mutagenesis and screening in primary neuronal culture to optimize genetically encoded sensors for neuronal activity. Our pipeline spans from biochemical characterization to *in vivo* validation with imaging experiments in mice, zebrafish and fruit flies. We will present the latest results of optimizing a set of genetically encoded indicators for voltage, calcium and neurotransmitter release.
SCaMP: Is a red calcium indicator based on the mScarlet scaffold currently undergoing extensive mutagenesis to identify variants with improved performance. Screening has identified single-site variants with a further ~5-fold increase in signal:noise, and rapid rise times compared to jRGECO and jRCaMP. These single-site candidates will be combined and evaluated for further performance improvements.
WHaloCaMP2: Is a hybrid chemigenetic calcium indicator composed of a genetically encoded calcium-sensing domain and a HaloTag-attached synthetic dye. This sensor enables imaging at near-infrared wavelengths, depending on the choice of the synthetic dye from the JF family. Our screening pipeline identified variants with 2-5x increased sensitivity to single action potentials, whose performance will now be tested in vivo.
iGluSnFR4: New variants of this indicator of glutamate release have higher sensitivity and diverse kinetic properties. Spontaneous synaptic release events (optical minis) in cultured neurons exhibit up to 5-fold improved signal:noise relative to iGluSnFR3[1]. In vivo testing is ongoing.
RubyACR: We have tested far-red-shifted optogenetic inhibitors A1ACR and HfACR ref.[2] in *Drosophila*. The action spectrum for these channels reaches out to wavelengths which penetrate Drosophila cuticle effectively, and elicit less behavioral response than the shorter wavelengths used for GtACR[3]. In vivo electrophysiology shows strong light-activated currents at 660nm, and

functional imaging indicates that neurons in the visual system are effectively inhibited. In freely behaving flies, these ACRs can acutely and reversibly inhibit ongoing production of courtship song.
(1).Aggarwal, A. et al. Nat. Methods 1-10 (2023).(2).Govorunova, E. G. et al. Proc. Natl. Acad. Sci. U. S. A. 117, 22833-22840 (2020).(3).Mauss, A. S., Busch, C. & Borst, A. Sci. Rep. 7, 13823 (2017).

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Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.08/Z7

Topic: I.04. Physiological Methods

Support: Allen Institute

Title: A platform for benchmarking genetically encoded neuromodulator indicators in vivo

Authors: *B. J. MACLENNAN[1], K. M. HAGIHARA[1], B. WYNALDA[2], S. NARAYAN[3], K. SVOBODA[4], K. PODGORSKI[1];
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Abstract: Neuromodulators (NMs) have been implicated in a variety of biological and behavioral functions. The development of genetically encoded fluorescent indicators for NMs has exploded in recent years, enabling molecularly specific, temporally precise optical measurements in targeted cell types. However, there is a need for systematic and quantitative evaluation of indicator performance in vivo, especially as multiple indicators exist for the same NM. In vitro assays do not always translate to in vivo performance because of differences in chemical environment, tissue architecture, and ligand concentration dynamics. A lack of systematic benchmarking makes it challenging for researchers to select the appropriate indicator for specific applications. Here, we present a platform to benchmark indicator performance in vivo. To achieve consistent expression level across indicators, indicators for norepinephrine (NE), serotonin (5-HT), acetylcholine (Ach), dhpamine(DA), and Histamine (His) were subcloned into a standardized pAAV plasmid backbone, “pND", and packaged into adeno-associated viruses (AAVs) for consistent expression across sensors. Optical fiber-based photostimulation of the cell bodies of NM neurons expressing soma-localized excitatory opsins was used to control neuromodulator release while measuring indicator activity in their projection

targets with fiber photometry. We evaluated indicators in at least two projection targets per NM with different axon innervation densities (e.g. the nucleus accumbens and basal amygdala for DA indicators), because the performance of indicators depends on affinities relative to NM concentration. To enable calibrated photostimulation we co-expressed soma-localized jGCaMP8s to monitor activity of NM neurons in response to photostimulation. This allowed us to calibrate photostimulation strength to physiologically-relevant activity levels by identifying photostimulation parameters that achieve an activity level matching the behaviorally-induced one. Epitope tags were attached to each indicator to quantitatively assess expression level using immunostaining. We assessed expression and surgical targeting with post-hoc slice-based histology and clearing-based whole-brain light-sheet microscopy, systematically registered to the Allen Institute's Common Coordinate Framework. In this presentation, we will share preliminary results and discuss strategies for disseminating data and benchmarking protocols. We aim to provide a reproducible, standardized, and trusted resource to guide design of in vivo optical measurements of NMs.

Disclosures: B.J. MacLennan: None. **K.M. Hagihara:** None. **B. Wynalda:** None. **S. Narayan:** None. **K. Svoboda:** None. **K. Podgorski:** None.

Poster

PSTR196: Optical Sensors for Neuronal Probing

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Topic: I.04. Physiological Methods

Support: NINDS U01NS120820

Title: Computationally aided redesign of a dopamine sensor from a serotonin-binding scaffold to investigate dopamine transporter-related modulation

Authors: *Y. HUA[1,2], D. LOPEZ MATEOS[3], J. L. ANDERSEN[4], R. DALANGIN[5], S. SINNING[4], V. YAROV-YAROVOY[3], L. TIAN[1];
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Abstract: Dopamine (DA) is a neuromodulator that is involved in reward, reinforcement, movement, and decision-making. There is an advanced knowledge of brain-wide dopaminergic innervations and their connections to degenerative diseases and addictions. The emergence of genetically encoded sensors has enabled the detection of neurotransmitter release with improved spatial and temporal resolution. Current DA biosensors are based on endogenous GPCRs as DA-binding scaffolds. While these sensors have facilitated the profound discoveries of DA release in various behavioral paradigms, there is still a limited understanding of the DA signaling inside the cell. The termination of DA transmission is mainly accomplished by dopamine transporter

(DAT) as well as other SLC family transporters-mediated reuptake from the synapses. DA reuptake has been indicated as a major therapeutic target for mood and movement disorders, and it is critical to further understand DA reuptake-targeting drugs' effects. Therefore, a sensor that can characterize the neuronal transmission by detecting the recycled dopamine is necessary for pharmacological studies. To achieve this goal, we are converting a periplasmic-binding protein (PBP) -based serotonin sensor, iSeroSnFR, to a DA sensor. PBP-based sensors are orthogonal to the endogenous receptors, which makes them less susceptible to the allosteric modulations affected by drugs. In addition, its high solubility expands the locations where the sensors can be expressed. To re-design iSeroSnFR to a DA sensor, we are using a synergic approach that involves feedback between computational modeling and semi-high-throughput screening of mutagenic libraries of variants to redesign the binding pocket. We use a combination of AlphaFold and Rosetta modeling strategies to help direct the mutagenesis around the binding pocket and simulate the docking of ligands to the binding domain. Then we introduce the suggested mutations to assess the new variants' properties and feedback on the assessment of the models. In all, this novel DA sensor has the potential to improve the understanding of the effects on transporters and DA signaling by psychostimulant drugs. Furthermore, the synergic design method used in this study will provide a general pipeline for fluorescent probe engineering for neurotransmitters and neuromodulators.

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Poster

PSTR196: Optical Sensors for Neuronal Probing

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Program #/Poster #: PSTR196.10/Z9

Topic: I.04. Physiological Methods

Support: NIH Grant 1R43MH134669-01

Title: A 30 um glucose microbiosensor for chronic in vivo recording

Authors: *D. AILLON, S. KAPLAN, D. A. JOHNSON;
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Abstract: Amperometric biosensors excel at monitoring changes in extracellular analytes in real time in the presence of interfering species. To maximize the efficacy of these sensors, they should be as small as possible and consume only minimal amounts of the analyte in question. This study demonstrates the use of a 30 µm diameter amperometric microbiosensor for chronic implantation in the brain. The 30 µm microbiosensor is manufactured as a pulled glass capillary electrode with a platinum wire at its core. The tip of the biosensor is ground to a disc, and then the platinum electrode is electrochemically etched to form a cavity with a depth of approximately 10 µm within the glass capillary. Enzyme is then deposited within the cavity and

electrochemically modified with a selective membrane to prevent any response to interfering compounds such as ascorbic acid. The 30 µm glucose biosensor has a linear range of over 5 mM with a sensitivity of approximately 200 to 300 pA/mM with excellent signal to noise. 30 µm glucose microbiosensors were implanted in sprague-dawley rats and monitored wirelessly using Pinnacle's Model 8172 Wireless Potentiostat. The gain of the potentiostat was modified from its standard factory setting (20M) to100M to accommodate the smaller signal levels of the microbiosensor. After surgery, rats were returned to their home cages. Daily injections (IP) of saline and glucose (1 g/kg) were administered starting approximately 24 hours after surgery. The amperometric current generated by the oxidation of enzymatically produced hydrogen peroxide was recorded at 1 Hz in Pinnacle's Sirenia Acquisition software. The chronically implanted glucose microbiosensors remained responsive to changes in glucose for over 7 days. These data demonstrate the utility of the new microbiosensor for chronic implantation. Besides their use in freely moving animals these microbiosensors are excellent candidates for use in anesthetized animals and brain slice preparations, and they can be combined in tetrode form for multi-analyte research.

Disclosures: D. Aillon: A. Employment/Salary (full or part-time):; Pinnacle Technology. **S. Kaplan:** A. Employment/Salary (full or part-time):; Pinnacle Technology. **D.A. Johnson:** A. Employment/Salary (full or part-time):; Pinnacle Technology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pinnacle Technology.

Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.11/Z10

Topic: I.04. Physiological Methods

Title: Subcellular cAMP signaling domains revealed with AKAP-targeted biosensors.

Authors: *S. MARTINKA, E. MERKEL, T. HUGHES;
Montana Mol., Bozeman, MT

Abstract: Different neurotransmitters and drugs can produce very different effects on the same neuron, even though they all signal through an increase in cAMP. There are now biophysical measurements and models that help explain how this occurs. It appears that cAMP is largely generated and destroyed in small (nanometers) domains referred to as "signalsomes". Diffusion of cAMP throughout the cell appears to be limited by an excess of binding sites in the cytosol. The question we face now is: how can we follow cAMP signaling in the subcellular signalsomes? To explore this, a variety of targeting motifs were added to the cADDis cAMP biosensor. One of the most successful strategies was to fuse AKAP proteins to the cADDis biosensor. The AKAP proteins organize the PKA enzymes and localize PKA activity in the cell. They also often bind adenylyl cyclases and/or phosphodiesterases, so they can be thought of as

an organizing protein for the signalosome. The constructs were tested for localization in living cells as well as their ability to respond to global cellular changes in cAMP. The AKAP1 localized sensor decorates the mitochondria beautifully, The smAKAP protein is exclusively localized to the plasma membrane, while the AKAP 79, AKAP18a, and AKAP18b and Gravin were localized to intracellular membranes. These sensors respond to receptor-driven changes in cAMP, but the kinetics of their response can be quite different, particularly at low levels of receptor activation. This is consistent with the signalosome signaling model. The preliminary characterization of the sensors was done in an immortalized cell line (HEK 293). Having identified the successful constructs, work is underway to characterize the function and localization in neuronal cells. While the initial results are intriguing, they raise new questions. How many different signalsomes are there in a neuron or glial cell? Which AKAPs are present at signalsomes crucial to neuronal function? Which G-protein coupled receptors act through which signalsomes?

Disclosures: S. Martinka: A. Employment/Salary (full or part-time):; Full time Salary, Scientist at company. **E. Merkel:** A. Employment/Salary (full or part-time):; Full time Salary, Scientist at company. **T. Hughes:** A. Employment/Salary (full or part-time):; Full time Salary, Scientist at company.

Poster

PSTR196: Optical Sensors for Neuronal Probing

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Program #/Poster #: PSTR196.12/Z11

Topic: I.04. Physiological Methods

Support: NSF CBET 1944846

Title: Fluorescence-guided micropipette platform for automated subtype-specific targeting

Authors: *E. MARSCHALL, J. ESHIMA, D. SMETANICK, C. MIRANDA, J. AKBARI-CARPENTER, Y. HABBABA, B. SMITH;
Arizona State Univ., Tempe, AZ

Abstract: Patch-clamp electrophysiology offers unparalleled temporal and spatial resolution recordings of excitable cells. To achieve real-time subtype-specific targeting, others have developed image-guided systems that use fluorescent labels and dyes in conjunction with high-powered microscopy techniques, such as confocal and two-photon microscopy[1]. However, there is currently no method for targeting specific subtypes beyond a tissue depth of 2mm, primarily due to limitations surrounding light scattering[2]. To bypass this limitation, waveguides have been utilized to precisely deliver light in deep tissue for applications such as imaging, optogenetics, and photometry[3,4]. Our lab recently employed integrated waveguide technology, aligning a traditional patch-clamping micropipette with a tapered fiber optic to localize fluorescence at the micropipette's tip[5,6]. This method integrates waveguides into micropipettes, granting them the

capability to target fluorescent cells towards high-resolution, subtype-specific recordings. In this work, we target and approach EGFP-transfected B35 neuroblastoma cells within an in vivo co-culture containing EGFP- and iRFP-transfected neurons and apply our microscope-free technology to ex vivo brain tissue. Enabling automated navigation for micropipette electrodes will improve cell targeting, enabling precise and high-resolution recordings at tissue depths previously difficult to access.

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Disclosures: E. Marschall: None. **J. Eshima:** None. **D. Smetanick:** None. **C. Miranda:** None. **J. Akbari-Carpenter:** None. **Y. Habbaba:** None. **B. Smith:** None.

Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.13/Z12

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

Support: NIH Grant R01DA05320001A1

Title: A Genetically Encoded Tools for Monitoring Dopamine Release in Animal Brain

Authors: J. WENG[1], ***J. DING**[2], I. SOLOWIEJ[1], W. WANG[3];
[1]Univ. of Michigan, Ann Arbor, MI; [3]Life Sci. Inst., [2]Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: Dopamine plays an important role in many physiological and pathological processes. Therefore, there is a need to study the release of dopamine with precise spatial and temporal

resolution in animal brains to uncover mechanisms of dopamine-regulated processes. Although real-time sensors can be used to monitor the dynamics of dopamine release, these sensors' transient signal makes separation of neurons related to the dopamine signaling impractical. Conversely an integration sensor, iTango, has been developed to leave permanent marks in the neurons within the dopamine-releasing area. However, iTango requires 1 hour of stimulation to see activation signal in the animal brain while dopamine release induced by a specific behavior occurs on the timescale of minutes or as briefly as seconds. To address this problem, we developed an integration sensor based on the SPARK (Specific Protein Association tools giving transcriptional Readout with rapid Kinetics) system to detect dopamine release within minutes. We showed this sensor can be activated in HEK cells and mouse brain with 1-minute dopamine- and light- stimulation providing high sensitivity and signal-to-background ratio for dopamine detection. We will further characterize this sensor in the HEK cells and neurons, including its ideal light- and drug-co-stimulation time, optimal light intensity, determining its EC50 for dopamine, as well as its dopamine specificity and selectivity. We will also validate the dopamine reporter in the mouse model and benchmark it against the iTango system. This new dopamine reporter will provide a robust platform for imaging dopamine release in animal models to facilitate the study of dopamine-regulated physiological and pathological processes.

Disclosures: J. Weng: None. **J. Ding:** None. **I. Solowiej:** None. **W. Wang:** None.

Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.14/Z13

Topic: I.04. Physiological Methods

Support: NIH Grant RO1NS123424-10
NIH Grant T32 GM008804

Title: A platform for simultaneous monitoring of long-term phasic and tonic dopamine dynamics

Authors: *N. C. WEINTRAUB[1], G. M. WINTER[2], J. R. SIEGENTHALER[1], S. L. COWEN[2], M. L. HEIEN[1];
[1]Dept. of Chem. and Biochem., [2]Dept. of Psychology, Univ. of Arizona, Tucson, AZ

Abstract: Long-term dysfunction in dopamine signaling is associated with many neurological disorders, such as Parkinson's disease, depression, and addiction making it important to monitor changes in dopaminergic dynamics over weeks and months. Dopamine neurotransmission has two signaling modalities that occur over different timescales, making concurrent monitoring technically challenging. Phasic signaling is characterized by rapid burst firing, occurring on the order of milliseconds to seconds, in response to salient stimuli, while tonic signaling is a continuous, steady-state release establishing the baseline level of dopamine activity and which changes over minutes to hours. To be able to monitor these dynamics, a technique requires

sufficient temporal, spatial, and chemical resolution to capture both modalities of dopamine release. The aim of this work is to develop a method to simultaneously monitor phasic and tonic neurotransmitter signaling stably over weeks to months, allowing for more powerful measurements to capture complex interactions and neurotransmitter dynamics. Fast-scan cyclic voltammetry (FSCV) is an electrochemical technique which monitors phasic dopamine release through oxidation and reduction of dopamine at the electrode surface. Fast-scan controlled adsorption voltammetry (FSCAV) is a complimentary technique which allows for quantification of tonic levels of extracellular dopamine using the same experimental design. To perform simultaneous FSCV-FSCAV, we developed a single board potentiostat capable of outputting multiple unique waveforms and new custom-built in-house software. Another issue for long-term electrochemical measurements is a loss of sensitivity due to biofouling of the electrodes, which degrades voltammetric performance, obscuring dopamine detection. To address this, we also developed a novel multichannel headstage based on a three-electrode configuration using a common Ag/AgCl reference, platinum counter, and multiple individually addressable working electrodes. The three-electrode design mitigates changes in the electrode signal due to biofouling occurring at the electrode surface, preserving dopamine sensitivity over long-term monitoring. *In vivo* FSCV-FSCAV allowed for the simultaneous quantification of tonic and phasic dopamine levels over 2 hours per day for 7 days. This platform lays the foundation for future research into complex dynamics of dopamine release and how dopamine signaling correlates with behavioral and physiological responses, while reducing the number of animal subjects required for future studies, contributing to more ethical and efficient research practices.

Disclosures: N.C. Weintraub: None. **G.M. Winter:** None. **J.R. Siegenthaler:** None. **S.L. Cowen:** None. **M.L. Heien:** None.

Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.15/Z14

Topic: I.04. Physiological Methods

Support: European Research Council
Autism Research Institute
Israeli Science Foundation
Teva's BioInnovators Fellowship

Title: Genetically encoded biosensor for fluorescence lifetime imaging of PTEN dynamics in the intact brain

Authors: *T. KAGAN[1], T. LAVIV[2];
[1]Tel Aviv Univ., Tel Aviv, Israel; [2]Physiol. and Pharmacol., Tel Aviv Univ., Tel Aviv, Israel

Abstract: The phosphatase and tensin homolog (PTEN) is a vital signaling protein which maintains an inhibitory brake that is critical for cellular metabolism, proliferation, and growth. The importance of PTEN signaling is evident from the broad spectrum of human pathologies associated with loss of function. Moreover, loss or gain of PTEN function in animal models leads to aberrant cellular morphology, function, and metabolic regulation. However, despite the important role of PTEN signaling, there is currently no method to dynamically monitor its activity in intact biological systems. Here, we describe the development of a novel PTEN biosensor, optimized for two-photon fluorescence lifetime imaging microscopy (2pFLIM) that is designed to measure PTEN activity within intact cells, tissues, and organisms. Our approach is based on monitoring FRET dependent changes in PTEN conformation, which serve as a proxy for the activity state in living cells. We identify point mutations that allow us to express this biosensor with minimal interference to endogenous PTEN signaling. We also demonstrate the utility of imaging PTEN signaling in cell lines, intact *C. elegans,* and in the living mouse brain. To complement this approach, we develop a red-shifted PTEN sensor variant that permits simultaneous imaging with GFP based sensors. Finally, we use in vivo PTEN imaging in the mouse brain to identify cell-type specific dynamics of PTEN activity in excitatory and inhibitory cortical cells. In summary, our approach enables dynamic imaging of PTEN activity in vivo with unprecedented spatial and temporal resolution.

Disclosures: T. Kagan: None. **T. Laviv:** None.

Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.16/Z15

Topic: I.04. Physiological Methods

Support: 1U01NS115588-01

Title: High-density, large-scale recording and stimulating neural probes with integrated ASIC

Authors: *Y. MA[1], Y. FAN[1], P. ZOLOTAVIN[1], W. WANG[2], T. CHI[1], C. XIE[1];
[1]Rice Univ., Houston, TX; [2]Electrical and computer engineering, Rice Univ., HOUSTON, TX

Abstract: Ultraflexible probes offer a revolutionary approach to stable neural recording and stimulation by creating a glial scar-free neuronal interface utilizing biocompatible materials in compact cross-sectional areas. However, these probes don’t have an equivalent number of electrodes within the same invasive footprint as the rigid probes have. This limitation stems from photolithography, which is used for fabricating ultraflexible probes, is not capable of yielding a similar high electrode density. Here, to record more neurons per implantation, we introduce a novel fabrication technique utilizing electron-beam lithography (EBL) to construct densely packed electrodes on the polyimide encapsulations. This method has enabled us to design and fabricate a 128-channel probe that presents the highest electrode density per cross-sectional area

among flexible probes. The oversampling electrodes can isolate highly clustered single units both locally and across brain regions over extended period for up to 6 months in mice cortex and 2 months in rats hippocampus along with populational neuronal tracking. The intracortical microstimulation (ICMS) triggered by dispensed microelectrodes can modulate the neural circuity and connectivity with a lower threshold at 1μA. Further, we propose a comprehensive system incorporating a large-scale flexible microelectrode array with 5,376 simultaneously recording sites into a customized application-specific integrated circuit (ASIC) with 1,344 ADCs. Multiple prototypes include penetrating multi-shank electrodes with a density of 100 electrodes/ mm depth and surface electrodes of 70 electrodes / mm^2. In summary, our work expands the toolkits for high-density, large-scale *in vivo* electrophysiology and advanced data acquisition technologies, providing unprecedented insights into studying brain functions.

Disclosures: Y. Ma: None. **Y. Fan:** None. **P. Zolotavin:** None. **W. Wang:** None. **T. Chi:** None. **C. Xie:** None.

Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.17/Z16

Topic: I.04. Physiological Methods

Support: NIH Grant 1R41NS113702-01
NSF Grant 1936173

Title: Multiplexed Detection of Neuropeptide Y and Glutamate using Fast-Scan Cyclic Voltammetry and Carbon Based Electrodes

Authors: *A. G. ZESTOS[1], ***A. G. ZESTOS**[2];
[1]Chem. and Ctr. for Neurosci. and Behavior, American Univ., Washington, DC; [2]Chem., American Univ., Washington, DC

Abstract: Carbon fiber microelectrodes (CFMEs) have been used to detect neurotransmitters and other biomolecules using fast-scan cyclic voltammetry (FSCV) for the past few decades. Carbon Fiber Multielectrode arrays have been utilized to measure multiple neurotransmitters in several brain regions simultaneously with multi-waveform application on each electrode. We have extended this work to measure larger molecule neuropeptides such as Neuropeptide Y and Oxytocin, a pleiotropic peptide hormone, is physiologically important for adaptation, development, reproduction, and social behavior. This neuropeptide functions as a stress-coping molecule, an anti-inflammatory agent, and serves as an antioxidant with protective effects especially during adversity or trauma. Here, we measure tyrosine using the Modified Sawhorse Waveform (MSW), enabling enhanced electrode sensitivity for the amino acid and peptide, decreased surface fouling, and codetection with other catecholamines. As both oxytocin and Neuropeptide Y contain tyrosine, the MSW was also used to detect these neuropeptides.

Additionally, we demonstrate that applying the MSW on CFMEs allows for real time measurements of exogenously applied neuropeptides on rat brain slices. These results may serve as novel assays for neuropeptide detection for *in vivo* measurements and further understanding of the role of Neuropeptide Y and oxytocin. We have also developed enzyme modified microelectrodes for the measurement of glutamate, which is an important excitatory amino acids and biomarker for epilepsy along with the inhibitory GABA. Since glutamate is not redox active at carbon electrodes, we modified CFMEs with glutamate oxidase enzyme to metabolize glutamate to hydrogen peroxide, which was then oxidized at carbon electrodes to produce readout CVs. The enzyme coating was optimized by varying the concentration of enzyme, chitosan binder, solvent, and deposition time. The coating was further analyzed electrochemically and imaged with scanning electron microscopy (SEM) for thickness and uniformity of surface coverages. Energy-Dispersive Spectroscopy (EDS/EDX) was utilized for chemical surface functionalization analysis. Glutamate oxidation was found to be adsorption controlled to CFMEs and characterized at various scan rates, concentrations, and stability times as well with an approximate 100 nM limit of detection. Glutamate was co-detected in complex mixtures with several monoamines such as dopamine, serotonin, norepinephrine, and others. It will furthermore be measured in several food samples and *ex vivo* in rat coronal brain slices and *in vivo* in anesthetized and freely behaving animals.

Disclosures: A.G. Zestos: None. **A.G. Zestos:** None.

Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.18/Z17

Topic: I.04. Physiological Methods

Support: BBSRC BB/S003894 to K.T. and T.C.

Title: Novel genetically encoded fluorescent sensor for EAAT2 function imaging

Authors: H. HUGHES[1], M. RENSHAW[2], T. NGUYEN[3], H. PUHL[3], G. MASHANOV[2], O. TRAN[1], S. VOGEL[3], *T. CARTER[4], **K. TÖRÖK**[1];
[1]St George's, Univ. of London, London, United Kingdom; [2]Imaging Sci. Technol. Platform, Francis Crick Inst., London, United Kingdom; [3]Lab. of Biophotonics and Quantum Biol., NIAAA/NIH, Rockville, MD; [4]St George's Univ. London, London, United Kingdom

Abstract: EAAT2 glutamate transporters play a critical role in maintaining the fidelity of glutamate neurotransmission in brain areas such as the cortex, hippocampus and striatum and impairment of their function is linked to neurodegenerative diseases. EAAT2 activity is routinely measured by steady-state radioligand assays or indirectly deduced from monitoring glutamate in the synaptic cleft. Here we present a genetically encoded FRET-based sensor system for visualising functioning EAAT2. We have used HEK293T cells as a model cell line to express the

FRET constructs and determined the plasma membrane localisation profile of the FRET constructs in comparison to EAAT2 by confocal microscopy. We measured glutamate uptake by visualising Cl^- intracellular concentration changes using mClY by wide-field microscopy and FRET changes by FLIM. Our data show that our FRET-EAAT2 constructs expressed in the plasma membrane with no significant difference compared to EAAT2. Concentration dependent glutamate uptake by EAAT2 and the FRET-EAAT2 construct was characterised by apparent K_d values of 13 ± 7 μM and 39 ± 26 μM by the Cl^- sensor and a saturating donor fluorescence lifetime shift of 141 ± 6 ps with a K_m for glutamate of 84 ± 11 μM. Single molecule imaging revealed a decrease in EAAT2 mobility in the absence of glutamate from K_{diff} of 0.096 ± 0.013 μms^{-1} to K_{diff} of 0.070 ± 0.014 μms^{-1} with glutamate present (*p*-value 0.00023). Interestingly, attempting to visualise glutamate uptake using intracellularly expressed $iGlu_u$ revealed that cellular metabolic changes accompanying EAAT2-mediated glutamate uptake preclude the use of cpEGFP-based genetically encoded fluorescent indicators due to fluorescence quenching of intracellularly expressed indicators. Our data introduce a FRET-based system by which EAAT2 transport can be visualised in cells. The quantitative power of the approach suggests that our FRET-based system will be suitable for distinguishing normal and impaired glutamate reuptake in brain cells and slices and may be suitable for in vivo FRET. Acknowledgement: BBSRC BB/S003894 to K.T. and T.C.

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Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.19/Z18

Topic: I.04. Physiological Methods

Support: NIH Grant NS133971
NIH Grant 1RF1NS128901
Klingenstein-Simons Award in Neuroscience

Title: Genetically encoded voltage indicators evolved for one- and two-photon illumination enable voltage imaging across microscopy methods

Authors: M. LAND[1], A. MCDONALD[1], S. YANG[2], Y. WANG[3], R. SIMS[4], I. BENDIFALLAH[5], S. BROWN[6], V. VILLETTE[7], S. DIEUDONNE[8], J. REIMER[9], I. M. RAMAN[10], V. EMILIANI[11], D. JAEGER[12], ***F. ST-PIERRE**[1];
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France; [9]Neurosci., Baylor Col. of Med., Houston, TX; [10]Neurobio., Northwestern Univ., Evanston, IL; [11]Photonics Dept., Vision Insitut, Paris, France; [12]Biol., Emory Univ., Atlanta, GA

Abstract: Understanding how spatiotemporal patterns of neuronal electrical activity underlie brain functions like sensory processing and decision making is a key goal in neuroscience. An emerging approach for tracking these electrical dynamics is voltage imaging using Genetically Encoded Voltage Indicators (GEVIs)—light-emitting proteins that change brightness based on voltage changes. GEVIs are promising tools for monitoring voltage dynamics with high spatiotemporal resolution in genetically defined cell types *in vivo*. Indicator performance has steadily progressed since GEVIs were first reported. Although GEVIs have evolved significantly since their inception, challenges remain in recording multiple cells deep within the brain or over extended periods, which restricts their application range. Here, we present a suite of GEVIs engineered by the St-Pierre lab for one- or two-photon imaging. These sensors couple a voltage-sensitive domain to an extracellular, circularly permuted GFP and were refined through extensive multi-parametric screening under varying illumination conditions. We illustrate how different groups have employed these improved GEVIs *in vivo* using both traditional and innovative optical imaging techniques such as laser-scanning, random-access, and scanless microscopy. We believe these advancements will encourage neuroscientists to adopt GEVIs for detailed investigations of neural activity, offering insights at the cellular level with millisecond precision.

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Poster

PSTR196: Optical Sensors for Neuronal Probing

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR196.20/Z19

Topic: I.08. Methods to Modulate Neural Activity

Support: CIRM DISC2-13483
1R43MH124563

Title: Graphene-mediated optical stimulation of cells: a non-genetic alternative for optogenetic stimulation biointerfaces

Authors: *A. SAVTCHENKO[1], T. ZHOU[2], V. P. CHERKAS[6], M. FERRAZ[3], P. MESCI[7], J. ADAMS[4], G. CHALDAIOPOULOU[3], F. DOWNEY[2], E. LAMONTAGNE[2], A. ALMENAR-QUERALT[3], J. SENA DE SOUZA[3], A. R. MUOTRI[5], E. MOLOKANOVA[8];
[1]Nanotools Biosci., La Jolla, CA; [2]Bioengineering, [3]Pediatrics, [5]Pediatrics/Cellular Mol. Med., [4]Univ. of California - San Diego, La Jolla, CA; [6]Inst. of Bioorganic Chem., Posnan, Poland; [7]Axiom Space, La Jolla, CA; [8]NeurANO Biosci., La Jolla, CA

Abstract: The need for modulation of functional activity of neurons is well-recognized. Currently, hiPSC-derived neurons are cultured in a “silent”, electrically-insulating environment of a plastic dish, while we know that the brain environment is electrically conductive and dynamically active.
Here, we present a pioneering graphene-mediated optical stimulation (GraMOS) technology for modulating the activity of neurons via an external light-controlled electric field near a graphene surface. This technology is rooted in unique optoelectronic properties of graphene and, specifically, in the ability of graphene to convert light into electricity on a femtosecond timescale via a hot-carrier multiplication process. GraMOS is exceptionally cell-friendly, does not change temperature of cell substrates due to excellent thermal properties of graphene, and does not interfere with either genetic make-up or structural integrity of neurons, thus providing truly non-invasive stimulation.
Specifically, in patch-clamp experiments, we demonstrated that by illuminating 2-D neurons on graphene using PhotonMaker LED-based light source, we can trigger either a single action potential (AP) (in response to light pulses) or a train of APs (in response to continued illumination). The reliability of optical stimulation of neurons at 1 -5 Hz was 100%, and the maximal frequency supported in our experiments was 50 Hz when the reliability of optically triggered action potentials was 47.55 ± 9.2 % (n = 5). Based on the temporal properties of action potentials, this limitation is likely due to properties of hiPSC-neurons rather than of GraMOS.
We also used the Maestro Pro MEA system (Axion Biosystems) equipped with the LUMOS optical stimulation module to perform GraMOS-empowered electrophysiological MEA assays using hiPSC-derived brain cortical organoids interfaced with graphene. In calcium imaging experiments on graphene-interfaced 2.5-D neuronal cultures and 3-D hiPSC-derived brain cortical organoids, we excited either (a) the entire neuronal network at once and visualized the activity of all cells using a wide-field fluorescent microscope or (b) activated a single neuron via a 5-μm-spot stimulation and monitored the excitation propagation throughout the neuronal networks using a confocal laser microscope. Due to unique optical properties of graphene, we were able to do so without optical crosstalk.
Based on these studies, we believe thatGraMOS has the potential to open new horizons in neuroscience by providing remote, lightning-fast, biocompatible stimulation of genetically intact neurons.

Disclosures: A. Savtchenko: A. Employment/Salary (full or part-time):; Nanotools Biosciece. **T. Zhou:** A. Employment/Salary (full or part-time):; NeurANO Bioscience. **V.P. Cherkas:** None. **M. Ferraz:** None. **P. Mesci:** A. Employment/Salary (full or part-time):; Axiom Space. **J. Adams:** None. **G. Chaldaiopoulou:** None. **F. Downey:** A. Employment/Salary (full or part-time):; Nanotools Biosciece. **E. LaMontagne:** None. **A. Almenar-Queralt:** None. **J. Sena de Souza:** None. **A.R. Muotri:** None. **E. Molokanova:** A. Employment/Salary (full or part-time):; NeurANO Bioscience.

Poster

PSTR197: High Density Electrode Arrays and Tissue Health

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.01/Z20

Topic: I.04. Physiological Methods

Title: Multimodal Brain Physiology: A Holistic Perspective through Multifunctional Intracortical Neural Interfaces

Authors: *J. R. LOPEZ RUIZ, D. YAN, M.-L. HSIEH, E. KO, Y. TIAN, W. CHEN, S. OH, V. LANZIO, P. MCCOMMONS, E. YOON;
Electrical Engin. and Computer Sci., Univ. of Michigan, Ann Arbor, MI

Abstract: Neuronal activity is the result of complex electrochemical events between highly diverse neuron populations that require a holistic approach to fully understand the underlying physiology of the neural processes. State-of-the-art intracortical neural interfaces are limited by device size and/or weight, number of recording sites, number and size of stimulation sites, and lack of additional physiological parameters being read. To address these limitations, we fabricated various functional modules and hybrid-integrated them on a flexible platform, by stacking multiple modules while maintaining a minimal cross-section area. The incorporation of cell sized micro-Light Emitting Diodes (μLEDs) onto a flexible substrate (polyimide) enabled long-term precise optogenetic stimulation and electrophysiological recordings in freely moving mice for up to 8 months. Neurotransmitter detection was implemented by functionalized electrodes for electrochemical sensing. We modified a large Pt electrode with GOx for dopamine detection while simultaneously recording the surrounding electrophysiological activity. We also developed a temperature control module, capable of focally lowering or increasing the temperature of the surrounding tissue by up to 7C, while simultaneously recording high-resolution extracellular activity. 3D origami probes were realized by wrapping multiple flexible sub-shanks connected with thin hinges on a cylindrical fiber. With the matching μLEDs stacked on the recording sites, directional optogenetic stimulation was achieved for true 360 degrees neural interface. Altogether, these developments represent a portfolio of tools that can be mixed-and-matched depending on user's specific requirements, enabling them to study physiology of the nervous system from a multiparametric perspective, resulting in a more comprehensive understanding of the neural processes.

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Poster

PSTR197: High Density Electrode Arrays and Tissue Health

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.02/Z21

Topic: I.04. Physiological Methods

Title: Flexthermo: an intracortical flexible microfluidics-based bidirectional temperature control module

Authors: *Y. TIAN[1], J. R. LOPEZ RUIZ[2], E. YOON[3];
[1]Electrical Engin. and Computer Sci., Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [2]Electrical Engin. & Computer Sci., Univ. of Michigan, Ann Arbor, MI; [3]Electrical Engin. and Computer Sci., Univ. of Michigan, Ann Arbor, MI

Abstract: Focal temperature control in the brain is emerging as a critical tool to understand the causal relationship between temperature and neural activity. Current Peltier-based cooling modules present many challenges, including uneven heat distribution due to the transfer method, and extensive tissue damage due to its large dimensions and rigidity. To address these, we developed FlexThermo, a polyimide-PDMS flexible microfluidic cooling module, that regulates local temperature of the target brain region. Here, we report the design and fabrication of FlexThermo, and evaluate its in-vivo performance in a head fixed setup when integrated with a high-density neural recording probe and a temperature sensor. Flexthermo demonstrated robust temperature control capabilities, achieving up to 12 degree c reductions in the rat hippocampus and 10 degree c in the deep brain of rats at a depth of 8 mm, respectively. By adjusting the flow rate within its microfluidic channel and utilizing feedback from the integrated temperature sensor, FlexThermo achieved precise and stable temperature adjustments. Additionally, the device's high-density neural recording probe effectively captured diverse responses in firing rates from three distinct cell types under varying temperature conditions. With a 3 degree c decrease, the firing rates of pyramidal neurons and narrow interneurons fell by around 40%, while wide interneurons showed minimal changes. Upon returning to baseline temperatures, firing rates stabilized. As temperatures dropped to 7 degree c, all cells exhibited reduced firing rates, alongside diminished ripple signals.The miniaturized microfluidic channel size, adaptable temperature control, and seamless integration with a temperature sensor and neural recording probes demonstrated the enhanced utility of FlexThermo. This tool is invaluable for neuroscientists seeking to understand the impact of temperature on brain activity and the correlation between brain and body temperature changes.

Disclosures: Y. Tian: None. **J.R. Lopez Ruiz:** None. **E. Yoon:** None.

Poster

PSTR197: High Density Electrode Arrays and Tissue Health

Location: MCP Hall A

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Program #/Poster #: PSTR197.03/Z22

Topic: I.04. Physiological Methods

Title: 3d origami µled optoelectrodes for 360 degree field-of-view neural interface

Authors: *M.-L. HSIEH, J. R. LOPEZ RUIZ, E. KO, D. YAN, E. YOON;
Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: Improving electrophysiological recording quality and longevity in chronic experiments are important for implantable neural probes. Recently, the use of flexible materials such as polyimide (PI) has gain attention due to its potential for mitigating tissue damage/reaction as compared with neural probes made of rigid materials such as silicon. The reduction of micromotion can lead to improved recording quality and longevity in chronic experiments. Neural probes with three-dimensional field-of-views can effectively increase recordable cell volumes. In this work, a novel, modular, self-assembled method was introduced to achieve 3D origami optoelectrodes on a non-planar surface for three-dimensional field-of-view. The proposed device consists of PI probe modules of two different functions (one for electrophysiology and the other for optogenetic stimulation from μLEDs) that were fabricated separately, and self-assembled on a cylindrical fiber (ϕ=80~125μm). This 3D origami optoelectrode possesses a near 360 degree field-of-view with precise spatiotemporal resolution. The self-assembled device was validated in vivo in mice hippocampus in acute experiments by conducting spatially controllable light delivery and spatially resolved electrophysiological recordings. Peristimulus time histograms show neurons in front of the triggered μLED responded within 6-8ms, likely due to direct optical modulation. Some radially opposite neurons to the triggered uLED (offsite neurons) also responded within the same time frame, suggesting potential antidromic activation of the offsite neurons. Lastly, longer latencies were observed for neurons radially neighboring the triggered μLED, likely due to polysynaptic activation. The optoelectrodes with three-dimensional field of view on a non-planar surface can provide significant improvements over conventional neural probes for studying the local circuitry of an intricate structure like the dorsal hippocampus.

Disclosures: M. Hsieh: None. **J.R. Lopez Ruiz:** None. **E. Ko:** None. **D. Yan:** None. **E. Yoon:** None.

Poster

PSTR197: High Density Electrode Arrays and Tissue Health

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.04/Z23

Topic: I.04. Physiological Methods

Title: A compact, lightweight headstage for high-density optoelectrodes for use in freely behaving experiments in rodents

Authors: *V. LANZIO[1], W. CHEN[1], J. R. LOPEZ RUIZ[1], P. MCCOMMONS[1], S. OH[1], S.-Y. PARK[2], E. YOON[1];
[1]Electrical Engin. and Computer Sci., Univ. of Michigan, Ann Arbor, MI; [2]Dept. of Electronics Engin., Pusan Natl. Univ., Busan, Korea, Republic of

Abstract: High spatiotemporal resolution implantable microprobes for simultaneous recording and modulation of neural activity have gained interest within the neuroscientist community for studying the underlying physiology of neural circuits and its relation to complex behaviors.

Recently, we have developed optoelectrodes that integrate 128 recording sites and 64 micro light-emitting diodes (micro-LEDs). These devices enable spatially confined optogenetic stimulation and simultaneous readout of neuronal responses to understand neural network connectivity. For freely behaving experiments in small rodents, it is critical to minimize the overall device dimensions, weight, and input/output connector pins. In this work, we present the development of a compact (18 x 18 mm^2), lightweight (1.7 g) headstage platform for simultaneously driving 64 micro-LEDs and 128 recording sites with minimal tethering. We hybrid-integrated a high-density optoelectrode onto a custom integrated circuit through an ultra-flexible polyimide cable. This allowed for a small headstage dimension with an 18-pin output connector, mitigating the tethering problem for chronic behaving animal experiments. The custom integrated circuit chip was developed in house for two functions: *(i)* amplification, multiplexing, and digitization of 128 recording channels with low RMS noise (<7 uV) and *(ii)* simultaneous and independent control of up to 64 light emitting diodes. With the same backend electronic circuits various optoelectrode configurations can be realized depending on the experimental needs, without the need for headstage redesign. Shank lengths and numbers of recording sites and micro-LEDs can vary as well as the electrode and micro-LED arrangements. In addition, we developed a custom user interface compatible with Open Ephys software, which drives any combination of micro-LEDs from the desired sub-millisecond activation patterns and custom waveforms. We validated the assembled headstage performance both at the benchtop and in-vivo on a Thy1-ChR2 mouse. Thus, we expect these devices to advance freely behavioral and bidirectional experiments in small rodents.

Disclosures: V. Lanzio: None. **W. Chen:** None. **J.R. Lopez Ruiz:** None. **P. McCommons:** None. **S. Oh:** None. **S. Park:** None. **E. Yoon:** None.

Poster

PSTR197: High Density Electrode Arrays and Tissue Health

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.05/Z24

Topic: I.04. Physiological Methods

Support: Army Research Laboratory Award #W911NF-24-1-0011

Title: Aerosol jet printed electrode arrays for simultaneous imaging and neural recording in vivo

Authors: *K. G. VOKT, C. KEMERE;
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Abstract: Neuronal ensemble and oscillatory activity in the hippocampus provide insight into learning and memory processes but occur at different timescales. We designed a unique approach combining electrophysiology with head-mounted fluorescence microscopy to simultaneously capture ensemble patterns and their high-speed oscillatory reactivations during natural behavior. Using aerosol jet printing, electrode arrays are fabricated using conductive and dielectric

nanoparticle inks onto a planar glass probe and on the curved face of a cylindrical GRIN imaging lens. We record acute in vivo rat cortical activity using the planar gold electrode array and measure impedance stability across days of electrodes soaked in saline. We demonstrate that aerosol jet printed gold and silver electrodes maintain an impedance under 5kOhm and that this fabrication process does not impair GRIN lens imaging resolution. This work presents crucial steps toward developing a versatile imaging and electrophysiology system to study the relationship between ensemble and oscillatory activity in the hippocampal neural circuit.

Disclosures: K.G. Vokt: None. **C. Kemere:** None.

Poster

PSTR197: High Density Electrode Arrays and Tissue Health

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.06/Z25

Topic: I.04. Physiological Methods

Support: U19NS123716
R01EY022979

Title: Longitudinal, chronic neuropixels recordings capture the evolution of multi-region population activity during learning of a perceptual decision-making task

Authors: *M. MELIN[1], A. K. CHURCHLAND[2], J. COUTO[2];
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Abstract: Understanding how distributed networks learn to accurately guide decision making requires measuring neural activity from many regions simultaneously over weeks or months as learning unfolds. There are many recent efforts to perform large-scale, multi-region recordings in expert animals, but studying the evolution of distributed networks across task learning has remained difficult, particularly in subcortical regions. Advances in optical technologies have enabled simultaneous sampling from many brain areas over extended periods, however, these methods are limited to cortical regions and lack sufficient temporal resolution for some experiments. Understanding how cortical and subcortical networks work together to guide learning requires a novel recording approach that can simultaneously target many cortical and subcortical regions for weeks to months at a time.
To overcome this limitation, we employed Neuropixels probes, chronically implanted with a device we recently engineered. The device enables months-long, simultaneous interrogation of neural activity from many brain regions during freely moving or head-fixed behaviors. Using this approach, we have chronically implanted up to 6 Neuropixels probes in one hemisphere of the mouse brain. Recordings are highly stable within sessions and across recording days: standard deviation of motion within sessions was less than 2 microns, and the standard deviation of motion across days was less than 4 microns. This approach also enables reliable implantation, extraction, and reuse of Neuropixels probes, with stable SNR and single-unit yield across reuses.

The implantation approach can also be combined with other modalities, such as optogenetics or widefield imaging.
Using this device, we performed simultaneous, multi-region recordings to understand the relative contributions of different corticostriatal circuits to the learning of a visual decision-making task. We recorded from multiple cortical regions and their corresponding striatal targets using at least 3 probes per animal, implanted in the same hemisphere. These regions included anterior cingulate cortex, secondary motor cortex, primary motor cortex, visual cortex, and multiple locations across the dorsomedial and dorsolateral striatum. These recordings (213 sessions, 1 h long each) were performed longitudinally while 4 mice learned a visual decision-making task over a period of 9-12 weeks. Each animal yielded hundreds of single units. This approach enabled us to capture simultaneous neural dynamics of many cortical and subcortical structures across task learning, and to quantify the evolving interactions between them.

Disclosures: M. Melin: None. **A.K. Churchland:** None. **J. Couto:** None.

Poster

PSTR197: High Density Electrode Arrays and Tissue Health

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.07/Z26

Topic: I.04. Physiological Methods

Support: NIH BRAIN Initiative U01NS113252
the Pew Biomedical Scholars Program
the Klingenstein-Simons Fellowship in Neuroscience

Title: Ultra-high density electrodes improve detection, yield & cell type identification in neuronal recordings

Authors: *Z. YE[1], A. M. SHELTON[2], J. SHAKER[1], J. BOUSSARD[3], J. COLONELL[4], D. BIRMAN[5], S. MANAVI[6], S. CHEN[7], C. WINDOLF[8], C. L. HURWITZ[9], T. NAMIMA[10], T. NAMIMA[11], F. PEDRAJA[12], S. WEISS[13], B. C. RADUCANU[14], T. V. NESS[15], X. JIA[16], M. CARANDINI[17], M. HAUSSER[18], G. T. EINEVOLL[19], G. J. LAURENT[20], N. SAWTELL[8], W. BAIR[5], A. K. PASUPATHY[21], L. M. PANINSKI[8], J. H. SIEGLE[22], C. KOCH[6], S. R. OLSEN[22], T. HARRIS[23], N. A. STEINMETZ[5];
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Kingdom; [19]Norwegian Univ. Life Sci., Aas, Norway; [20]Max-Planck Inst. for Brain Res., Frankfurt/Main, Germany; [21]Biol. Structure, Univ. Washington, Seattle, WA; [22]Allen Inst., Seattle, WA; [23]HHMI JRC, Ashburn, VA

Abstract: To understand the neural basis of behavior, it is essential to sensitively and accurately measure neural activity at single neuron and single spike resolution. Extracellular electrophysiology delivers this, but it has biases in the neurons it detects and it imperfectly resolves their action potentials. To minimize these limitations, we developed a silicon probe with much smaller and denser recording sites than previous designs, called Neuropixels Ultra (NP Ultra). This device samples neuronal activity at ultra-high spatial density (~10 times higher than previous probes) with low noise levels, while trading off recording span. NP Ultra is effectively an implantable voltage-sensing camera that captures a planar image of a neuron's electrical field. We use a spike sorting algorithm optimized for these probes to demonstrate that the yield of visually-responsive neurons in recordings from mouse visual cortex improves up to ~3-fold. We show that NP Ultra can record from small neuronal structures including axons and dendrites. Recordings across multiple brain regions and four species revealed a subset of extracellular action potentials with unexpectedly small spatial spread and axon-like features. We share a large-scale dataset of these brain-wide recordings in mice as a resource for studies of neuronal biophysics. Finally, using ground-truth identification of three major inhibitory cortical cell types, we found that these cell types were discriminable with approximately 75% success, a significant improvement over lower-resolution recordings. NP Ultra improves spike sorting performance, detection of subcellular compartments, and cell type classification to enable more powerful dissection of neural circuit activity during behavior.

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Poster

PSTR197: High Density Electrode Arrays and Tissue Health

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.08/Z27

Topic: I.04. Physiological Methods

Support: NINDS 1R21NS116519-01
NIAAA 5R01AA016852-13

Title: An innovative approach for dispersed electrode placement to measure brain wide dynamics

Authors: *D. KLORIG[1], D. W. GODWIN[2];
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Abstract: Current state-of-the-art recording methods in neuroscience are optimized to provide high density single unit recordings from as many neurons as possible. However, there is a growing recognition that due to the highly interconnected nature of neural circuits, understanding neural activity requires capturing of the network dynamics that coordinate such activity on a moment to moment basis. Unfortunately, methods that rely on high density probes do not scale well when moving from a single to many brain areas, especially for chronic applications. New approaches are needed in order to gain long-term access to brain wide dynamics. We have developed a novel approach that allows for individual placement of microwire electrodes in a dispersed configuration targeting specific anatomical areas of interest through microcraniotomies to keep the skull intact. This approach trades density for coverage, offering network wide access while minimizing brain damage and head mounted hardware. The result is a chronic preparation for freely moving animals that provides long-term (> 1 yr) network wide recording in a low cost, low profile, minimally invasive package. Our approach is tailored, and most advantageous, for use in mice but can be adapted to work with larger experimental models. Here, we demonstrate the components of our integrated system for chronic multisite recordings including durable and biocompatible 3D printed hardware that facilitates the implantation process and protects the connectors from dust and wear for stable long-term chronic recordings. A compliant electrode holder allows for precise alignment, an array mounting device provides a stable platform for electrode placement, a skull mounted retractor provides clean margins, a coiling device allows for orderly cable management, a magnetic headstage connector with integrated guide-pins provides reliable connections that reduce wear on electrical contacts, and a removable magnetic dust cap protects the implant when not in use. In addition to enabling multisite implantations manually, our hardware is designed to be forward compatible with an automated surgical solution currently under development. Our open-source design will enable widespread adoption by the neuroscience community.

Disclosures: D. Klorig: None. **D.W. Godwin:** None.

Poster

PSTR197: High Density Electrode Arrays and Tissue Health

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.09/Z28

Topic: I.04. Physiological Methods

Support: NIH Grant F060746
Gilmore Fund University of Michigan GO28528

Title: Integrating chronic electrophysiology into rodent digital phenotyping: a novel approach for studying long-term behavioral dynamics and brain function

Authors: *E. GOLDIEZ, D. KIM, A. GHIMIRE, N. OGNJANOVSKI, B. O. WATSON; Dept. of Psychiatry, Univ. of Michigan Med. Sch., Ann Arbor, MI

Abstract: We have developed the “Digital Homecage” model, where we gather multidimensional behavioral data, to discover novel associations between behaviors and pathologic states. We call this recording of detailed spontaneous behavior “rodent digital phenotyping” to match human digital phenotyping that has similar goals. However, a key advantage of doing such phenotyping in rodents is to use powerful rodent neuroscience tools to link long-term behavioral dynamics to brain function. Spiking in a neural network is the ultimate mediator of sensory input perceptions and motor outputs from the brain; therefore, we seek to incorporate electrophysiologic network activity signatures into our behavioral paradigm.We seek to link this long-term behavioral measurement with long-term electrophysiologic metrics or “Electrophysiologic background state” (EBS). EBS differs from standard spiking analyses in that it is not triggered by events or behaviors, but rather is an ongoing multi-metric quantification of brain-state over minutes to hours. Therefore it is the perfect complement for digital phenotyping of homecage activity over days and weeks. We recorded in the medial prefrontal cortex (mPFC), a part of the brain important in a variety of behaviors which also shows circadian modulation of gene-expression. Moreover, prior work from our group showed important changes in EBS in mPFC in sleep in wildtype rats, but these metrics have yet to be explored in mice.To record chronic electrophysiology for such long periods, we modified our “Digital Homecage” model to interface with custom 64-channel tetrode implants using parts from Open Ephys, Intan, and others. We find that optimizing for a low-profile implant design allows for chronic recordings with less disruption to naturalistic animal behavior. To create an implant, we paired a low-profile headstage and EIB with a custom 3-D printed tetrode dome to record from 15 tetrodes and 4 EEGs simultaneously. For the mPFC, we modified the tetrode dome for two tetrode bundles to allow for bilateral recording from left and right hemispheres. The dome can be further modified to support a single tetrode bundle or two or more different outputs to different brain regions. We modified standard 12-pin SPI cables to generate more rotational torque against the commutator, which we find permits less-inhibited mouse movement. The millisecond behavioral resolution coupled with the high sampling rate of electrical recording facilitates meticulous investigation of spiking activities that encode behavioral information across the brain.

Disclosures: E. Goldiez: None. **D. Kim:** None. **A. Ghimire:** None. **N. Ognjanovski:** None. **B.O. Watson:** None.

Poster

PSTR197: High Density Electrode Arrays and Tissue Health

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.10/Z29

Topic: I.04. Physiological Methods

Support: NIH NINDS 1RF1NS133972-01

Title: High-density and customized depth array integration enabled by aerosol jet printing

Authors: *X. BAI[1], J. LIM[1], P. PATHIRANA[2], A. ABREGO MANCILLA[1], R. SHORES[2], Y. KAJIKAWA[3], A. NUNEZ[1], E. BRIZUELA[1], C. MATTHEWS[1], J. CAO[2], B. ANDAM[2], C. E. SCHROEDER[4], N. TANDON[5], J. P. SEYMOUR[6];
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Abstract: Objective: Deep brain recording with high-density microelectrodes offers a unique set of packaging challenges. Current techniques limit the contact density or have a large, fragile backend. This study sought to develop miniaturized electronics mated with depth arrays capable of recording directional local field potentials.
Methods: We microfabricate polyimide high-density arrays achieving 128-channels in a single metal layer. Critical to our approach is a novel packaging scheme that allows the thin film to be fully wrapped along the entire length. We demonstrate a reliable way to pattern a uniform, low-CTE ramp material so that aerosol jet printing(AJP) can be used to connect the PCB to the thin-film bond pads.
Main Results: We present the yield, testing, and in vivo demonstration of this compact depth array having directional sensitivity. We also fabricated and assembled several new form factors for use in acute and chronic settings. The information density of directional sensitivity was demonstrated in a sensory classification task in macaques, high-resolution seizure onset activity in rats, and interictal epilepsy discharges in humans.
Significance: We demonstrate an elegant electro-mechanical solution to high-density depth arrays.

Disclosures: X. Bai: None. **J. Lim:** None. **P. Pathirana:** None. **A. Abrego Mancilla:** None. **R. Shores:** None. **Y. Kajikawa:** None. **A. Nunez:** None. **E. Brizuela:** None. **C. Matthews:** None. **J. Cao:** None. **B. Andam:** None. **C.E. Schroeder:** None. **N. Tandon:** None. **J.P. Seymour:** None.

Poster

PSTR197: High Density Electrode Arrays and Tissue Health

Location: MCP Hall A

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Program #/Poster #: PSTR197.11/Z30

Topic: I.04. Physiological Methods

Support: NIH NBIB DP2-EB029757
NIH R01NS123655-01
NIH UG3NS123723-01
NIH 5R01NS109553-03

Title: Establishing Thresholds for Tissue Damage from Pulsed Electrical Stimulation

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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 in acute and chronic setups, mimicking the typical clinical setups. 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. **Results:** Using both electrochemical and 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 characterize the observed damage to the tissue in response to pulsed stimulation, correlating the stimulation design parameters to the observed damage. We compare these safety limits to the currently established damage thresholds to understand the limitations of the current approaches.

Disclosures: R. Vatsyayan: None. **K.J. Tonsfeldt:** None. **K. Shukla:** None. **A. Bourhis:** None. **S. Dayeh:** None.

Poster

PSTR197: High Density Electrode Arrays and Tissue Health

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR197.12/Z31

Topic: I.04. Physiological Methods

Support: R44NS105500
NSF CBET 1943906
R01NS129632

Title: Vibrational insertion reduces dimpling and improves chronic electrophysiological performance for angled electrode implantation

Authors: *T. THAI[1], J. GALLEGO[2], F. LI[3], R. PATEL[3], K. W. GHERES[4], R. BAGWELL[4], M. L. MULVIHILL[4], T. D. KOZAI[3];
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Abstract: Intracortical microelectrode arrays (MEA) are vital for neuromodulation and studying neural circuitry. Yet, mechanical failure and device longevity is a current obstacle for brain-computer interfacing. Variations in insertion equipment between studies can lead to discrepancies in electrophysiology performance. Perpendicular insertion is often used to investigate MEA performance across cortical depth, while angled insertions paired with multi-photon imaging allow for continuous tracking of cell-specific activity post-injury. Comparisons across studies seldom account for different insertion arrangements. Vibration of electrodes along the axis of insertion has been shown to lower the force required to puncture the pial surface and friction forces during insertion compared to traditional insertion techniques, reducing buckling of implants, tissue dimpling, and acute tissue damage. In this study, we used a vibrational insertion method (20kHz, ~10µm axial displacement) to implant 16-channel Michigan-style multi-shank MEAs at a 30-degree angle or single-shank MEAs perpendicularly into the mouse primary visual cortex to investigate differences in electrophysiological performance for 28 days. Implanting across a depth of 1600µm into the visual cortex at a speed of 0.05mm/s, dimpling depth during insertion significantly decreased for the perpendicular vibrational group (N=6, 108.3±34.4µm) compared to the control (N=6, 391.7 ±34.4µm, unpaired Welch's t-test, $p<0.0001$). Across days, there was a similar mean signal-to-noise ratio (SNR) between vibrational and control groups for perpendicular (4.3±0.12 vs 4.5 ± 0.12, Two-way ANOVA, $p=0.36$) and angled insertion (3.5±0.49 vs 5.5±2.8, Two-way ANOVA, $p=0.65$). However, there was an increase in signal detectability for angled insertion (SU yield 18.8± 0.078% vs 85.4± 0.17%, Two-way ANOVA, $p<0.0001$) as early as day 7. Detected units with trough-to-peak latencies <0.43ms were classified as putative inhibitory units and putative excitatory units had latencies ≥0.43ms. For the absolute percent change in the inhibitory-to-excitatory (I/E) count ratio, there was a decrease on days 3 and 7 for perpendicular vibrational insertion, indicating a more stable I/E balance. The control group had decreased stability, with an increase in the I/E ratio from day 14 on. Together, these results indicate that differences in single-unit analysis using perpendicular vibrational insertion are comparable to traditional methods with reduced dimpling. Further, angled vibrational insertion improved electrophysiological performance and positively altered the visual cortex's I/E balance.

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Poster

PSTR198: Network Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.01/Z32

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

Support: CIHR PJT-168980

Title: Modeling brain network dynamics in early childhood with The Virtual Brain

Authors: L. ROKOS[1], S. L. BRAY[2], J. WANG[3], *K. SHEN[4], A. R. MCINTOSH[4];
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Abstract: “The Virtual Brain” (TVB, thevirtualbrain.org) is a neuroinformatics platform that was developed to investigate whole-brain dynamics through the incorporation of multimodal neuroimaging data and neural mass models (Sanz Leon et al., 2013). Subject-specific, whole-brain network models provide a means to integrate various scales and modalities, offering insight into potential mechanisms linking structural and functional development. Modeling early developmental stage brain networks can further enhance TVB’s utility in characterizing individual developmental trajectories. In adult populations, anatomical data and dynamic functional connectivity (FCD) have been used to create simulated data that reflect resting-state networks and their “metastability”, or switching behaviour (Lavanga et al., 2022; Deco et al., 2017). The relationship between structural connectivity changes observed in early development and brain network dynamics in children remains to be further explored with network modeling. In this study, we describe the generation and evaluation of simulated network dynamics that align with empirical features of children’s brain networks. Neuroimaging data (i.e., T1-weighted, diffusion-weighted MRI, passive viewing state functional MRI scans) were collected at an initial baseline and one-year follow-up visit from typically developing children between the ages of 4 and 8. TVB was used to produce brain network models using data from 39 children (20 girls), resulting in 78 simulations. The models were optimized by maximizing the combined rank of the: 1) maximum FCD variance, 2) maximum correlation between the empirical and simulated FC matrices, and 3) minimum Kolmogorov-Smirnov distance between the empirical and simulated FCD matrices. We used linear mixed effects models to assess the relationship between the optimal model parameter values (i.e., global coupling and noise variance) with age. We found a significant, longitudinal increase in the fitted noise variance with age ($p=0.02$). Additionally, region-wise metrics of bistability were computed from the biophysiological model outputs. The models exhibited a spatial distribution of bistability that was also replicated in a separate developmental dataset of typically developing children (i.e., Calgary Preschool MRI dataset; Reynolds et al., 2020), suggesting the models capture a spatial aspect of brain dynamics established in early development. This work illustrates the utility of TheVirtualBrain for

neurodevelopmental research and provides a framework to characterize developmental trajectories that can serve as a reference for studying atypical development.

Disclosures: L. Rokos: None. **S.L. Bray:** None. **J. Wang:** None. **K. Shen:** None. **A.R. McIntosh:** None.

Poster

PSTR198: Network Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.02/Z33

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

Support: Horizon EBRAINS2.0 (101147319), Virtual Brain Twin (101137289), EBRAINS-PREP 101079717, AISN – 101057655, EBRAIN-Health 101058516, Digital Europe TEF-Health 101100700, EU H2020 Virtual Brain Cloud 826421, Human Brain Project SGA2 785907
Human Brain Project SGA3 945539, ERC Consolidator 683049;
German Research Foundation SFB 1436 (project ID 425899996)
SFB 1315 (project ID 327654276);
SFB 936 (project ID 178316478
SFB-TRR 295 (project ID 424778381)
SPP Computational Connectomics RI 2073/6-1, RI 2073/10-2, RI 2073/9-1
DFG Clinical Research GroupBECAUSE-Y 504745852, PHRASE
Horizon EIC grant 101058240
Berlin Institute of Health & Foundation Charité, Johanna Quandt Excellence Initiative; ERAPerMed Pattern-Cog2522FSB904

Title: Runaway synaptic modifications in Alzheimer's Disease: Investigating mechanisms of excessive plasticity with The Virtual Brain

Authors: *L. BRANDT[1,2], L. STEFANOVSKI[1,2], P. RITTER[1,2];
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Abstract: Plasticity is the fundamental mechanism that allows the brain to learn and form memories by modifying neuronal connections. Despite its central role, many aspects of how plasticity operates on network levels remain poorly understood, particularly in pathological conditions. Runaway synaptic modifications, characterized by the exponential growth of undesired synaptic connections, arise from unrestricted plasticity, i.e., Hebbian learning (Hasselmo et al. 1994). This phenomenon is linked to the loss of inhibitory cholinergic synapses,

which are responsible for the regulation of synaptic plasticity, a fundamental characteristic of the progression of Alzheimer's disease (AD). In the modeling of AD, the primary focus often lies on the role and toxic effects of amyloid-beta and Tau protein, particularly on the spread and eventual cause of cell death. However, impaired plasticity mechanisms, together with the pathological runaway synaptic modifications can explain memory deficits observed in AD and directly contribute to the disease progression through excitotoxic effects, an effect still poorly understood at the network level. Our study, using the whole-brain simulation framework The Virtual Brain (www.thevirtualbrain.org), allows exploring plasticity effects on the network level, unlike previous research limited to spike-time-dependent plasticity in spiking neuron models, this proof-of-concept explores excessive Hebbian learning within neural mass models in TVB (Roy et al., 2014). We integrate an additive Hebbian learning mechanism in TVB to induce structural network changes depending on resting-state BOLD functional connectivity. For the structural connectivity, we use healthy subject data from the Human Connectome Project, as well as for the functional connectivity (n=103) which serves as a baseline metric. We demonstrate the emergence of runaway synaptic modifications on the temporal level of synaptic potentials and alterations of functional network properties. Furthermore, we introduce an inhibiting factor that simulates the cholinergic inhibition (Coronel-Oliveros, 2021) in the neural mass model (Jansen-Rit model) and successfully postpones the emergence of runaway synaptic modifications. Lastly, we show how stimulating the area M1 can lead to pronounced runaway synaptic modifications suggesting a mechanism of how overactivity might accelerate the process of runaway synaptic modifications and therefore, the progression of AD. Finally, our findings indicate that excessive Hebbian learning can lead to network alterations like those observed in AD, providing a potentially contributing factor to the pathogenesis of AD.

Disclosures: L. Brandt: None. **L. Stefanovski:** None. **P. Ritter:** None.

Poster

PSTR198: Network Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.03/Z34

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

Support: ERAPerMed Pattern-Cog 2522FSB904; PHRASE Horizon EIC grant 101058240; EU Horizon Europe program Horizon EBRAINS2.0 (101147319)
Virtual Brain Twin (101137289);
AISN – 101057655
EBRAIN-Health 101058516; EBRAINS-PREP 101079717
Digital Europe TEF-Health 101100700
EU H2020 Virtual Brain Cloud 826421
Human Brain Project SGA2 785907; Human Brain Project SGA3 945539
German Research Foundation SFB 1436 (project ID 425899996); SFB 1315 (project ID 327654276); SFB 936 (project ID 178316478; SFB-TRR

295 (project ID 424778381);
ERC Consolidator 683049;
SPP Computational Connectomics RI 2073/6-1, RI 2073/10-2, RI 2073/9-1;
DFG Clinical Research Group BECAUSE-Y 504745852;
This work was supported by the Virtual Research Environment at the Charité Berlin – a node of EBRAINS Health Data Cloud.
Part of computation has been performed on the HPC for Research cluster of the Berlin Institute of Health.
Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu).
Data collection and sharing for the Alzheimer's Disease Neuroimaging Initiative (ADNI) is funded by the National Institute on Aging (National Institutes of Health Grant U19 AG024904).

Title: Learning candidate genetic mechanisms from machine learning prediction models in neuroimaging

Authors: *K. DHINDSA[1,2], P. BEY[4,3], L. MARTIN[1,3], K. BÜLAU[5,3], L. STEFANOVSKI[1,3], P. RITTER[1,3];
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Abstract: Introduction: The use of predictive machine learning (ML) as a diagnostic tool is increasingly common in neuroscience. However, gaining scientific and clinical insights about diseases from ML remains a major challenge. Even with current advances in explainable ML (Chaddad 2023), opening the "black box" only extends as far as the input training data . We introduce a model-agnostic approach to extend the reach of ML explainability from a prediction model's input data to deeper biological knowledge. As an example use case, we link our Alzheimer's disease (AD) prediction model trained on PET-derived amyloid beta and tau protein distributions (Triebkorn 2022) to gene expression data in the Allen Human Brain Atlas (Shen 2012), thereby identifying candidate genetic mechanisms that make accurate prediction possible . Methods: Our approach learns a mapping function from the discriminating patterns learned by ML (target maps; TMs) to biological knowledge maps (KMs) in the same brain parcellation. The mapping is learned via multitask regression to jointly select a sparse set of genes that reconstructs the TMs from the KMs (Thung2018). The selected genes were then ranked by their t-statistic when comparing to fit weights of null models (obtained by permuting brain regions in TMs). The final output is a list of genes ranked by estimated explanatory power.
Results: Our method shows a tradeoff between strict selection pressure on genes and reconstruction accuracy of feature importance maps, from R^2=0.92 with 1338 of 15633 genes, to an optimum on the R^2 curve of R^2=0.66 with only 710 genes . Coefficient analysis shows consistent explanatory power of selected genes (62% consistency in either up- or down-

regulation; stable weighting with coefficient of variation = 0.24). A cutoff of $p<0.01$ compared to null models resulted in 79 top candidate genes . The output genes were referenced against the literature , revealing genes with known associations with AD (APOE, EP300, LRIG1) and genes associated with mechanisms known to be impaired in AD (PMF1, CERS5, PAM).
Conclusions: We developed an interpretable model-agnostic approach to hypothesize biological mechanisms explaining ML prediction models based on neuroimaging features . This approach is widely applicable due to its minimal input requirements. Unlike most current methods for linking biological mechanisms to brain disorders, our approach does not require direct access to any patient data or the prediction model itself. It is therefore a safe and effective option for gaining additional value from ML by probing models for potential evidence towards discoveries about the biological nature of how diseases manifest in the brain.

Disclosures: K. Dhindsa: None. **P. Bey:** None. **L. Martin:** None. **K. Bülau:** None. **L. Stefanovski:** None. **P. Ritter:** None.

Poster

PSTR198: Network Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.04/Z35

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

Support: EU Horizon Europe program Horizon EBRAINS2.0 (101147319)
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EBRAINS-PREP 101079717
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EU H2020 Virtual Brain Cloud 826421
Human Brain Project SGA2 785907
Human Brain Project SGA3 945539
ERC Consolidator 683049; German Research Foundation SFB 1436 (project ID 425899996)
SFB 1315 (project ID 327654276), SFB 936 (project ID 178316478
SFB-TRR 295 (project ID 424778381), SPP Computational Connectomics RI 2073/6-1
RI 2073/10-2, RI 2073/9-1; DFG Clinical Research GroupBECAUSE-Y 504745852, PHRASE Horizon EIC grant 101058240; Berlin Institute of Health & Foundation Charité, Johanna Quandt Excellence Initiative; ERAPerMed Pattern-Cog2522FSB904
Data collection and sharing for the Alzheimer's Disease Neuroimaging Initiative (ADNI) is funded by the National Institute on Aging (National Institutes of Health Grant U19 AG024904).
A complete listing of ADNI investigators can be found at:

http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

Title: Towards hypothesis-driven drug design with The Virtual Brain

Authors: *L. STEFANOVSKI[1,2], M. DA COSTA ZEMSCH[1,2], L. MARTIN[1,2], R. SCHMITT[1,2], K. BÜLAU[1,2], P. RITTER[1,2,3,4];
[1]Berlin Inst. of Hlth. at Charité – Universitätsmedizin Berlin, Berlin, Germany; [2]Brain Simulation Section, Department of Neurology with Experimental Neurology, Charité - Universitätsmedizin Berlin, Berlin, Germany; [3]Bernstein Center for Computational Neuroscience Berlin, Berlin, Germany; [4]for the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu) - ADNI investigators contributed the underlying data but not participated in writing this abstract, San Francisco, CA

Abstract: INTRODUCTION. As our technological advances may grow as fast as our knowledge about the brain, most neurological diseases remain too poorly understood to provide an effective causal treatment. In Alzheimer's Disease (AD), recent developments have resulted in novel therapeutic options against Amyloid-beta, but the effects remain mild, and the treatment stratification is controversially discussed in the field. Computational Neuroscience offers the tools to remodel a disease mechanistically and, combined with knowledge technology and artificial intelligence, provides new avenues for drug development besides the known paths.
METHODS. We present a novel methodology based on The Virtual Brain (thevirtualbrain.org), showcasing how mechanistic brain simulation can be leveraged to generate treatment hypotheses for AD. By integrating an established model of Amyloid-beta-induced hyperexcitation (Stefanovski et al. 2019) with a deeply phenotyped subset of the Alzheimer's Disease Neuroimaging Initiative, we can delve into the interplay between inflammation and excitotoxicity in the early disease stage. Furthermore, we combine current knowledge graphs and ontologies of biomedicine with cutting-edge developments in large language models to unravel the entire mechanistic chain underlying the observed association in real-world data.
RESULTS. Our Amyloid-dependent simulation has revealed a unique co-occurrence between excessed excitation on a cellular level and increased Tumor-Necrosis-Factor-alpha in cerebrospinal fluid in the group of mild cognitive impairment, suggesting excitotoxic neuroinflammation in the early disease stage of AD that may be addressed by pharmacologic inhibitors. Further analysis with large language models and biomedical ontologies uncovers a complete chain of influence factors to the biology of Tumor-necrosis-factor-alpha, illuminating new pathways for intervention in the complex mechanisms at play in AD.
DISCUSSION. This work demonstrates how to use a dataset that spans from in vivo Amyloid imaging to in vitro immunological markers and finally to mechanistic in silico biomarkers of a neurodegenerative disease for therapeutic research. Whole-brain simulation based on multimodal data in combination with artificial intelligence and knowledge technology has allowed the proposal of a promising new approach towards hypothesis-driven drug design in the era of supercomputers.
REFERENCES: Stefanovski, L., et al., Linking Molecular Pathways and Large-Scale Computational Modeling to Assess Candidate Disease Mechanisms and Pharmacodynamics in Alzheimer's Disease. Frontiers in Computational Neuroscience, 2019.

Disclosures: L. Stefanovski: None. **M. da Costa Zemsch:** None. **L. Martin:** None. **R. Schmitt:** None. **K. Bülau:** None. **P. Ritter:** None.

Poster

PSTR198: Network Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.05/Z36

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

Support: The Virtual Research Environment at the Charité Berlin – a node of EBRAINS Health Data Cloud
HPC for Research cluster of the Berlin Institute of Health
EU Horizon Europe program Horizon EBRAINS2.0 (101147319)
Virtual Brain Twin (101137289)
EBRAINS-PREP 101079717
AISN – 101057655
EBRAIN-Health 101058516
Digital Europe TEF-Health 101100700
EU H2020 Virtual Brain Cloud 826421
Human Brain Project SGA2 785907, SGA3 945539
ERC Consolidator 683049
German Research Foundation SFB 1436 (project ID 425899996)
SFB 1315 (project ID 327654276); SFB 936 (project ID 178316478; SFB-TRR 295 (project ID 424778381)
SPP Computational Connectomics RI 2073/6-1, RI 2073/10-2, RI 2073/9-1
DFG Clinical Research GroupBECAUSE-Y 504745852; PHRASE
Horizon EIC grant 101058240; Berlin Institute of Health & Foundation Charité; Johanna Quandt Excellence Initiative; ERAPerMed Pattern-Cog2522FSB904

Title: Investigating brain mechanisms of fast acting therapies for depression - ketamine and TSD

Authors: *M. DA COSTA ZEMSCH[1,2], A. HALIMI[1,2], M. SCHIRNER[1,2], L. STEFANOVSKI[1,2], P. RITTER[1,2];
[1]Berlin Inst. of Hlth., Berlin, Germany; [2]Department of Neurology, Charité Berlin, Berlin, Germany

Abstract: Depression affects 280 million people worldwide, constituting 5% of adults and ranking in the global top 10 burdens (World Health Organization). Current treatments face challenges like inefficiency, delayed onset, and unpredictability (Gumnick et al., 2000). Ketamine and Total Sleep Deprivation (TSD) offer novel therapies with immediate antidepressant effects. Our aim is to investigate their impact on Major Depressive Disorder (MDD) brain dynamics, seeking insights in their mechanisms of action and potential therapy biomarkers. We used data from the Human Connectome Project investigating fast acting therapies for depression (Tozzi et al., 2021), including behavioral data, structural and resting-

state functional MRI (186 subjects, 102 female, age 20-75). We investigated the mean functional connectivity (FC) between networks (Ji et al., 2019) to achieve three objectives: 1) compare the mean FC of MDD patients and healthy controls (HC), 2) assess subject-wise changes in FC after ketamine therapy, and 3) underline the impact of TSD on MDD patients compared with HC. Connectivity analysis highlights lower global connectivity in MDD compared to HC. Ketamine therapy shows increased connectivity in visual, language, and frontoparietal networks, and a decreased connectivity in default-mode and auditory networks. The connectivity increases in MDD patients after TSD across all networks and is notably higher in HC. This effect even surpasses MDD-HC differences. In conclusion, our study reveals a reduction in global connectivity in MDD, which subsequently increases following therapy, consistent with findings in MDD (Weckmann et al., 2019) and ketamine (Vasavada et al., 2021) literature. Our novel results highlight TSD's high impact on connectivity increase in patients with MDD and HC, despite symptoms return post-sleep. This prompts the hypothesis that the remarkable high increase in connectivity may be associated with the low sustainability of fast therapies. Future research directions focus on exploring connectivity and network-specific biomarkers for therapy outcome, utilizing simulated dynamics with The Virtual Brain (www.thevirtualbrain.org).

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Poster

PSTR198: Network Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.06/Z37

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

Support: Michael Smith Health Research BC / Alzheimer Society of BC Research Trainee Award RT-2023-3242
CIHR Grant PJT-168980

Title: Brain criticality changes in healthy human aging

Authors: *S. G. J. DOBRI[1], J. SOLOMON[2], P. SORRENTINO[4], K. SHEN[3], P. R. JOHNSTON[5], A. R. MCINTOSH[2];
[1]Biomed. Physiol. and Kinesiology, [3]Inst. for Neurosci. and Neurotechnology, [2]Simon Fraser Univ., Burnaby, BC, Canada; [4]Aix-Marseille Univ., Marseille, France; [5]Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: The brain criticality hypothesis holds that the human brain is a complex dynamical system operating close to a critical point at which its processing and computation properties are optimized. Theoretical arguments understand neuronal avalanches, defined as sequences of brain activity substantially above baseline, as an observable quantity related to underlying regimes operating near criticality. At the critical point, incoming brain activity is approximately

preserved as it passes through neural networks, which constrains the duration of neuronal avalanches as well as the number of regions involved, i.e. size. Away from the critical point, the brain may be in a subcritical state where incoming activity is dampened, leading to shorter and smaller avalanches, or in a supercritical state where incoming activity is amplified, leading to longer and larger avalanches. In this study, we investigated how brain criticality changes across human adulthood and into older age. We analyzed neuronal avalanches in resting-state MEG recordings from 612 participants (303 female, 309 male) from the Cambridge Centre for Aging and Neuroscience (Cam-CAN) dataset, all of whom were cognitively healthy and ranged in age from 18-88 years. We found that the number of unique combinations of brain regions involved in neuronal avalanches increased with age. Also, the distribution of the avalanche duration showed an increase in longer avalanches, and the distribution of the avalanche size showed an increase in large avalanches. Finally, for a system at the critical point, there is a defined relationship between the distributions of the avalanche sizes, durations, and average size for a given duration. We used this relationship to estimate the distance from the critical point for each participant. We found that this distance increased with age. We did not find any evidence for sex differences in these effects. Together, our findings suggest the brain shifts slightly towards a supercritical state in healthy aging.

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Poster

PSTR198: Network Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.07/Z38

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

Support: Virtual Research Environment at the Charité Berlin – a node of EBRAINS Health Data Cloud
Digital Europe Grant TEF-Health # 101100700
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H2020 Research and Innovation Action Grant Human Brain Project SGA3 945539
H2020 Research and Innovation Action Grant EOSC VirtualBrainCloud 826421
H2020 Research and Innovation Action Grant AISN 101057655
H2020 Research Infrastructures Grant EBRAINS-PREP 101079717
H2020 European Innovation Council PHRASE 101058240
H2020 Research Infrastructures Grant EBRAIN-Health 101058516
H2020 European Research Council Grant ERC BrainModes 683049
JPND ERA PerMed PatternCog 2522FSB904
Berlin Institute of Health & Foundation Charité, Johanna Quandt

Excellence Initiative
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German Research Foundation SFB 1315 (project ID 327654276)
German Research Foundation SFB-TRR 295 (project ID 424778381)
German Research Foundation SPP Computational Connectomics RI 2073/6-1, RI 2073/10-2, RI 2073/9-1.

Title: Semantic Annotation of Computational Neuroscience Knowledge: The Virtual Brain Ontology for Reproducible Whole-Brain Models

Authors: *L. MARTIN[1], K. BÜLAU[2], C. HUETTL[1], R. SCHMITT[1], D. PERDIKIS[1], H. TAHER[2], L. DOMIDE[3], L. STEFANOVSKI[1], P. RITTER[1];
[1]Berlin Inst. of Hlth. at Charité Univ. Med. Berlin, Berlin, Germany; [2]Charité Univ. Med. Berlin, Berlin, Germany; [3]Codemart, Cluj-Napoca, Romania

Abstract: Recent advances in brain network modeling have refined our understanding of neural function and disease. However, integrating multimodal study outcomes into a unified framework that explains brain dynamics mechanistically remains challenging. Inconsistencies in metadata dissemination, terminology, mathematical notations, and software complicate model reproducibility and knowledge synthesis. Additionally, the highly interdisciplinary nature of brain modeling requires extensive expertise in biology, data analysis, and mathematics, raising entry barriers for new researchers. The Virtual Brain Ontology (TVB-O) is an expandable knowledge framework for large-scale brain network models based on Web 3.0 standards. It systematically annotates all elements of the neuroinformatics platform TVB, building a knowledge graph with their mathematical relationships and biological meanings. TVB-O includes a comprehensive metadata schema for computational models and a detailed provenance model logging each computational step of a simulation experiment. It is also interoperable with established neuroscience data models like Brain Imaging Data Structure, Brainlife.io, and the Neuroimaging Data Model, facilitating the integration of metadata and provenance information of empirical model inputs into a complete and transparent experiment report. TVB-O supports the automatic generation of simulation-ready code for computational infrastructures in languages like Python, Fortran, Julia, or Jax using symbolic mathematics derived from the ontology. TVB-O's knowledge base currently hosts definitions for 1180 entities with 18034 relationships and has been integrated into 24 local neural mass models within TVB, with plans to extend to more models. The framework includes eight coupling functions for inter-regional connectivity and nine numerical integration schemes to solve model equations. We annotated ten current peer-reviewed modeling studies and reproduced their main results with our code-generation pipeline.TVB-O facilitates the development and sharing of whole-brain network models, guides scientists from model choice to code generation, and aims to enhance transparency and interpretability of brain modeling results. It enables systematic, collaborative simulation of complex brain dynamics. However, its full functionality will be realized as more studies are implemented and the collaborative aspect fully engages. Potential future applications for large language models are discussed.

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Poster

PSTR198: Network Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.08/AA1

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

Support: CIHR PJT-168980

Title: Modeling changes in the spatial distribution of network dynamics across the adult lifespan using The Virtual Brain

Authors: J. WANG[1], L. ROKOS[2], K. SHEN[1], *A. MCINTOSH[1];
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Abstract: The Virtual Brain (TVB) is a brain modeling platform that simulates brain activity using an individual's own neuroimaging data (Sanz Leon et al., 2013). TVB models enable the description of local and global brain dynamics using simulation-derived measures and model parameters. The Virtual Aging Brain (VAB) has been used to generate simulated data that reflect network dynamic changes with age for an older adult population (ages 55-85) from the 1000BRAINS dataset (Lavanga et al., 2023). Work has yet to be done that 1) examines the full adult lifespan (18+) and 2) describes the spatial distribution of dynamics. In this study, we aim to determine the relationship between region-specific brain dynamics and age. The Cambridge Centre for Ageing and Neuroscience (Cam-CAN) dataset consists of neuroimaging data and cognitive measures from individuals across the adult lifespan. We generated individualized TVB models for a subset of the Cam-CAN dataset that underwent extensive quality assurance for multimodal neuroimage processing (ages 18-87, N=69). Using the Montbrió-Pazó-Roxin mean-field approximation for each region in the model, we simulated resting-state BOLD-fMRI dynamics (Montbrió et al., 2015). We optimized each model by tuning parameters (noise variance and global coupling) in search of maximal variance in functional connectivity dynamics (FCDvar), a metric of neural dynamics that quantifies switching between low and high activity states. For each region within each individual, we generated 5 minutes of simulated neural population-mean firing rate, membrane potential, and BOLD signal. We replicated findings from older adults in VAB, finding that global coupling is positively correlated with age ($r = 0.33$, $p = 0.006$). This suggests a modulatory effect overlaid on structural connectivity to maintain network dynamics. We extend these findings by developing metrics of bistability, a measure of switching behaviour for each region. Bistability metrics were extracted from simulated firing rates and membrane potentials. We found that adult brains exhibit spatial patterns of bistability that change significantly with age ($p = 0.003$). Regions in the dorsal attention, default mode, and control networks exhibit the most prominent decreases in bistability with age. This loss of switching behaviour with age may be associated with decreased connectivity, anticorrelation, and flexibility of connectivity patterns among these regions, potentially impacting cognitive function (Avelar-Pereira et al., 2017). These generative models can support our understanding of aging, by providing insight into the biophysical properties that change across the adult lifespan.

Disclosures: J. Wang: None. **L. Rokos:** None. **K. Shen:** None. **A. McIntosh:** None.

Poster

PSTR198: Network Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.09/AA2

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

Support: Digital Europe Grant TEF-Health # 101100700
H2020 Research and Innovation Action Grant Human Brain Project SGA2 785907
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H2020 Research and Innovation Action Grant EOSC VirtualBrainCloud 826421
H2020 European Innovation Council PHRASE 101058240
H2020 European Research Council Grant ERC BrainModes 683049
JPND ERA PerMed PatternCog 2522FSB904
Berlin Institute of Health & Foundation Charité
Johanna Quandt Excellence Initiative
German Research Foundation SFB 1436 (project ID 425899996)
German Research Foundation SFB 1315 (project ID 327654276)
German Research Foundation SFB 936 (project ID 178316478)
German Research Foundation SFB-TRR 295 (project ID 424778381)
German Research Foundation SPP Computational Connectomics RI 2073/6-1, RI 2073/10-2, RI 2073/9-1
DFG Clinical Research Group BECAUSE-Y 504745852; Horizon Europe: EBRAINS 2.0 101147319, Virtual Brain Twin 101137289, Research and Innovation Action Grant AISN 101057655, Research Infrastructures Grant EBRAINS-PREP 101079717, Research Infrastruc

Title: Neuroimmunology of Psychosis in The Virtual Brain

Authors: *C. HUETTL[1,2], K. BÜLAU[1,2], R. SCHMITT[1,2], M. SCHIRNER[1,2], L. STEFANOVSKI[1,2], P. RITTER[1,2,3];
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Abstract: Psychosis is one of the most common and severe psychiatric syndromes. The long-term quality of life is most adversely affected by negative symptoms of Psychosis, like avolition or anhedonia, and cognitive symptoms impairing cognition and memory. Besides a genetic

component and the effects of Cannabis and other hallucinogens, the literature points in the direction of an inflammatory imbalance, i.e. increased pro-inflammatory cytokines in blood and cerebrospinal fluid, as an important factor in the development of Psychosis. Functional connectivity (FC) changes have been found to be predictive of different types of psychotic symptoms. Prior clinical trials using immunomodulatory therapies aiming to mitigate these symptoms, have found significant improvements e.g. in cognition. At the same time our understanding of the underlying mechanisms is incomplete and thus the targeting of the relevant pathways in each individual may be improved. Therefore, this project aims at integrating the molecular mediators of inflammation e.g. cytokines and enzymes at the microscale, with macroscale FC and behavioral manifestations of psychotic symptoms. The cohort from the Human Connectome Project for Early Psychosis (HCP-EP) dataset is categorized into two groups based on inflammatory biomarkers: individuals with an inflammatory state (IS) and those with a non-inflammatory state (NIS), as determined by comparing similarity measures between imaging data and gene expression maps of inflammatory pathways. FC is derived by measuring the temporal correlation between different brain regions' activity. The HCP-EP fMRI data are the basis for brain simulation derived network metrics like integration and segregation predicting psychotic symptoms. The brain simulations are performed using the neuroinformatics platform The Virtual Brain (TVB, www.thevirtualbrain.org). The behavior is evaluated using comprehensive questionnaires provided by the HCP including the NIH Toolbox Cognitive Function Battery. The present study for the first time integrates three mechanistic scales in psychosis focusing on neuroinflammatory factors. This results in a more fine-grained understanding of the underlying mechanisms of psychotic symptoms, enabling a prediction of psychotic symptoms based on virtual biomarkers. It sheds light on the question which psychotic symptoms are closely related to inflammatory processes and therefore might be suitable targets for future immunomodulatory clinical trials. This approach may also enhance our understanding of Psychosis as a concept, adding to a growing body of evidence suggesting different mechanisms underlying this one diagnostic label.

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Poster

PSTR198: Network Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.10/AA3

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: Efficiency and reliability in biological neural network architectures

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Abstract: Neurons in a neural circuit exhibit astonishing diversity in terms of the numbers and targets of their synaptic connections and the statistics of their spiking activity. We hypothesize that this diversity is the result of an underlying tension in the neural code between reliability - highly correlated activity across trials on the single neuron level - and efficiency - highly uncorrelated activity between neurons within a trial. Specifically, certain architectures of connectivity foster efficient activity while others foster the opposite, i.e., robust activity. Both coexist in a neural circuit, leading to the observed long-tailed and highly diverse distributions of connectivity and activity metrics, and allowing the robust subpopulations to promote the reliability of the network as a whole. To test this hypothesis we developed a notion of the complexity of the connectivity of a subpopulation and used it to analyze several openly available connectomes. The electron microscopy reconstructions of the full brain connectivities of the adult C. elegans (Witvliet et al. 2021), the Drosophila larva (Winding et al., 2023) and of $1mm^3$ volume of mouse primary visual cortex (MICrONS Consortium et al., 2021) as well as a morphologically detailed model of $2mm^3$ of rat somatosensory cortex (Markram et al., 2015). Our analysis revealed that they all exhibited wide complexity distributions. Using co-registered functional data and simulations in case of the detailed network model, we found that low complexity subnetworks were indeed characterized by efficient spiking activity, and high complexity subnetworks by reliable but inefficient activity. Moreover, for neurons in cortical input layers, the focus was on increasing reliability and for output layers on increasing efficiency. To progress from describing correlations to establishing causation, we manipulated the connectivity in the model and showed that complex subnetworks indeed promote the reliability of the network as a whole. Our results improve our understanding of the neural code, demonstrating that the code itself is as diverse as the neuronal connectivity and activity, and must be understood in the context of the efficiency-reliability tradeoff.

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Poster

PSTR198: Network Models II

Location: MCP Hall A

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Program #/Poster #: PSTR198.11/AA4

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.
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Title: Dendritic architecture differentiates human from mice neuronal networks

Authors: *L. KANARI[1], A. ARNAUDON[1], N. BARROS-ZULAICA[1], J. S. COGGAN[1], R. BENAVIDES-PICCIONE[2], J. DEFELIPE[2], J. MEYSTRE[3], R. PERIN[3], M. PEZZOLI[1], R. STOOP[4], I. SEGEV[5], H. MARKRAM[1], C. P. DE KOCK[6];
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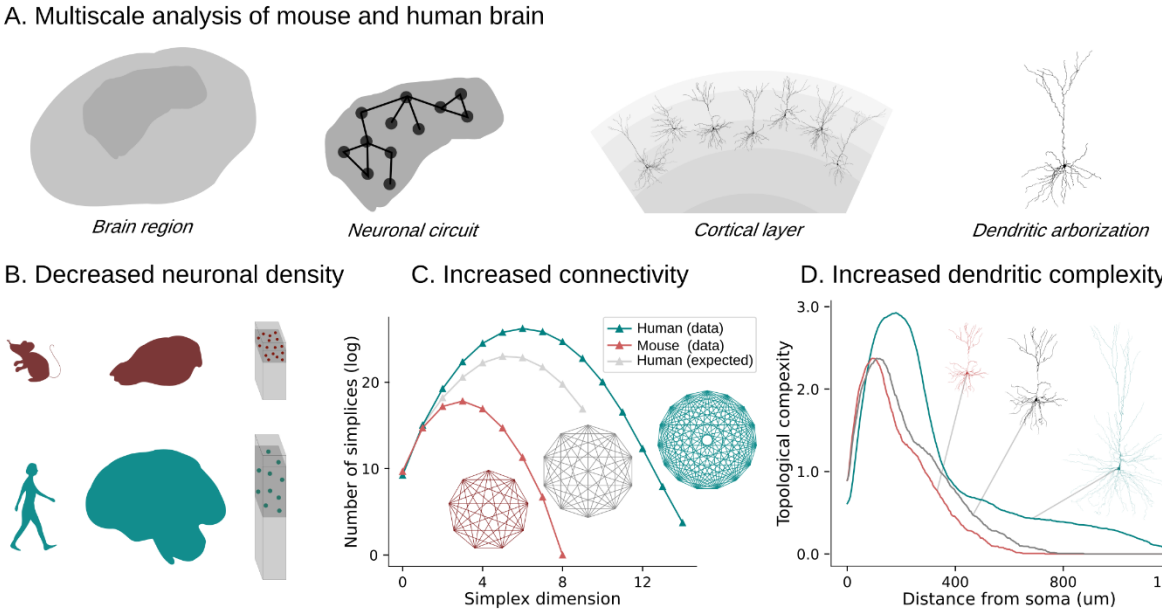
Abstract: The organizational principles that distinguish the human brain from other species have been a long-standing enigma in neuroscience. Focusing on the uniquely evolved cortical layers 2 and 3 in humans, human pyramidal neurons show more intense connectivity among themselves compared to the mouse. This is surprising because human L2 and 3 neurons are much sparser. We show that the number and size of neurons fail to account for this connectivity difference, suggesting that another property of neurons is a key determinant of human network connectivity. Topological comparison of human and mouse dendrites reveals significant morphological differences between the two species. In particular, dendrites of human pyramidal cells exhibit much higher perisomatic (basal and oblique) branching density than mouse dendrites, which can be attributed to the increased space between human neurons due to decreased neuronal density. These differences persist across cortical layers (layers 2, 3, and 5) and brain regions (hippocampus), suggesting they are a defining feature of human cells, effectively preserving the density of dendritic processes and the connectivity between neurons. In addition, we show that dendritic structure directly impacts network-level topological complexity, including the formation of a rich subnetwork structure. Higher dendritic complexity contributes to higher numbers and dimensions of simplices in networks of human pyramidal cells. The increased structural complexity generated by the sophisticated shapes of dendrites suggests that a network is not merely a simple sum of its components. The complex branching patterns of human pyramidal cells enable more robust and coordinated neural computations in the human cortex. This result reinforces the view that dendritic complexity plays a crucial role in biological networks, as Santiago Ramon y Cajal proposed. We conclude that greater dendritic complexity, which is a defining attribute of human neurons, may provide enhanced computational capacity and cognitive flexibility to the human cortex.



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Poster

PSTR198: Network Models II

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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: Multiscale data integration to generate atlas-based biophysical modeling of mouse striatal nuclei

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Abstract: The striatum is a critical component of the basal ganglia and plays a significant role in controlling motor behavior and reward processing. Dysfunctions in the striatum have been associated with several neurological disorders, including Parkinson's disease, Huntington's disease, addiction, etc. Dopamine loss is a hallmark of Parkinson's disease and has been implicated in the pathogenesis of striatal dysfunction. However, the precise mechanisms by which dopamine loss leads to dysregulated striatal outputs, dopamine-acetylcholine chemical imbalances, pathological local field potential oscillations, and the interplay between these changes must be fully comprehended. By understanding these mechanisms, we can gain insight into the neural dynamic pathology in the basal ganglia and demonstrate that these pathological oscillations originate in the striatum. To study these phenomena, we developed an atlas-based, anatomically-constrained model of the mouse dorsal striatum.The model integrates various layers of data, including cellular, synaptic, electrophysiological, and morphological information. Expanding upon the prior work on the striatal microcircuit (Hjorth et al., 2020), the model integrates data-driven enhancements to the Blue Brain Cell Atlas (Erö et al., 2018), specifically addressing cell density and composition. The structural and functional intrastriatal connectivity were constrained and validated with experimental data while populating the dorsal striatum volume with synthesized morphologies. Through this approach, the study provides a comprehensive computational tool for exploring differences in striatal phenomena between normal and pathological states, including dopamine modulation, rhythmic oscillations, excitation-inhibition balance, and multisensory integration.The model represents a significant advancement in understanding the nuanced function of the striatum in neural processes. Through iterative validation, it lays the groundwork for an in-depth exploration of striatal phenomena and their interaction with other brain regions, in both normal and pathological conditions. This research contributes to our understanding of neurological disorders associated with striatal dysfunction and provides a framework for future investigations into therapeutic interventions targeting the basal ganglia.

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Poster

PSTR198: Network Models II

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Support: ETH board
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Title: Network connectivity of human vs rat: a comparative topological study of biologically detailed models

Authors: *N. BARROS-ZULAICA[1], D. EGAS SANTANDER[2], A. ARNAUDON[3], D. MANDGE[4], R. PERIN[5], J. DEFELIPE[6], R. BENAVIDES-PICCIONE[7], L. KANARI[8], H. MARKRAM[9], M. W. REIMANN[10];
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Abstract: The human neocortex stands out as the brain region that sets us apart from other species, primarily by imparting unique cognitive skills, such as spoken language. Even though Ramon y Cajal was already studying the neocortex in the 19th century, much remains unknown about fundamental structural characteristics. A recent study found that differences in morphology between human and mouse neurons affect the network topology even when only basic connectivity rules are considered [1]. Specifically, the human neurons that have complex dendritic arborizations promote strongly connected subnetworks in the network of pyramidal cells, demonstrated by a considerable increase in the number of non-random neuron motifs, and higher memory capacity.It has also been shown that the use of biologically detailed computational models can help speed up the process of unraveling the mysteries of brain circuits. By comparing computational models of different species, we can analyze the functional and structural differences between them, therefore enhancing our understanding of the human cortex. For this study, we have built a detailed model of a human cortical microcircuit following the approach used in [2] to build a rat microcircuit. To this end, we collected new human histological and anatomical data, such as bouton densities and morphological reconstructions from experiments and the literature. We also developed various strategies to overcome missing data, such as generalizing or adapting data from other species.In this study, we built a comprehensive model of the human cortex, taking into account detailed biological connectivity data, such as bouton densities and the number of touches per pair of connected neurons. Crucially, connectivity in the model was derived from appositions between the dendrites and axons of reconstructed human neurons, placed in biologically realistic positions. We performed complex connectivity and topological analysis, such as common neighbor bias, and degree distribution of motives, and we compared these results against the rat model published in 2015 [2]. We characterized the topological structure of human connectivity in terms of robustness, degree distributions, symmetry, and related measurements.Therefore, we gained insights into the effect of morphological differences on the network structure of the two species that have implications for the advanced cognitive abilities of humans.1.Of Mice and Men: Increased dendritic complexity gives rise to unique human networks Lida Kanari, et al., bioRxiv 2023.09.11.557170;2.Reconstruction and simulation of neocortical microcircuitry. H. Markram, et al. Cell, 163:456-492, 2015.

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Poster

PSTR198: Network Models II

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Title: DU15NET: An Open, Available Set of Atlases for Human Neuroimaging

Authors: *J. DU[1], L. M. DINICOLA[1], P. A. ANGELI[2], N. SAADON GROSMAN[1], W. SUN[1], S. KAISER[1], J. LADOPOULOU[1], A. XUE[3], T. YEO[3], M. ELDAIEF[4], R. L. BUCKNER[1,4,5]; [1]Dept. of Psychology, Ctr. for Brain Sci., Harvard Univ., Cambridge, MA; [2]Psychological and Brain Sci., Dartmouth Col., Hanover, NH; [3]Natl. Univ. of Singapore, Singapore, Singapore; [4]Dept. of Psychiatry, [5]Athinoula A. Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA

Abstract: The cerebral cortex is populated by specialized regions that are organized into networks. Using resting-state fixation fMRI data from 15 intensively sampled participants (each scanned 8 or more times), we recently applied a multi-session hierarchical Bayesian model to delineate 15 distinct networks (Du et al., 2024, *J Neurophysiol*). To provide group atlases based on the 15-network model, we constructed a set of atlases in multiple surface and volume-based formats, named the DU15NET atlases. These atlases utilized the model solutions from individual participants to construct atlases that reflect the spatial central tendencies across the group (Dworetsky et al., 2021, *NeuroImage*). Specifically, the overlap of network estimates across the 15 participants was used to create (1) a network consensus atlas, (2) a series of thresholded network agreement atlases, and (3) a tripartite hierarchical atlas, each described in detail and available at https://freesurfer.net/fswiki/CorticalParcellation_DU15NET. The primary atlas, DU15NET-Consensus, represents the assignment at each vertex to the most common network among the 15 participants collected here. It reflects the fine-scale idiosyncratic features and spatial registrations of the data from these 15 participants, including small regions that are absent in group-averaged estimates. For example, there is a clear previously under-appreciated representation of the Salience / Parietal Memory Network in the medial prefrontal cortex. As another example, spatial details separating the canonical Salience and Cingulo-Opercular

networks are maintained. The agreement atlases are thresholded at various levels, leaving only vertices with agreement across at least a given number of participants. Agreement atlases may be most useful when the goal is to construct regions-of-interest with a high probability of being in one network and not another. Initially computed in FreeSurfer fsaverage6 space, the atlases were further sampled to the fsaverage5 and fsaverage spaces, and projected to HCP surface spaces and MNI152 volume space, broadening their utility across various neuroimaging contexts.

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Poster

PSTR198: Network Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR198.15/Web Only

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

Title: Causal functional connectivity from neural dynamics

Authors: *R. BISWAS[1], S. MUKHERJEE[2], S. SRIPADA[3], E. SHLIZERMAN[1];
[1]Electrical and Computer Engin., Univ. of Washington, Seattle, WA; [2]Statistics & Data Sci., Natl. Univ. of Singapore, Singapore, Singapore; [3]Ctr. for Sci. & Consciousness, Bellevue, WA

Abstract: Functional connectivity represents brain network interactions and is fundamental to the translation of neural structure to brain function. While multiple approaches have been proposed for mapping functional connectivity based on statistical associations between neural activity, association does not necessarily incorporate causation. Additional approaches have been proposed to incorporate aspects of causality to turn functional connectomes into causal functional connectomes, however they focus on specific aspects of causality. This warrants a systematic statistical framework to causal functional connectomics to evaluate existing approaches and guide the development of further causal methodologies. In this work, we first establish such a statistical guide. We particularly focus on the introduction of directed graphical models as a framework, which defines the directed Markov property as an essential criterion for capturing causality in the proposed functional connectomes. Based on these notions, we perform a comparative study of existing approaches for inferring causal functional connectivity from neural time series. However, the common formulation of directed graphical modeling is not ideal for neural time series since it was developed for variables with independent and identically distributed samples. Therefore, we develop a novel methodology, coined the Time-aware PC (TPC) algorithm, that adapts directed graphical modeling to the time series scenario. We establish the mathematical guarantee of the TPC algorithm in inferring causal relationships from time series data under standard time series conditions. We then demonstrate the utility of the methodology in simulated and public benchmark datasets, and recent Neuropixels recordings from the mouse visual cortex under different visual stimuli. Lastly, we compute the causal

functional connectivity in the human domain in Alzheimer's disease from resting-state functional magnetic resonance imaging (fMRI) data and perform an exploratory analysis of alteration of causal functional connectivity edges between subjects with Alzheimer's disease compared to cognitively normal subjects. The corresponding brain regions are found to be in agreement with medical literature on brain regions impacted by Alzheimer's disease.

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Poster

PSTR198: Network Models II

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Program #/Poster #: PSTR198.16/AA8

Topic: D.06. Vision

Support: CNS2022-135870 funded by MCIN/AEI/ 10.13039/501100011033 and by "European Union NextGenerationEU/PRTR"

Title: Transfer learning for the classification of electrically evoked neural responses in visual prosthetics employing murine and feline models

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Abstract: Over the last 40 years, the cochlear implant has helped more than one million people to successfully recover functional audition. Retinal prostheses aim to follow the same path. However, one of the main obstacles to overcome relates to the difficulty of replicating the neural patterns of healthy vision. Along these lines, researchers have demonstrated that preferential activation of different types of retinal ganglion cells is possible using kilohertz-frequency electrical stimulation. In this context, machine learning can be a valuable tool to inform whether these stimulation strategies are able to mimic physiological neural patterns. Here we describe a preliminary study to determine if artificial neural networks (ANN) can discern between visually and electrically evoked neural patterns in different animal models. An ANN architecture with long short-term memory (3 units), convolutional (15 filters) and dense network layers (3 units) was built. The ANN was then trained using mouse cortical responses from different types of visual stimuli using data from de Vries et al (2020). Subsequently, we implemented a transfer learning process using neural data recorded from the superior colliculus in a rat model (Barriga-Rivera et al 2018). These neural patterns were the responses to visual (flash) and electrical (biphasic pulses) stimulation. Further tuning and testing of the ANN were done using cortical recordings from a feline model (Barriga-Rivera et al, 2017). The action potential spikes from each animal were taken from 0-0.25 s after the stimulus was presented, and binned into 20 equal-width bins. Initial test showed that the ANN could predict whether a mouse was given a visual stimulus with a significantly higher accuracy than chance (n_{mice} = 13, accuracy = 98.3%). A test

using rat recordings demonstrated ability to predict whether the rat was given light, electrical, or no stimulus with satisfactory accuracy (n_{rats} = 6, accuracy = 80%). Finally, training using cat recordings and testing across animals showed a lower prediction accuracy, but still higher than chance ($n_{cats,\ train}$ = 4, $n_{cats,\ test}$ = 2, accuracy = 65%). In the context of visual prosthesis technology, this preliminary work shows the ability of the ANN algorithm to quantify the difference between electrically- and visually-induced neural responses, which can be used in the optimization process of the electrical stimulus delivered. Moreover, the potential transferability of the ANN to predict the responses in higher mammals by training using data from rodents was confirmed. This approach can help reducing the number of animals required.

Disclosures: A. Pratiwi: None. **H. Guzmán-Miranda:** None. **A. Barriga-Rivera:** None.

Poster

PSTR199: Network Computations: Data Analytics and Statistics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.01/AA9

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

Support: German Federal Ministry of Edcation and Research (Cluster4Future SEMECO, 03ZU1210FB)

Title: Cnns improve decoding of selective attention to speech in cochlear implant users

Authors: *C. JEHN[1], A. KOSSMANN[2], N. VAVATZANIDIS[2], A. HAHNE[2], T. REICHENBACH[1];
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Abstract: Cochlear implants (CIs) are neural prostheses that use artificial electrical stimulation of the cochlea to restore hearing in severely hearing-impaired individuals. While modern CIs enable a majority of users to achieve good speech understanding in quiet environments, background noise and competing speech streams pose significant challenges. Auditory attention decoding (AAD) seeks to decode the attention of a listener in a multi-talker situation from electroencephalography (EEG) data. AAD may be used in the development of a neuro-steered CI, which aims to help CI users in challenging listening situations by amplifying the target speaker and attenuating background sounds. A variety of methods for AAD in normal-hearing individuals have been developed and evaluated over the past years, with deep neural networks (DNNs) proving superior to linear models in terms of decoding performance. However, although the feasibility of AAD in CI users has been demonstrated by several studies, the advantages of DNNs remain to be proven for CI users. Here we demonstrate how the implementation of a convolutional neural network (CNN) improves the decoding of selective attention to speech in CI users. First, we collected a substantial selective attention dataset from 25 bilateral CI users

(15 female 10 male, median age 56 years ± 11.1), where stimuli were presented in a free field environment and EEG was measured simultaneously. Second, we implemented a CNN as well as a linear backward model for AAD. The CNN emerged as the superior method, as measured by the achieved decoding accuracy on all studied decision windows ranging from 1s to 60s. In conjunction with a learnable Support-Vector-Machine for speaker classification, the CNN achieved a maximal decoding accuracy of 74% (± 11%) on the population level and thereby significantly outperformed the linear backward model. These findings underscore the potential of DNNs with adaptable speaker classification as promising candidates for neuro-steered CIs, translating advancements made in AAD for normal-hearing individuals to benefit CI users.

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Poster

PSTR199: Network Computations: Data Analytics and Statistics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.02/AA10

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

Title: Hierarchical communities in the larval Drosophila connectome: Links to cellular annotations and network topology

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Abstract: One of the longstanding aims of network neuroscience is to link a connectome's topological properties--i.e. features defined from connectivity alone--with an organism's neurobiology. One approach for doing so is to compare connectome properties with maps of metabolic, functional, and neurochemical annotations. This type of analysis is popular at the meso-/macro-scale, but is less common at the nano-scale, owing to a paucity of neuron-level connectome data. However, recent methodological advances have made possible the reconstruction of whole-brain connectomes at single-neuron resolution for a select set of organisms. These include the fruit fly, *Drosophila melanogaster*, and its developing larvae. In addition to fine-scale descriptions of neuron-to-neuron connectivity, these datasets are accompanied by rich annotations, documenting cell type and function. Here, we use a hierarchical and weighted variant of the stochastic blockmodel to detect multi-level communities in a recently published larval *Drosophila* connectome. We find that these communities partition neurons based on function and cell type. We find that communities mostly interact assortatively, reflecting the principle of functional segregation. However, a small number of communities interact non-assortatively. The neurons that make up these communities also form a ``rich-club", composed mostly of interneurons that receive sensory/ascending inputs and deliver outputs along descending pathways. Next, we investigate the role of community structure in shaping neuron-to-neuron communication patterns. We find that polysynaptic signaling follows specific trajectories

across modular hierarchies, with interneurons playing a key role in mediating communication routes between modules and hierarchical scales. Our work suggests a relationship between the system-level architecture of an organism's complete neuronal wiring network and the precise biological function and classification of its individual neurons. We envision our study as an important step towards bridging the gap between complex systems and neurobiological lines of investigation in brain sciences.

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Poster

PSTR199: Network Computations: Data Analytics and Statistics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.03/AA11

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

Title: Predicting synaptic connections from pretectal responses to optokinetic stimuli

Authors: *S. CHALYSHKAN[1], T. BISWAS[1], F. KUBO[2], H. BAIER[3], J. E. FITZGERALD[1]; [1]Neurobio., Northwestern Univ., Evanston, IL; [2]RIKEN Ctr. for Brain Sci., Saitama, Japan; [3]Genes - Circuits - Behavior, Max Planck Inst. of Neurobio., Planegg, Germany

Abstract: Studying the link between neural network structure and function is crucial in understanding the principles underlying brain activity. With the growing use of large-scale activity recordings and advances in anatomical circuit-level reconstructions, researchers can now posit numerous wiring diagrams that tie observed neural activity with synaptic connectivity. While various theoretical frameworks have been developed to narrow down plausible models of circuits, existing approaches are yet to be rigorously tested on suitable neural datasets. Here, we examined the framework developed by Biswas et al. (2023) in making synaptic predictions on the prior work by Svara et al. (2022), which used optokinetic stimuli to identify functional neuronal response types in an anatomically reconstructed pretectum of the larval zebrafish. First, we constructed a matrix consisting of the average fluorescence activity of 11 major pretectal response types and an approximation of retinal activity. Using numerical methods detailed in Biswas et al. (2023), we then calculated the smallest synaptic weight norm that reproduces the neuronal responses, W-min, and compared it to the smallest weight norm required for a given synapse to be absent, W-critical. The higher the W-critical to W-min ratio, the more consistently the synapse is present across solutions. This method predicted consistent excitatory connections from the retina to four monocular response types, in line with the previously hypothesized circuit model. This approach also predicted that monocular response types send consistent excitatory and inhibitory connections to certain binocular response types. Altogether, we have shown preliminary evidence that the Biswas et al. (2023) method might yield compelling structural predictions from functional neuronal types to optokinetic stimuli. Further comparisons with the anatomically reconstructed synapses will provide a clearer picture of the accuracy of the theoretical framework predictions.

Disclosures: S. Chalyshkan: None. **T. Biswas:** None. **F. Kubo:** None. **H. Baier:** None. **J.E. Fitzgerald:** None.

Poster

PSTR199: Network Computations: Data Analytics and Statistics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.04/AA12

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

Support: NSF Grant DGE 2137420

Title: The Split-Trial Analysis: Efficient Inference of Information Limiting Noise

Authors: *D. LE[1], X. WEI[2];
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Abstract: It has long been known that correlated activity in neural population responses has the potential to limit the coding fidelity of a network.This is especially notable considering a common approach to understanding network activity is to perform decoding based analyses of the population response.In light of this, it is important to identify bounds on decoding error in biological networks, which is analogous to an information limit for the population response.Recent theoretical examinations propose that shared noise along the stimulus encoding direction in the neural response space is the primary determinant for the precision of the network.Noise of this form has thus been termed information-limiting noise, which is studied through examination of information-limiting noise correlations.The intuition for such correlations limiting precision is as follows:shared noise in the stimulus direction leads to neural responses from the population that are indistinguishable from a neural response encoding a stimulus that is different from the underlying ground truth stimulus.Despite the practical importance of these ideas, application to experimental data has been challenging due to difficulties involved in direct approaches to calculating information.To overcome this major limitation, we have developed a new method based on partitioning of the neural population.Specifically, we split the neurons into subpopulations, then perform decoding analysis on each subpopulation.Intuitively, the decoding error for each subpopulation contains both information-limiting noise and private noise along the encoding direction that is specific to that sub-population.We devise an appropriate statistical procedure to infer the shared noise, which serves as an estimator of the information-limiting noise.Notably,our method goes beyond popular single-trial analysis because it crucially relies on the splitting of a single-trial to create repeated measurements.We thus refer to our method as the``split-trial analysis".We have performed theoretical analyses and extensive numerical validation using synthetic and recorded data.We find that our method has several key advantages:(i)compared to prior methods, the split-trial analysis achieves a given accuracy with a much smaller number of neurons;(ii)it is reliable across a wide range of parameter regimes;(iii)importantly, it does so without directly estimating

the noise covariance matrix, which is a major hurdle to existing techniques.Our method is simple yet general, and should be generally applicable for understanding neural coding under different experimental conditions in many brain regions.

Disclosures: D. Le: None. **X. Wei:** None.

Poster

PSTR199: Network Computations: Data Analytics and Statistics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.05/AA13

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

Support: NSF Grant 1931249

Title: Predicting nonstationary spike-spike correlations with local fields

Authors: *Z. TAJIK MANSOURI[1,2], J. DION[1,3], M. A. ESCABI[4,2,3], I. H. STEVENSON[5,2,6]; [1]Univ. of Connecticut, Storrs, CT; [2]Biomedical Engineering, University of connecticut, Storrs, CT; [3]Psychological Sciences, University of connecticut, Storrs, CT; [4]Electrical and Computer Engin., Univ. of Connecticut, Storrs, CT; [5]Dept. of Psychological Sci., Univ. of Connecticut, Storrs, CT; [6]Connecticut Institute for Brain and Cognitive Science, University of connecticut, Storrs, CT

Abstract: Local field potentials (LFPs) are often correlated with neural spiking activity, and previous statistical techniques have shown how stationary spike-spike correlations can be predicted by the spike-LFP relationships of individual neurons. Here we evaluate methods for fitting spike-spike correlation from LFP using large-scale multi-electrode recordings in the mouse hippocampus and visual cortex from the Allen Institute – Neuropixels dataset. We compare a single-channel Fourier model, a multi-channel filter-bank model, and models based on generalized phase estimation. We find that multi-channel models can be more accurate and LFP frequency bands contribute differentially to predicting spike-spike correlations. We then extend these models to describe nonstationary spike-spike correlations. Although both cortical and hippocampal spike-spike correlations vary over the recording as brain state changes, these changes are, at least partially, predicted by models that assume a fixed spike-LFP relationship. In analyses of neural coding, spike-spike correlations can be used as features to better decode sensory variables. This work may help to partition spike-spike correlations and disentangle the contributions of external variables and internally generated, ongoing oscillatory activity.

Disclosures: Z. Tajik Mansouri: None. **J. Dion:** None. **M.A. Escabi:** None. **I.H. Stevenson:** None.

Poster

PSTR199: Network Computations: Data Analytics and Statistics

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Program #/Poster #: PSTR199.06/AA14

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

Support: SyBBURE Undergraduate Research Program

Title: Analyzing stimulus richness across the mouse visual hierarchy using higher-order neuronal interactivity

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Abstract: The brain is a highly complex system with many higher-order interactions. We propose that basic methods such as rate-coding and pairwise analysis fall short of fully characterizing these complex interactions. To test this, we leveraged integrated information theory to measure higher-order interactions in response to stimuli of varying degrees of complexity and richness. Using data collected by the Allen Institute, we calculated an experimental measure of higher-order neural integration (Phi hat) across six visual areas of the mouse cortex with varied visual stimuli from basic artificial stimuli (flashes) to complex artificial stimuli (an array of varied static gratings) and naturalistic stimuli (natural images and natural movies). Complex artificial stimuli had a higher degree of complexity than the natural stimuli as measured by changes in pixel intensity over time but should be less contextually relevant to the mice and therefore less rich than the natural stimuli. The order of putative stimulus richness goes from simple artificial to complex and then natural stimuli whereas the order of complexity goes from simple artificial to natural and then finally to complex artificial stimuli. We analyzed gamma-power local field potentials (LFP) and current-source density (CSD) and found that Phi hat increased with stimulus richness across all visual areas for both signal types (Repeated measures ANOVA, $p < .001$; partial eta-squared = .724 and .332 for CSD and LFP respectively). The increase with stimulus richness was not seen in the base LFP and CSD gamma power signals for the same time interval. There were significant differences between visual areas, but the effect size was small, and the differences did not scale with the visual hierarchy (Repeated measures ANOVA, $p < .001$; partial eta-squared = .075 and .167 for CSD and LFP respectively). Higher-order interactions as measured by Phi hat better reflected the richness of stimuli with naturalistic stimuli yielding greater neuronal interactivity compared to artificial stimuli. This was not the case for the base signals where static gratings evoked a greater neural response than natural movies. Our study suggests that quantifying higher-order interactions better reflects stimulus meaningfulness than rate coding and pairwise analysis techniques. Our analysis of higher-order interactions sheds light on how brain areas encode for stimulus richness and complexity.

Disclosures: P.G.L. Poggi: None. **B.M. Carlson:** None. **B. Mitchell:** None. **A.V. Maier:** None.

Poster

PSTR199: Network Computations: Data Analytics and Statistics

Location: MCP Hall A

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Program #/Poster #: PSTR199.07/AA15

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

Support: National Key R&D Program of China (N0. 2020AAA0130400)

Title: Reconstructing Circuit Connectivity from in vivo Spike Trains Using Deep Domain-adaptive Matching

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Abstract: Inferring the connectivity of neural circuits from in vivo experimental data is essential for understanding the neural architectures that underpin behavior and cognition. However, advanced machine learning approaches in this domain, particularly deep learning, are significantly constrained by incomplete in vivo observations. This limitation restricts the ability to assign “ground-truth” labels to in vivo data, compelling researchers to rely on synthetic data generated by biophysical neural models for initial training. Yet, this strategy introduces a widely perceived insurmountable challenge: "model mismatch", where synthetic model dynamics fail to accurately match true neural dynamics. To tackle these interrelated challenges, we introduce DeepDAM (Deep Domain-Adaptive Matching), a robust, adaptive, and versatile framework that fundamentally transforms the training of deep neural networks (DNNs) for inference tasks using both synthetic data and unlabeled in vivo data. DeepDAM fine-tunes DNNs on a fusion of synthetic and unlabeled in vivo data, adaptively aligning the DNN's feature space with in vivo neural dynamics, thereby effectively mitigating the model mismatch challenge. Impressively, we validated the framework with extracellular recordings in the hippocampal CA1 region of freely behaving mice, which achieves an exemplary Matthews correlation coefficient of ~1.0 in connectivity inference accuracy, significantly outperforming existing methods (~0.6-0.7). Importantly, our framework can well adapt to diverse experimental conditions and a broad spectrum of neural properties and scales, demonstrating its high generalizability across various in vivo and ex vivo scenarios. This marks a significant step towards the accurate and comprehensive reconstruction of functional mammalian brains using data-driven methodologies.

Disclosures: K. Sheng: None. **M.J. Beau:** None. **K. Du:** None.

Poster

PSTR199: Network Computations: Data Analytics and Statistics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.08/AA16

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

Title: Unraveling Mitochondrial Bioenergetics: Advanced Modeling of Complex IV Dynamics

Authors: *M. EINI KELESHTERI[1,2], C. CADONIC[4,3], T. GHAFOURIAN[5], W. SNOW[4], J. DJORDJEVIC[4], P. FERNYHOUGH[1], A. ADLIMOGHADDAM[4,6], S. PORTET[2], **B. C. ALBENSI**[5,4,1];
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Abstract: Mitochondrial bioenergetics, particularly the electron transport chain (ETC) and Complex IV, are vital for cellular function and energy production. Mathematical models provide insights into normal mitochondrial function and disease, including neurodegenerative disorders. Our research focuses on refining a mathematical model, with an emphasis on Complex IV in the ETC. Objectives include incorporating mitochondrial activity modulation using inhibiting and uncoupling reagents, akin to oxygen consumption experiments. Rigorous validation, calibrated against Oroboros Oxygraph-2k data from C57BL/6 mouse mitochondria, ensures accurate reproduction of dynamic bioenergetic activities. The developed graphical user interface (GUI) complements objectives, providing an *in silico* platform for hypothesis testing. Employing an innovative kinetic methodology, we integrate inhibiting reagents (oligomycin, rotenone, antimycin A, FCCP) into the computational model to simulate bioenergetic responses across varied physiological conditions. Optimization of the Mean Square Error (MSE) objective function using multiple algorithms, including the genetic algorithm, and calibration against Oroboros Oxygraph-2k data from freshly isolated mitochondria of C57BL/6 mice, ensures rigorous validation of the model's precision under both unperturbed and perturbed scenarios. These outcomes confirm the model's efficacy in accurately simulating Complex IV contributions to bioenergetics. Our refined mathematical model effectively simulates mitochondrial bioenergetics, validated against experimental data, and offers insights into mitochondrial dysfunction and neurodegenerative diseases. The integration of inhibiting and uncoupling reagents, along with the user-friendly GUI, enhances accessibility and usability. Our research contributes to advancing medical understanding, highlighting the role of computational models in deciphering mitochondrial complexities in neurological disorders.

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Poster

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Program #/Poster #: PSTR199.09/AA17

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

Support: NIH Grant R00MH128772

Title: Predicting the Resting-State Functional Connectome from Regional Gene Expression in Human Population Datasets

Authors: *A. RATZAN[1], J. DONG[2], S. FAIZAL[2], R. RAJ[2], E. VAROL[2];
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Abstract: A longstanding goal of systems neuroscience is to uncover the molecular mechanisms underlying large-scale brain network organization. Resting-state functional connectivity and tissue-specific RNA-sequencing capture distinct, yet complementary information about the steady-state nature of the human brain. Linking these modalities can provide insight into genetic aspects of diverse functional properties of the brain including disconnectivity patterns in several disorders (Arnatkeviciute, 2023). Moving beyond correlative studies in single datasets, we propose a prediction based framework analogous to models fit on synaptic connectivity data, to capture more complex interdependencies between gene expression and connectivity. Two comprehensive brain-wide transcriptomic and connectomic datasets are the Allen Human Brain Atlas (AHBA; n = 6) and Human Connectome Project (HCP; n = 207). These datasets are parcellated into 8 networks across 114 cortical and subcortical regions and population averaged to yield a single transcriptome and connectome per dataset. A gradient-boosted ensemble method (XGBoost) is trained on this data to predict how strongly regions functionally coactivate based on gene expression profiles. To isolate the information gain of gene expression, we first fit a baseline model to predict connectivity strength between two regions based on their connectivity profiles with other regions. Over 100 random 75-25 splits, the baseline connectivity model predicts connectivity strength between regions of the test set with Pearson r = 0.87, the gene expression model predicts the test set with r = 0.91, and a combined model using both gene expression and connectivity profiles predicts the test set with r = 0.95. A simpler partial-least squares (PLS) model is then fit and tested on the same splits. Compared to XGBoost, the simpler connectivity and gene expression based models perform weaker, but combining the two modalities yields a Pearson r of 0.88. PLS models are then used to predict subsets of the HCP connectome using gene expression from two independent datasets, GTEx (subcortical; n = 368) and UT Southwestern (frontal cortex; n = 6). Models fit to the additional datasets perform on par or better than comparable models trained on AHBA. Results across several models and datasets suggest that gene expression patterns are highly relevant for inter-region connectivity and may vary in different subnetworks. Leveraging higher and lower-order statistical patterns unveiled by these models in tandem with gene ontology and clinical data offers promise towards understanding the intricate influence of genes on neural circuitry in health and disease.

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Poster

PSTR199: Network Computations: Data Analytics and Statistics

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Program #/Poster #: PSTR199.10/AA18

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

Title: Characterizing the geometry of representations in mouse PPC with non-linear encoding manifolds

Authors: A. VISHNUBHOTLA[1], P. RAVISHANKAR[2], M. T. KAUFMAN[3], *R. NOGUEIRA[4];
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Abstract: Adaptive behavior requires the representation of external world variables by populations of neurons in different areas of the brain. Populations of neurons represent specific combinations of these variables as points in the neural state space, the space where each axis corresponds to activity of an individual neuron. Population representations can form manifolds with different geometries, which support different computations by downstream neurons. For instance, low-dimensional representations have been shown to generalize knowledge better, whereas higher-dimensional representations are useful for flexible behaviors (Rigotti et al. 2013; Bernardi et al. 2020; Nogueira et al. 2023). Linear encoding models have been proven as a powerful method to characterize and denoise the relationship between continuous variables and population activity (Musall et al. 2019). However, there is increasing evidence that this relationship can be better explained with non-linear functions of these variables (Nogueira et al. 2023). Here, we used a novel method to characterize the non-linear geometry of the representational manifold when the task is complex and continuous. We analyzed the geometry of representations in the Posterior Parietal Cortex (PPC) of mice performing a vision-to-movement task. Mice used a 2D joystick to cancel out the motion of a visual stimulus that drifts diagonally. On each trial, the drift direction was drawn randomly and the instantaneous position of the joystick added a velocity component to the drifting stimulus. Crucially, only one axis of the joystick was coupled to the (vertical or horizontal) visual stimulus drift. Mice learned to use only the relevant visual information to inform movements of the 2D joystick. Once the animals became experts (65% correct performance), we performed two-photon calcium imaging in PPC. (L2/3, 14,000 pyramidal neurons). We found that the best model of external variables and PPC activity was a non-linear encoding model composed by a feed-forward architecture of one fully-connected hidden layer. To characterize the shape of this encoding manifold, we analyzed the gradient and hessian of the manifold by differentiating the implicit relationship with respect to the input variables. We also were able to artificially synthesize the combinations of input variables that would maximally activate the populations of neurons in the PPC by using gradient descent on the activations of our encoding model. Our method constitutes a general and function-agnostic approach to characterizing the geometry of the representational manifold for complex environments that need to be described by several continuous variables.

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Poster

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Program #/Poster #: PSTR199.11/AA19

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

Support: R01NS120850
R01NS129608
R01NS079518

Title: Population-level analysis of saccadic modulation of visual representations

Authors: *S. HANSON[1], J. HUNT[2], A. BUTEAU[3], A. POLEG-POLSKY[4], G. FELSEN[5]; [1]Dept. of Physiol. and Biophysics, The Univ. of Colorado Anschutz Med. Campus, Aurora, CO; [2]Univ. of Colorado, Anschutz Med. Campus, Aurora, CO; [3]Physiol. & Biophysics, Univ. of Colorado, Anschutz Med. Neurosci. Grad. Training Program, Aurora, CO; [4]Physiol. and Biophysics, Univ. of Colorado Sch. of Med., Aurora, CO; [5]Dept. of Physiol. and Biophysics, U. of Colorado Sch. of Med., Aurora, CO

Abstract: Animals perform saccades - rapid eye movements - when navigating and exploring their visual environment. Saccades modulate visual perception and neural activity representing visual stimuli including in the superior colliculus (SC) a midbrain sensorimotor structure. However, whether and how populations representations of visual stimuli, which likely underlie visual perception, are modulated by saccades has not been studied. We used Neuropixels to record the activity of populations of visual SC neurons (20-50 neurons/session) in mice presented with visual probes and making saccades. Some probes were presented synchronously with a saccade (perisaccadic) and some were presented alone (extrasaccadic). To compare perisaccadic and extrasaccadic population responses, we examined state-space trajectories, during the 500 ms following probe presentation, under both conditions.The trajectories follow qualitativelydifferent paths, suggesting that the structure of visual population representations was altered by saccades. We are currently quantifying this effect and examining how it depends on saccade magnitude and direction, and on the saccade-probe latency.

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Poster

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

Support: NSF Grant IIS-2123781
NSF 1830881
NSF OAC-1818253

Title: Optimization of In Vitro Reservoir Computing informed by Volume Electron Microscopy and Simulation

Authors: *Z. DOU[1], S. KIM[1], G. UPADHYAY[1], K. KAZEMI[1], X. ZHANG[1], H. GRITTON[2], M. GAZZOLA[1];
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Abstract: The Reservoir Computing (RC) paradigm is motivated by the mechanistic underpinnings of how biological neural networks handle multimodal, dynamic, and real-time inputs. Our goal is to explore using in vitro neural cultures to implement energy-efficient reservoirs endowed with the inherent parallelism, scalable hypernetworks, and plasticity of biological systems.
We applied RC to in vitro neural networks assembled from mouse embryonic stem cell-derived motoneurons cultured on microelectrode arrays. Our results reveal that reservoir qualities, including kernel rank and memory capacity, are strongly influenced by the strength of synaptic connections and the distribution of neuronal density.
To better understand the relation between network structure and reservoir qualities, we employ volume electron microscopy (vEM) here to reconstruct the 3D topology of in vitro networks from serial block-face scanning electron microscopy (SBF-SEM) images. Obtained morphology data and connectivity statistics are used to instantiate in silico simulations capturing experimentally observed population-wise excitatory activities. In turn, in silico experiments provide insights for optimizing in vitro reservoir qualities.

Disclosures: Z. Dou: None. **S. Kim:** None. **G. Upadhyay:** None. **K. Kazemi:** None. **X. Zhang:** None. **H. Gritton:** None. **M. Gazzola:** None.

Poster

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Program #/Poster #: PSTR199.13/AA21

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

Support: Schmidt Futures

Title: mapping neuronal connectivity: investigating approaches from function to structure

Authors: *S. J. IHLE[1], C. WEIS[1], N. D. MEDINA[3], X. HUANG[4], G. WILDENBERG[5], P. LITTLEWOOD[1], N. B. KASTHURI[2];
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Abstract: Unlocking the enigma of how the brain learns and preserves information sis one of the great mysteries in neuroscience. This intricate process hinges on the dynamic interplay of neurons, continuously adjusting their connections: plasticity. Despite significant advances, the precise mechanisms governing these connections and their role in learning and memory remain elusive.
To deepen our grasp of memory and plasticity, discerning the link between neuronal structure and function is imperative. Leveraging the recent strides in connectomics, structural connectivity of vast networks can now be reconstructed[1,2]. However, understanding the relationship between the connectome and the network spiking behavior has not yet been uncovered.
In this work we investigate various methodologies bridging function to structure. Generalized linear networks, as showcased by Pillow et al., have successfully deciphered connectivity within retinal neurons[3]. Ising models, grounded in robust mathematics, offer potent reconstruction capabilities[4]. Hidden Markov models also hold promise[5]. Additionally, causal coupling inference[6] and other machine learning, particularly symbolic models integrated with deep learning, present viable avenues[7].
Here, we are presenting an approach to study the feasibility of these methodologies using simulated networks. Our ongoing inquiry aims to unveil the potential to map structure from function, probing for any emergent phase transitions and assessing scalability. With this work, we strive to develop an understanding of how to connect network structure and function, advancing our comprehension of memory and plasticity in the brain.
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[7] M. Cranmer et al., Advances in neural information processing systems, vol. 33, pp. 17429-17442, 2020.

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Poster

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

Support: NIH/NINDS Grant 1UF1NS115779

Title: Neural dynamics in gustatory cortex during taste mixture-based perceptual decision-making

Authors: *L. LANG, J. BLACKWELL, Y. ZHENG, S. WANG, G. LA CAMERA, A. FONTANINI;
Neurobio. & Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: Neural activity in gustatory cortex (GC) evolves as a sequence of abrupt transitions between ensemble-coordinated firing rate patterns called metastable states. Previous work has shown metastable dynamics underlie GC's role in mediating taste-based decision-making. However, it is unclear exactly what properties of metastable states allow for encoding of decision-making task-relevant variables; moreover, it is unknown how this framework applies when taste stimuli are mixtures that vary along a continuum rather than a set of categorical values. Here we address these questions by analyzing high-density electrophysiological recordings from GC neural ensembles in mice performing a sucrose/NaCl binary taste mixture-based decision-making task. In this task, animals (1) sample a sucrose/NaCl mixture (ranging, in % sucrose/% NaCl, from 0/100 to 100/0) from a central spout, (2) wait for a delay period, then (3) lick a lateral spout for a water reward based on the predominant mixture component (i.e., % sucrose > % NaCl --> lick left; else --> lick right). On the single-unit level, analyses of tuning curves over time revealed mostly linear representations of stimulus information during the early trial period and mostly binary representations of perceptual, cognitive, and decision variables during the later trial period. On the ensemble level, we used Hidden Markov Models to extract metastable states from simultaneously-recorded units. We classified each state based on the shape of its mean duration vs. stimulus profile (analogous to the single-unit tuning curve) and found that, over the time course of a trial, ensemble states are most likely to initially reflect sensory information linearly, then perceptual/abstract cue information categorically, and then decision/choice information categorically, consistent with the single-unit findings. Altogether, our results suggest that linear coding of the stimulus dynamically gives rise to categorical coding of other task-relevant variables during taste mixture-based decision-making and implicate metastable state durations as a key property for mediating this transition. Ongoing work is directed at explaining these dynamics in terms of circuit functional connectivity changes via computational modeling with predictive coding graphs.

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Poster

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Title: Decoding Neuronal Activity in Freely Moving Rodents Using Machine Learning: Identification of Calcium Transients via miniScope-Captured Signals

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Abstract: Advances in imaging technology and fluorescent Ca^{2+} indicators have allowed analyses of the neuronal activity with high temporal resolution underlying a variety of behaviors. Excitingly, our team has successfully collected data *via* this *in vivo* Ca^{2+} imaging procedure in rodents during intravenous drug taking or seeking sessions in operant chambers. However, when we transitioned to the data analysis stage, we realized that, although there were multiple constrained nonnegative matrix factorization (CNMF) algorithm-based tools to extracting the Ca^{2+} transients from the original video files, there were no quality evaluations of the CNMF-detected cells and their Ca^{2+} transients, nor any tools to efficiently and reliably identify Ca^{2+} transients as a downstream step of CNMF-extracted traces of Ca^{2+} influx. To address these issues, our team has developed a GUI using the python package PYQT5. In the first component, all pertinent output from the CNMF process (i.e., calcium traces, cell footprints, processed videos) were synchronized in visualization *via* a user-friendly window. Together with the acceptable rate of Ca^{2+} traces, an evaluation of the CNMF process was made with 3 further options, including (1) continue with Ca^{2+} transient identification, (2) optimize the CNMF parameters, and (3) improve original image quality. In the second component, we first developed two non-machine learning tools, including (1) Ca2+ transient kinetics (e.g., Amplitude threshold, inter-transient interval)-based auto-identification and (2) manual identifications of Ca2+ transients, which can be used to generate the data sets in training, validation and testing the machine learning model. Specifically, two variants of Recurrent Neural Networks machine learning models (i.e., LSTM and GRU) and two versions of the Transformer model (i.e., the original model and one using local attention) were tested. Our data demonstrated the high reliability of the GRU-based machine learning model, by which F1 score (i.e., a statistical measure of predictive performance) was achieved beyond 0.9 using a limited dataset comprising Ca^{2+} traces from just 10 cells over a 15-minute window to train the machine learning model. We also developed a third component, in which the big data with details of Ca2+ transients can be exported to excel spreadsheet or visualized by scatter plots or column figures. This comprehensive platform that we have developed offers a highly efficient tool with wide accessibility and broad expandability. It bridges the gap between CNMF-based extraction of Ca^{2+} traces and the final analysis of the biological significance of Ca^{2+} influx-based neuronal activity.

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Poster

PSTR199: Network Computations: Data Analytics and Statistics

Location: MCP Hall A

Time: Monday, October 7, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR199.16/AA24

Topic: H.10. Human Learning and Cognition

Title: Enhancing Spiking Neural Networks with Reference Spikes as New Plastic Parameters to Improve Temporal Information Processing

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Abstract: Spiking Neural Networks (SNNs) can model information processing of biologically plausible neurons that communicate through trains of spikes. During unsupervised or supervised learning, SNNs can adapt their plastic parameters to perform various tasks similar to Artificial Neural Networks (ANNs). However, there may be more plastic parameters from the inherent complexity of the biological neural system than just the traditional weights and time delays, which may help SNNs improve their performance and achieve brain-like functions. Here, we propose a new type of plastic parameter called reference spikes. Reference spikes come from a hypothesized upstream network independent from the input. The number and timings of reference spikes are plastic and modified by learning rules. Reference spikes are delivered to a neuron through synapses, which provide reference information to help the neuron learn by modulating the integration of incoming spikes at a detailed level. Through computational experiments, we show that with unsupervised learning (STDP), reference spikes can help SNNs recover the time sequence of spikes in a pattern hidden in noise signals. With supervised learning, reference spikes improve the memory capacity of SNNs to map input spike patterns to target output spike patterns and increase classification accuracy on the MNIST, Fashion-MNIST, and SHD datasets, where both input and target output are temporally encoded. Our results demonstrate that reference spikes improve the performance of SNNs by enhancing their temporal information processing ability.

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