SfN Boston Chapter

SOCIETY *for* NEUROSCIENCE

Boston Area Neuroscience Group
neuroboston.org

Graduate Program for NEUROSCIENCE
BOSTON UNIVERSITY

November 4th, 2022

Boston University Photonics Center
Colloquium Room, 9th Floor

Keynote Speaker:

Eve Marder, PhD
Victor and Gwendolyn Beinfield Professor of Biology
Brandeis University
Member, National Academy of Sciences

Chapter Meeting:

Friday, November 4th at 10:00 am

Doors open at 9:30 am

Boston University

Featuring:

Keynote Speaker

Poster Session

Career Panel

Poster Prizes

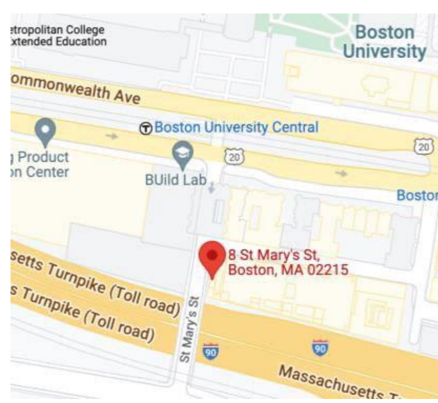
Lunch will be served!

@NeuroBoston

Location:

8 St. Mary's Street, Boston

BOSTON UNIVERSITY



Meeting is easily accessible from the Green Line (BU East and BU Central), with additional garage and [limited] metered spots.

Questions? Email us at BeantownBANG@gmail.com

Welcome to NeuroBoston and Boston University!

We are thrilled that we can share the excitement of the science coming from laboratories all around New England with you today at our campus. It is so wonderful to see the passion of our students, postdocs, and faculty members fill the rooms with new possibilities for discovery and its translational application. There is no better way to spend a Friday than with our dear colleagues at BU and our new neuroscience friends from around New England.

Special thank you to Professor Eve Marder for her generous offer to give the Keynote Address as well as our neuroscience colleagues in Pharma, Melanie Shackett Brennan and Jacobi Cunningham. Also thanks to the rest of the members of our Career Panel for setting aside time to provide an invaluable service to our New England student community. Thank you also goes out to Dr. Camron Bryant, Chair of the organizing committee for NeuroBoston 2022, and to the members of the NeuroBoston Council and GPN's Assistant Director Sandi Grasso that kept the ball rolling!

The Boston Area Neuroscience Group Council

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(**Bolded were our heavy hitters for the event!**)

Enjoy!

Shelley

Shelley J. Russek, Ph.D.
Professor of Pharmacology
Director, BU Graduate Program for Neuroscience
Center for Systems Neuroscience
Boston University Charles River Campus
Chobanian and Avedisian School of Medicine

NeuroBoston Fall Symposium 2022

Program of Events:

9:30 a.m. **Doors open**: Coffee and light pastries available at Photonics. Please pick up your badges at the registration table from our student hosts (Anosha Khawaja-Lopez, Patrick Bloniasz).

10:00 a.m.-10:15 a.m. **Welcome address** (Dr. Shelley Russek, Director BU Graduate Program for Neuroscience, Professor of Pharmacology) and introduction of Keynote Speaker by Ms. Audrey Teasley (PhD student in BU Computational Neuroscience).

10:15 a.m.-11:10 a.m. **NeuroBoston Keynote Lecture by Dr. Eve Marder**, Victor and Gwendolyn Beinfield, Professor of Biology, Brandeis University, Member National Academy of Sciences, **"Resilience to Perturbation in Degenerate Neurons and Networks: Relevance to Climate Change"** (Q & A, Lucas Carstensen.)

11:10 a.m.-11:55 a.m. **Lightning Talks** (Moderated by Dr. Camron Bryant, BU Associate Professor of Pharmacology; Q &A Graduate Student Will Lynch.)

11:10-11:20: Undergraduate Researcher: **Petra Dujmic**, Brandeis, "A Possible Role for Satellite Glia in Regulating Sympathetic Fiber Growth to The Heart"

11:20-11:30: Graduate Researcher: **Abigail Roper**, UMass Boston, "Valproic acid improves anxiety-like behavior and amphetamine hyper-reactivity in isolation reared trait anxiety rats but reverses the benefits of environmental enrichment"

11:30-11:40: Postdoctoral Researcher: **Dr. Elvia Mena Avila**, University of Rhode Island, "Primary Afferent Depolarization in a rabbit model of Cerebral Palsy"

11:40-11:55: Faculty Researcher: **Dr. Stephanie Puig**, Boston University "Opioid Use Disorder is associated with alterations in circadian pathways: proteomics analysis of human postmortem brains"

12:00 p.m.-1:00 p.m. **Lunch at Photonics Center**

1:00 p.m.-2:30 p.m. **Poster Session** Photonics Center (Numbers 1-40) and Rajen Kilachand Center for Integrated Life Sciences & Engineering (Numbers 41-60). Coffee will be available at both sites.

2:30 p.m. – 3:15 p.m. **Career Panel** (Moderator: Graduate Student Lucas Carstensen)

Dr. Melanie Shackett Brennan, Principal Scientist, AbbVie Inc.
Dr. Jacobi Cunningham, Director of Pharmacology and Scientific Operations, Apellis Pharmaceuticals
Dr. Kelsea Gildawie, Postdoctoral Fellow, Tufts University
Dr. Heidi Meyer, Assistant Professor, Boston University

3:15 p.m. – 3:30 p.m. **Trainee Awards** (Presented by Dr. Camron Bryant)

Lightning Talk Presenters

Petra Dujmic is an undergraduate student at Brandeis University pursuing a combined Bachelor's and Master's degree in neuroscience and biology. Her work in the laboratory of Dr. Susan Birren focuses on understanding the development of sympathetic neurons contacting the heart. She is specifically interested in characterizing the cellular and molecular mechanisms regulating sympathetic neuronal outgrowth, and thus identifying how structural plasticity is facilitated at the neuron-myocyte interface. Aberrant increases in sympathetic fiber growth to the heart is thought to promote the development of hypertension in the spontaneously hypertensive rat (i.e., SHR), the most commonly used model of cardiovascular disorders. Thus, the work of Dr. Birren's laboratory is important to understanding the pathogenesis of high blood pressure. In the future, she hopes to pursue an MD/PhD in effort to identify therapeutic interventions for cardiovascular disorders.

Abigail Roper is a PhD student in the Developmental and Brain Sciences program at the University of Massachusetts, Boston. She received her B.A in Psychology from Iona College in 2019 and M.S in Neuroscience: Systems, Behavior, and Plasticity from Temple University in 2021. She currently does research under the supervision of Dr. Tiffany Donaldson investigating the cognitive and affective consequences of early life stress and addiction related to neuroinflammation and neural changes.

Dr. Elvia Mena Avila is a Neuroscientist from Mexico. After finishing her MD in 2012, she got a master's degree in Neuropharmacology, and a PhD in 2020. Her doctoral work elucidated the noradrenergic role in sensory-motor integration in the mouse. For the last two years she has been working as a postdoctoral fellow on sensory-motor integration in a rabbit model of cerebral palsy in Katharina Quinlan's lab at the University of Rhode Island. She hopes to continue working to find possible therapeutic interventions to relieve spasticity in cerebral palsy.

Dr. Stephanie Puig is an Assistant Professor of Pharmacology at Boston University Chobanian & Avedisian School of Medicine. She received her Master's degree in Physiology and Neuroscience from the University of Pierre et Marie Curie in Paris, and her Ph.D. in Neuropharmacology and Neurobiology of Addiction from the University of Paris Descartes. During her postdoctoral training in the Pittsburgh Center for Pain Research at the University of Pittsburgh, she studied the mechanisms mediating opioid long term side-effects, with a focus on involvement of receptor tyrosine kinases in analgesic tolerance. Her research now aims at finding safer strategies to treat chronic pain by preserving long term opioid analgesia and preventing opioid addiction.

Lightning Talk Abstracts

A POSSIBLE ROLE FOR SATELLITE GLIA IN REGULATING SYMPATHETIC FIBER GROWTH TO THE HEART ***Petra Dujmic, Undergraduate, Brandeis University,*** Melda M. Buyukozturk, MS, Susan J. Birren, Ph.D.

Due to a technical glitch we are unable to share the full text of Petra's abstract, but be sure to catch her lightning talk as this is really interesting work! Please accept our sincere apology for the error.

VALPROIC ACID IMPROVES ANXIETY-LIKE BEHAVIOR AND AMPHETAMINE HYPER-REACTIVITY IN ISOLATION REARED TRAIT ANXIETY RATS BUT REVERSES THE BENEFITS OF ENVIRONMENTAL ENRICHMENT ***Abigail Roper, MA, Graduate Student, University of Massachusetts, Boston,*** Juliet Buteme, BS; Vanessa Romero, BS; Joseph Lusse, BS; Tiffany Donaldson, PhD. (POSTER#

Valproic acid (VPA), a deacetylase inhibitor, has been shown to improve functioning in a number of animal models of early life stress (ELS) and mood disorders. The enriched environment (EE) also promotes recovery following ELS, anxiety and depression working at least through epigenetic mechanisms. In the present study, we determined the interaction of VPA with housing environment using 8th generation outbred trait anxiety animals bred along high (HAn) and low anxiety (LAn)-like behavior lines. Animals were housed for 30 days in EE, standard (SE) or isolated environments (IE), tested for anxiety and amphetamine sensitivity, given a 2-week VPA treatment (7 mg/ml) in drinking water, and re-tested on each of the behavioral measures. Results indicate that HAn animals reared in IE and SE showed greater anxiety-like behavior on the elevated plus-maze and open field, and hyper-activity to amphetamine, relative to HAn EE and all LAn groups. VPA decreased the benefits of EE but significantly improved the adverse effects of IE on anxiogenic tests and amphetamine-induced locomotion. Immunohistochemical analysis of corticotropin-releasing hormone (CRH)-immunoreactivity in the central amygdala revealed a significant decrease in CRH-ir in LAn EE-reared rats compared to HAn EE. Collectively, the data suggest benefits of EE and VPA treatment in modulating trait anxiety and isolation rearing behavioral consequences. Moreover, the EE shifts in emotional responding and drug-sensitivity likely work through mechanisms other than regulation of CRH.

PRIMARY AFFERENT DEPOLARIZATION IN A RABBIT MODEL OF CEREBRAL PALSY ***Elvia Mena Avila, PhD, MD, Postdoctoral Fellow, University of Rhode Island,*** Emily Reedich PhD, Landon Genry BS, Katharina Quinlan PhD. (POSTER#

Cerebral palsy (CP) arises from a variety of perinatal insults and results in permanent, lifelong motor deficits, most often spasticity. Pain is the most common comorbidity. Despite the seriousness and prevalence, there have been few recent advances in therapeutics. Spastic CP is thought to arise from damage to the cortex and downstream disinhibition of spinal motoneurons, although the specific mechanism has never been directly tested. Perinatal hypoxia-ischemia (HI) is a factor in many CP-causing injuries. Rabbits subjected to prenatal HI at 70% gestation show motor deficits similar to spastic CP including hypertonia and hyperreflexia, thus allowing a deeper investigation into mechanisms of spasticity. HI not only damages cortical neurons, but also directly impacts the spinal cord including cell death and expansion of nociceptor projections. In addition to pain, nociceptor sprouting has implications for spasticity. In the spinal cord, peptidergic nociceptors and corticospinal projections converge onto GAD2+ GABAergic interneurons (GABAaxo) that mediate primary afferent depolarization (PAD). We propose that loss of descending modulation of PAD (from damaged corticospinal tracts) results in less finely tuned phasic (short duration) PAD and permits larger amplitude tonic PAD, underlying chronic over-activation of stretch reflexes driving hyperreflexia in CP. We show here that HI rabbits have more reflex irradiation and larger amplitude tonic PAD from activation of cutaneous and nociceptive afferents which likely increases spasticity. Increased nociceptor-driven tonic PAD could diminish normal sensory modulation of PAD through a ceiling effect or shunting. This investigation could lead to better therapies to prevent spasticity and perhaps better understanding of the mechanisms of chronic pain in the context of CP as well.

OPIOID USE DISORDER IS ASSOCIATED WITH ALTERATIONS IN CIRCADIAN PATHWAYS: PROTEOMICS ANALYSIS OF HUMAN POSTMORTEM BRAINS ***Stephanie Puig, PhD, Assistant Professor of Pharmacology, BU Chobanian and Avedisian School of Medicine,*** Ryan Salisbury (BA), Anastasia Yocum (PhD), Jill Glausier (PhD), David Lewis (MD), Bernie Devlin (PhD), Zachary Freyberg (MD, PhD), Marianne Seney (PhD), Matthew MacDonald (PhD), and Ryan Logan (PhD).

Prevalence rates of opioid use disorder (OUD) have increased dramatically, and many patients being treated for OUD relapse within first year. Vulnerability to opioid relapse is associated with severe disruption to sleep and circadian rhythms. Thus, improving circadian rhythms may be an effective intervention for reducing relapse. Unfortunately, while relationships between OUD and circadian rhythm alterations have been studied in rodents, an understanding of the molecular alterations that occur in human brains of people diagnosed with OUD remains limited. Here, we used proteomics to investigate protein alterations in human postmortem brains of OUD and unaffected subjects. We focused on the nucleus accumbens (NAc), a striatal structure, and on the dorsolateral prefrontal cortex (dlPFC), two structures strongly interconnected and heavily implicated in opioid addiction.To enable recreation of rhythms of protein expression, we used time of death information to build cohorts distributed across 24-hour timescale. Quantitative proteomics with TMT was used to measure protein in NAc and dlPFC tissue homogenates and synaptosomes fractions. Limma was used to analyze differentially expressed proteins with adjusted and unadjusted p-values and log-fold-changes (logFC). Identification of rhythmic proteins showed that, in homogenates, the total number of rhythmic proteins was reduced in OUD subjects in both the NAc and the dlPFC (NAc: 115 rhythmic proteins in unaffected controls vs. 56 OUD subjects; dlPFC: 88 rhythmic proteins in unaffected controls vs. 53 in OUD subjects). Conversely, in synaptosomes of OUD subjects, total rhythmic proteins in NAc and dlPFC were decreased or increased, respectively. Interestingly, rhythmic proteins were largely different between groups in both, homogenates and synaptosomes. Notably, in synaptosomes, pathways enriched in OUD rhythmic proteins included membrane potential, vesicle-mediated transport, and GPCR signaling, primarily involved synaptic function. Circadian pathways were also identified as top enriched pathways in synaptosomes. Analysis of differential rhythmicity that compares periods phases, amplitudes and variance (R2), highlighted proteins that strictly change rhythmicity between unaffected and OUD subjects. In the NAc 25 and 23 proteins changed rhythmicity in homogenates and synaptosomes respectively and the majority lost rhythmicity. In the dlPFC, only 10 and 18 proteins changed rhythmicity in homogenates and synaptosomes, respectively. Interestingly, changes were equally distributed towards gain or loss of rhythm. In NAc synaptosomes, top pathways enriched in OUD proteins that changed rhythm included membrane trafficking, response to light stimulus and signaling by Rho GTPases. In dlPFC synaptosomes, they included platelet-derived growth factor beta signaling, tyrosine phosphorylation and GTPase signal transduction. Our study is the first to analyze changes in protein rhythms caused by OUD in human postmortem brains at a large scale. Our findings demonstrate significantly altered rhythms in synaptic function and signaling in the NAc and dlPFC associated with OUD. These results provide insight on processes mediating alterations in circadian rhythms and sleep/wake cycles caused by OUD. Ongoing studies are investigating the possible functional links between these pathways in mouse models of opioid addiction.

Career Panel

Dr. Melanie Shackett Brennan received her PhD in Neuroscience in 2014 from Boston University. She is currently a Principal Scientist at AbbVie Inc. in Cambridge, MA. From 2016-2020, she was a Senior Scientist at Yumanity Therapeutics. Prior to Yumanity, she was a Research Scientist at Biogen, Research Labs, where she played an instrumental role in the development of a major drug for the treatment of intractable MS.

Dr. Jacobi Cunningham is currently the Director of Pharmacology and Scientific Operations at Apellis Pharmaceuticals. He received his PhD in Pharmacology from BU School of Medicine in 2010. Rose through the ranks at Alkermes to become a Principal Scientist in 2020.

Dr. Kelsea Gildawie, received her PhD in 2021 from Northeastern University and is currently a postdoctoral fellow at Tufts University where her research is focused on the use of animal models to investigate the neuromolecular drivers of addiction.

Dr. Heidi Meyer is a new Assistant Professor at Boston University in the Department of Psychological and Brain Sciences. She received her PhD in 2016 from Dartmouth College, and was a Postdoctoral Fellow at Weill Cornell Medicine with Francis Lee until 2021. Her laboratory leverages behavioral, systems, and molecular neuroscience techniques to examine the cognitive and neurodevelopmental underpinnings of affective regulation.

Poster Session Booklet

Author List (Alphabetical by First Name)

Abigail Parakoyi (17)
Abigail Roper (13) *Lightning Talk Abstract
Aidan Evans-Strong (33)
Alekhya Rekapalli (19)
Benjamin R. Williams (47)
Britahny Baskin (1)
Caitlyn Cody (16)
Cassandra Hartsgrove (7)
Catherine Bostian (44)
Chantal Aaron (45)
Clarissa Fantin Cavarsan Muller (3)
Daniel Peters (24)
Douglas K. Reilly (40)
Eden Maness (43)
Elaine Ruiz (23)
Elvia Mena Avila (6) *Lightning Talk Abstract
Emmett Bergeron (10)
Ethan Wong (25)
Gabriela Rodriguez (39)
Garrett Scarpa (18)
Gayathri Kondakath (11)
Hannah Bues (32)
Jinyoung Choi (42)
Johan S. Martinez-Fuentes (28)
Julia R. Mitchell (9)
Kaitlyn Dorst (12)
Kelly Wingfield (46)
Kelsea Gildawie (48)
Kıvılcım Kılıç (30)
Landon Genry (4)
Laura Marshall (22)
Lauren Granata (14)
Lucy Peterson (56)

Mariusz Furmanek (20)
Masen Boucher (2)
Michaela Fanikos (15)
Mikaela Laine (8)
Natalie Fomin-Thunemann (29)
Noell Cho (36)
Patrick Doran (27)
Que Anh Pham (26)
Rebecca Shteyn (53)
Rebecca Suthard (37)
Renee Cauchon (35)
Robert Alan Brown (38)
Ryan Ashiqueali (5)
Ryan Logan (51)
Shruti P Gajjar (41)
Sophia Miracle (52)
Spencer Jacobs-Skolik (34)
Stephanie Puig (54)
Stephanie Puig (55) *Lightning Talk Abstract
Sukhneet Bhogal (31)
Sundararaman Rengarajan (21)
Victoria Lombardi (57)
Will Lynch (50)
Zoe Irving (49)

Author List by Stand Number

Britahny Baskin (1)

Masen Boucher (2)

Clarissa Fantin Cavarsan Muller (3)

Landon Genry (4)

Ryan Ashiqueali (5)

Elvia Mena Avila (6) *Lightning Talk Abstract

Cassandra Hartsgrove (7)

Mikaela Laine (8)

Julia R. Mitchell (9)

Emmett Bergeron (10)

Gayathri Kondakath (11)

Kaitlyn Dorst (12)

Abigail Roper (13) *Lightning Talk Abstract

Lauren Granata (14)

Michaela Fanikos (15)

Caitlyn Cody (16)

Abigail Parakoyi (17)

Garrett Scarpa (18)

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Kelly Wingfield (46)
Benjamin R. Williams (47)
Kelsea Gildawie (48)
Zoe Irving (49)
Will Lynch (50)
Ryan Logan (51)
Sophia Miracle (52)
Rebecca Shteyn (53)
Stephanie Puig (54)
Stephanie Puig (55) *Lightning Talk Abstract
Lucy Peterson (56)
Victoria Lombardi (57)

Poster Abstracts by Stand Number

1

BIOMARKERS, BEHAVIORS, AND BATTLE-RELATED INJURIES: SEX DIFFERENCES IN ACUTE AND CHRONIC OUTCOMES FOLLOWING REPETITIVE BLAST TRAUMA

Britahny M. Baskin, PhD, Postdoctoral Fellow, Boston University, Aric F. Logsdon, PhD; Sujung Janet Lee, BS; Brian D. Foresi, MBA; Elaine R. Peskind, MD; William A. Banks, MD; David G. Cook, PhD, Abigail G. Schindler, PhD.

Mild traumatic brain injury (mTBI) is a leading cause of disability worldwide. Approximately 75% of mTBIs among Operation Iraqi Freedom/Operation Enduring Freedom/Operation New Dawn (OID/OED/OND) are associated with exposure to high explosive detonations. In addition to the potential for persistent post-concussive symptoms, 50-75% of these individuals experience a constellation of comorbid psychiatric and behavioral symptoms (e.g., anxiety, depression, risk taking, aggression). Despite their prevalence, the mechanism(s) mediating such outcomes is well not understood. Adding significantly to this knowledge gap, the vast majority of blast-related mTBI research has been conducted only in males, yet a growing number of females are now serving with military occupational specialty (MOS) codes which may increase their risk for blast exposure. This highlights the importance of understanding sex as a biological variable (SABV) in relation to adverse outcomes following blast exposure. Using a well-accepted electronically-controlled pneumatic shock tube that accurately models battlefield-relevant open field blast forces, we exposed adult male and female mice to repetitive (3x) sham or blast exposure. Acutely following repetitive exposure (0-72 hours), we measured serum and brain cytokine levels, blood-brain barrier (BBB) disruption, fecal microbial abundance, and locomotion and anxiety-like behavior in the open field assay. Chronically (1 month post-blast), we assessed behavioral correlates of mTBI and PTSD-related symptoms commonly reported by Veterans with a history of blast-mTBI using the elevated zero maze, acoustic startle, and conditioned odorant aversion paradigms. Repetitive blast exposure resulted in a mix of both similar and disparate patterns of acute serum and brain cytokine as well as gut microbiome changes in male and female mice yet blast mice acute BBB disruption following repetitive blast exposures was evident in both. While female and male blast mice both exhibited acute locomotor and anxiety-like deficits in the open sexes field assay however, only male mice exhibited chronic adverse behavioral outcomes demonstrating some divergent patterns of blast-induced dysfunction. This novel survey of sex differences following exposure to blast overpressure waves highlight the importance of timing in assaying behavioral dysfunction and confirms the need for more research dedicated to understanding the resulting sequelae following blast exposures in diverse populations. Finally, it suggests novel targets for future diagnosis and therapeutic developments.

2

DOSE-DEPENDENT DIFFERENCES IN SPATIOTEMPORAL PROFILE OF GENE EXPRESSION FOLLOWING SINGLE AND REPEATED MILD TRAUMATIC BRAIN INJURY

Masen Boucher, Research Assistant II, Boston Children's Hospital, Kaitlyn Warren, MS; Jianhua Qiu, MD, PhD; William P. Meehan, MD; Rebekah Mannix, MD.

Traumatic brain injury (TBI) is a leading cause of disability and mortality in young people, yet lacks effective interventions. One of the most significant challenges in designing such interventions is the heterogeneity of injury types and injury response. There is evidence to suggest that injury patterns not only vary with injury dose (severity and frequency of injuries), but also vary by brain region and time point post-injury. In order to design effective treatments for TBI, thorough characterization of these differential responses is needed. Using the NanoString mouse neuroinflammation RNA-based gene panel, we quantified 770 genes to understand how expression patterns change in response to our mouse weight drop model of both single mild TBI and repetitive mild TBI. We also examined differences in brain region (cortex vs. hippocampus) and time point following injury (3 days post-injury vs. 10 days post-injury). Analyses were performed using R Studio and Ingenuity Pathway Analysis (IPA). Investigation of the effects of, and interactions between, the predictors (injury dose, brain region, time point) revealed four functional nodes – inflammation and immunity, metabolism and energy homeostasis, phagocytosis and apoptosis, and cell morphology, movement, and communication. While modification of these nodes was present in each injury condition, the specific genes and pathways, and their degree of activation or inhibition, changed as a result of the other predictors. Decision trees were also generated to identify genes and gene profiles that could discriminate between injury conditions. While this method is statistically different from IPA, it yielded accurate modeling and identified similar targets from the four functional nodes. Principal component analysis revealed clustering of samples based on brain region, while hierarchical clustering yielded clustering of samples based on time point post-injury, followed by brain region and then by injury dose. Genes utilized by the first three principal components mirrored those in the four nodes highlighted by IPA as well. Together, these results both highlight the relevance of the four functional nodes—inflammation and immunity, metabolism and energy homeostasis, phagocytosis and apoptosis, and cell morphology, movement, and communication—as well as indicate the way genes and pathways are differentially modified by injury dose, brain region, and time point. Further, these analyses provide both specific gene expression and pathway targets for further investigation, and underscore the importance of spatiotemporally targeted treatments for TBI.

3

BKCA CHANNEL ACTIVATORS IMPROVES OPEN FIELD BEHAVIOR CHANGES IN 3XTG-AD MOUSE MODELS. ***Clarissa Fantin Cavarsan Muller, PhD, Research Associate IV, University of Rhode Island,*** Scarlett Huck, BA; Wenzhuo Ma, MD-PhD; John K. Robinson, PhD; Richard Clements, PhD.

Introduction: Alzheimer’s disease (AD) is associated with mitochondrial structural and functional alterations in numerous brain cell types. Therapeutic strategies to mitigate these cellular metabolic alterations in AD are lacking. BKCa channels are Ca++ and voltage regulated K+ channels that are expressed on the plasma membrane as well as the mitochondrial inner membrane in neurons. Aberrant function of BKCa in neurons has been associated with brain disorders such as AD. Our group and others have previously shown that activators of BKCa channels are associated with improvements in mitochondrial function and cell survival in multiple cell types. We tested if the BKCa activator rottlerin (a.k.a. mallotoxin) could modulate behavioral deficits in the 3xTg-AD mouse model of AD which exhibits plaque and tangle pathology associated with synaptic dysfunction. Objectives: We aimed to investigate if rottlerin was able to improve behavioral function in an AD transgenic animal model (3xTg-AD). Methods: Wild type (WT; $n = 21$) mice and 3xTg-AD transgenic mice ($n = 20$), both 24 weeks old, were treated with rottlerin or vehicle for 2 weeks. Behavioral test groups had approximately 10 animals/group by each treatment. After habituation (handling), animals started drug treatment. After two weeks of treatment the mice were submitted to motor function behavior tests: Open Field, RotaRod and Barnes Maze. Brains were harvested after the treatment and behavior for histopathological and molecular biology analysis. (Institutional Animal Care and Use Committee (IACUC) number: # AN1920-006) Results: BKCa channel expression was confirmed in both the plasma membrane and mitochondrial of harvested mouse cortex by standard fractionation methods. Using Open Field, we found that control 3xTG animals were less active than WT animals. 3xTG spent more time resting (WT: 162 ±14 s vs. 3xTg-AD: 212 ±16 s, $p = 0.035$) and showed less moving/ambulatory time (WT: 136 ±14 s vs. 3xTg-AD: 87 ±16 s $p = 0.035$ and WT: 17 ±2 vs. 3xTg-AD: 9 ±2 s $p = 0.024$, respectively) when compared to control mice. However, this difference disappeared when 3xTg-AD animals were treated with rottlerin (resting time: 171 ±14 s; moving time: 128 ±14 s; ambulatory time: 15 ±2 s) and were not significantly different than control animals. Rottlerin was able to bring Alzheimer's animals to levels of motor function similar to control animals. Rottlerin-treated 3xTg-AD animals had a higher latency to fall from RotaRod as compared to vehicle-treated 3xTg-AD animals (Vehicle: 121 ±21 s vs. Rottlerin: 171 ±19 s; $p = 0.019$). In the Barnes maze, at the day 5 (last day of Barnes Maze task) 3xTg-AD animals treated with vehicle had a higher latency to the first entry of the animal's head to the escape hole as compared to WT animals (WT: 25.4 ±28.7 s vs. 3xTg-AD: 177.0 ±33.7 s, $p = 0.002$) however, rottlerin treatment was able to reduce this latency for the 3xTg-AD animal and they had similar behavior as the WT animals (WT: 72.1 ±24.6 s vs. 3xTg-AD: 90.1 ±38.9 s, $p = 0.699$).. Conclusion: We showed that BKCa activator could normalize 3xTg-AD animal’s behavior function similar to WT animals. Our preliminary results point to the need to further evaluate the potential for BKCa activators as novel therapeutics for AD.

4

PRENATAL HYPOXIA-ISCHEMIA RESULTS IN PERSISTENT NOCICEPTIVE BEHAVIOR IN A RABBIT MODEL OF CEREBRAL PALSY. ***Landon Genry, University of Rhode Island,*** Emily J Reedich, PhD; Elvia Mena Avila, PhD; Clarissa F Cavarsan, PhD; Brianna Taylor; Kendra Gever, Titiana Tambi, Aly M Garrett, Lisa Dowaliby, Megan Detloff, PhD; Katharina A Quinlan, PhD.

Cerebral palsy (CP) results in lifelong impairments in motor control. The most common comorbidity is pain. Many injuries that give rise to CP involve perinatal hypoxia-ischemia (HI). It is well documented that the cerebral cortex and white matter of the descending corticospinal tracts are affected in patients with CP, but it is less appreciated that the spinal cord is also vulnerable. We utilized a rabbit model of CP based on prenatal exposure to either 40 minutes of HI or sham surgery at 70-80% gestation, followed by a natural term birth. To measure nociception, we used noxious mechanical and thermal stimuli (von Frey and Hargreaves, respectively) from birth until postnatal day (P) 18 in HI and sham kits of both sexes. Open field testing was also performed after rabbits opened their eyes and movements held behavioral significance. At P18 we sacrificed animals in which behavior was measured and examined the nociceptive afferents within the spinal cord dorsal horn. Both mechanical and thermal nociception was enhanced in kits throughout the duration of measurement, without concomitant changes in the open field test. Anatomical measurement of the peptidergic and nonpeptidergic afferents is still underway. In summary, our results suggest that developmental injuries related to CP may also result in nociplastic pain.

5

HISTONE DEACETYLASE 3 INHIBITION DECREASES CEREBRAL EDEMA AND PROTECTS THE BLOOD-BRAIN BARRIER AFTER STROKE. ***Ryan Ashiqueali, Graduate Research Assistant, Beth Israel Deaconess Medical Center,*** Hui Lu, MD, Chin I Lin, MSc, Aashlesha Walchale, MSc, Victoria Clendaniel, BS, Rudy Matheson, BS, Marc Fisher, MD, Eng H. Lo, PhD, Magdy Selim, MD, PhD, Amjad Shehadah, MD.

Introduction: We have previously shown that selective inhibition of histone deacetylase 3 (HDAC3) decreases infarct volume and improves long-term functional outcomes after stroke. In this study, we examined the effects of HDAC3 inhibition on cerebral edema and blood-brain barrier (BBB) leakage and explored its underlying mechanisms. Methods: Adult male Wistar rats were subjected to 2-h middle cerebral artery occlusion (MCAO) and randomly treated i.p. with either vehicle or a selective HDAC3 inhibitor (RGFP966) at 2 and 24 hours after stroke. Modified neurological severity scores (mNSS) were calculated at 2 hours, 1 day, and 3 days. H&E , Evans blue dye (EBD) assay, and fluorescein isothiocyanate(FITC)-dextran were employed to assess cerebral edema and BBB leakage. Western Blot for matrix metalloproteinase-9 (MMP9), MMP-9 zymography, and immunostaining for HDAC3, GFAP, Iba-1, albumin, aquaporin-4, claudin-5, ZO-1, and NF-kB were performed. Results: Early RGFP966 administration decreased cerebral edema ($p=0.002$) and BBB leakage, as measured by EBD assay, FITC-dextran, and albumin extravasation ($p<0.01$). RGFP966 significantly increased tight junction proteins (claudin-5 and ZO-1) in the peri-infarct area. RGFP966 also significantly decreased HDAC3 in GFAP+ astrocytes, which correlated with better mNSS ($r=0.67$, $p=0.03$) and decreased cerebral edema ($r=0.64$, $p=0.04$). RGFP966 decreased aquaporin-4 in GFAP+ astrocytes ($p=0.002$), as well as, the inflammatory markers Iba-1, NF-kB, and MMP9 in the ischemic brain ($p<0.05$). Conclusions: Early HDAC3 inhibition decreases cerebral edema and BBB leakage. BBB protection by RGFP966 is mediated in part by the upregulation of tight junction proteins, downregulation of aquaporin-4 and HDAC3 in astrocytes, and decreased neuroinflammation.

7

ESTROGEN ADMINISTRATION IMPROVES RECENT BUT NOT REMOTE FEAR EXTINCTION MEMORY RECALL IN FEMALE RATS. ***Cassandra Hartsgrove, Graduate Student, University of Massachusetts, Boston,*** Genelle Walker, Kendall Yuknewicz-Boisvert, McKenzie Askler, Sally Lee, Zach Grandinetti, and Lisa Y. Maeng, PhD.

Anxiety and stress-based disorders, such as post-traumatic stress disorder, are reported to be more prevalent in women (Kessler et al., 2012). However, the neurobiological mechanisms underlying sex differences in these disorders are not fully understood. It is hypothesized that gonadal hormones influence brain circuits involved in fear regulation, which is significantly impaired in stress-related disorders. Fear extinction is the ability to reduce or control fear responses when a threat is no longer present, and it was previously found that higher estrogen levels can facilitate memory recall for this type of learning 24h after training (Maeng et al., 2017). The current study further explored the persistence of estrogen's memory-enhancing effect up to 14 days following extinction training. Briefly, 11 naturally cycling female Sprague-Dawley rats underwent a 3-day fear conditioning paradigm. On Day 1, all animals in estrus completed a habituation phase (5 tone-alone trials) and conditioning phase (7 tone-shock paired trials). Extinction training (20 tone-alone trials) took place on Day 2 while subjects were in metestrus (a period of low circulating estrogen levels). 30 minutes prior to extinction training, 5 rats were administered estrogen (15µg/kg) and 6 were administered vehicle. On Day 3, the recent extinction recall test took place with 3 tone-alone trials. The remote extinction recall test took place at least 1 week after the extinction training. Freezing behavior across all behavioral phases was scored manually by 4 blinded raters and averaged as two-trial blocks. Estrogen-treated females showed significantly lower freezing behavior compared to vehicle-treated females during the recent recall memory test ($t(5.55) = 2.146$, $p = 0.04$); however, there was no difference in freezing between treatment groups when tested at least 1 week after training ($t(3) = -0.398$, $p = 0.359$). Administration of estrogen was found to result in short-term facilitation of fear extinction memory consolidation, as observed by lower average freezing behavior during recent extinction recall, but not during remote recall. Future analyses will examine the molecular mechanisms underlying estrogen's effects on fear neurocircuitry to enhance short-term extinction recall.

8

LESSONS FROM EAVESDROPPING ON RATS: SEX DIFFERENCES IN RAT ULTRASONIC VOCALIZATIONS DURING FEAR CONDITIONING AND EXTINCTION ***Mikaela Laine, PhD, Postdoctural Fellow, Northeastern University,*** Julia R Mitchell, MSc; Johanna Rhyner, BS; Rose Clark, BS; Akshara Kannan; Jack Keith, BS; MaryClare Pikus; Emmett Bergeron; Isabella Ravaglia, BS; Ece Ulgenturk; Ashwini Shinde; Rebecca M Shansky, PhD.

Pavlovian fear conditioning is a powerful tool for studying aversive learning, a key component of stress-related psychiatric disorders. We often rely on specific behaviors as readouts of emotional states of non-verbal animals. For example, rats exhibit various threat-related behaviors, including freezing, locomotion and ultrasonic vocalizations, but little is known about how these behaviors relate to one another or differ across sexes. To draw translationally relevant conclusions it is crucial that we understand situational and individual determinants of how such behaviors are expressed. Here we recorded a diverse set of motor and vocal behaviors during cued fear conditioning and extinction. Using machine learning-based tools we found, for example, that both male and female rats emitted short audible vocalizations in response to foot shocks scaling with shock intensity, but males emitted more 'alarm calls' (long 22 kHz calls) with a clearer association with shock intensity than did females. Alarm calls were also observed during extinction, with females showing faster extinction of this response than males despite comparable extinction rates for freezing. In males, alarm call rate during fear conditioning predicted recall of the CS-US association 24 hours later, while in females locomotor and vocal behaviors manifested as largely independent traits. Collectively our data suggest considerable sex-dependence of how similar-appearing behaviors relate to innate and conditioned threat responses. Our findings show the advantages of multidimensional behavioral neuroscience; relying on any single behavior could mislead us, while embracing the richness of behavioral repertoires opens avenues for new discoveries and ultimately improved evidence-based treatment strategies.

9

THE ROLE OF INFRALIMBIC-PERIAQUEDUCTAL GRAY CIRCUITRY IN PAVLOVIAN FEAR CONDITIONING ***Julia R. Mitchell, MS, Graduate Student, Northeastern University,*** Leilani Potgeiter, BS; Emmett Bergeron; Rose Clark, BS; Rebecca M. Shansky, PhD.

In Pavlovian fear conditioning, darting, an escape-like response elicited by the conditioned stimulus (CS), occurs more frequently in females and is predictive of heightened unconditioned responses and enhanced extinction retention. The neural circuit responsible for driving darting behavior is unknown. The dorsal periaqueductal gray (dPAG) is active during escape-like threat responses and receives direct input from the infralimbic cortex (IL), which plays a role in emotionally regulated behavior and is also important for extinction learning. Activation of the IL-dPAG circuit could increase an animal's likelihood to engage in darting and promote any associated behaviors. To test this hypothesis, we used a cre-based intersectional DREADDs approach to activate the IL-dPAG pathway before Pavlovian fear conditioning. Using ScaredyRat, a custom Python tool designed in our lab to analyze raw Ethovision data files, we quantified conditioned (freezing, darting) and unconditioned responses (shock and post-shock response), as well as behavior during extinction learning and retention. Although excitation of the IL-dPAG circuit did not influence an animals' conditioned or unconditioned responses during fear conditioning, it did affect females' behavior during extinction retention: females with the excited circuit freeze less than females without, suggesting that although the circuit might not be involved in the motor aspects of darting, it is involved in the predictive effects seen during extinction retention. Because of the IL's involvement in extinction learning and its apparent role in this circuit during fear conditioning, we measured cFos expression in the IL following fear conditioning. Females had higher cFos expression in the IL than males, and this heightened expression was not dependent on conditioned response type. This suggests that activity in the IL during fear conditioning is potentially a driver of the enhanced extinction retention seen as a result of darting as a conditioned response. Taken together, these results highlight activation in the IL-dPAG pathway as a key component of the enhanced behavioral flexibility resulting from darting and indicate that activation in the IL during fear conditioning contributes to the sex-biased nature of darting.

10

LINKING PAIN AND FEAR: A BEHAVIORAL AND NEURONAL APPROACH ***Emmett Bergeron, Undergraduate, Northeastern University,*** Genevieve Hulshof; Akshara Kannan; MaryClare Pikus; Julia Mitchell, MS; Rebecca Shansky, PhD.

Fear is studied utilizing a classical conditioning approach, in which a neutral, conditioned stimulus (often an auditory tone) is paired with an aversive, unconditioned stimulus (a footshock). Fear is measured quantitatively through conditioned responses such as freezing and darting. The aversive footshock also causes pain, and sensitivity to this pain is currently an unexplored variable in fear research. This study investigated the relationship between pain and conditioned fear response, and if this relationship contributes to any sex differences seen during fear conditioning. To assess pain threshold, rodents were exposed to the hot plate assay. Four days later, rodents underwent auditory cued fear conditioning and received either a 1 mA or 0.3 mA shock, or a tone with no shock. Tissue extractions were then performed 90 minutes after fear conditioning, and stained for cfos+ cells. cFos expression was analyzed in the prefrontal cortex (PFC), periaqueductal gray (PAG), amygdala, and spinal cord. These areas were selected because of their involvement in pain processing and perception, as well as fear response. Hot plate hind paw withdrawal was found to correlate with both unconditioned and conditioned responses, but these effects were dependent on sex and conditioned response type. Sex differences were also observed in correlations between conditioned and unconditioned responses and cFos levels. Overall, these results suggest that sex-driven differences exist both behaviorally and neuronally within the processing of aversive stimuli.

11

NOCICEPTION IN TOBACCO HORNWORM ***Gayathri Kondakat, MS, PhD Candidate,Tufts University,*** Barry Trimmer, PhD.

Defensive behaviors are ubiquitous in animals, often mediated by nociception, the sensory system that responds to noxious stimuli. In the tobacco hornworm (Manduca sexta) caterpillar, this consists of sudden movements in response to strong thermal or mechanical stimuli. Manduca has two distinct defensive behaviors: movement of the anterior body away from the stimulus (‘withdrawal’) or a rapid turn of the head and thorax towards the stimulated site (‘strike’). Using brief pulses of heat applied to the body wall we observed that the likelihood of withdrawal is strongly associated with stimuli towards the head or thorax, whereas strike resulted from stimuli on posterior abdomen segments. Interestingly, stimulation of the anterior abdominal segments can initiate either behavior, even in a single individual, suggesting that the two motor programs are similarly connected to noxious stimuli. Additionally, we are studying the correlation between the behavioral program selection and the role of sensory context and behavioral state. For example, if Manduca is disturbed prior to a stimulus it can “freeze” into a sphinx posture. In this state, Manduca is much less likely to strike or withdraw in response to a thermal stimulus. We are studying the mechanisms that underlay this modulation of nociception and considering how it relates to the survival of caterpillars in natural settings. Nociception is the first step of conserved pain perception pathways across the animal kingdom and Manduca offers a tractable and ethical model system for studying pain processing and its alleviation in animals. We plan to follow up our results with electrophysiology readings from muscles that evoke nocifensive behaviors, as well as in vitro preparations to demonstrate the underlying nociceptive circuit.

12

HIPPOCAMPAL ENGRAMS ALTER BOTH BEHAVIORAL AND BRAIN FEAR STATES **Kaitlyn Dorst, PhD Candidate, Boston University,** Ryan Senne, BA; Anh Diep, Heloise Leblanc, BA; Antje de Boer; Evan Ruesch, BA; Sara Skelton, BA; Olivia McKissick, BS; Steve Ramirez, PhD.

Animals utilize a repertoire of defensive behaviors to avoid predators and other noxious stimuli. Successful implementation of these behaviors depends on both the external environment and the internal state of the animal as the brain makes a series of computations to integrate and use such information. Importantly, cognitive dysfunction can disrupt this integration and generate maladaptive defensive behaviors. Memory systems, in particular, play a highly influential role in mediating defensive strategies based on previous experiences. Yet, how cells that drive memory expression modulate downstream neural systems to properly gate defensive behaviors is unknown. To address this, we use activity-dependent labeling strategies to leverage optical control over populations of cells (i.e., an engram) within the dorsal dentate gyrus (dDG) that encode information of a fearful experience. Here, we optogenetically reactivate a dDG fear engram under varying environmental sizes to test for its capacity to mediate defensive behaviors in a manner contingent on spatial demands placed on the animal. We found an inverse relationship between the size of the environment and the amount of light-induced freezing: mice significantly froze during fear engram reactivation in the smallest arena, but not in the largest arena. This suggests that a dDG engram is not fixed to generate one behavioral output, but can be integrated into brain-wide pathways to elicit adaptive defensive behaviors. Our current work uses network analyses to 1) examine correlations in endogenous cFos across brain regions to identify putative hub regions that mediate these state-dependent alterations in defensive strategies during fear engram reactivation, and 2) delete these regions in silico and in vivo to test for their causal contributions to light-induced memory expression. By identifying and manipulating areas supporting memory, our work seeks to resolve systems-level biological mechanisms mediating memory’s capacity to modulate maladaptive behavioral states.

14

ALTERED DEVELOPMENT OF NEUROENDOCRINE GENE EXPRESSION DURING EARLY LIFE ADVERSITY: RELATIONSHIP WITH ULTRASONIC VOCALIZATIONS AND ACCELERATED FEMALE PUBERTAL TIMING
Lauren Granata, MS, Graduate Student, Northeastern University, Grace Liu; Alekhya Rekapalli; Prachi Thakur; Isabel Roman; Abigail Parakoyi, BA; Heather Brenhouse, PhD.

Early life adversity (ELA) increases the risk of developing anxiety disorders later in life, and accelerated pubertal timing mediates the association between ELA and anxiety in women. Using the maternal separation paradigm in rats, ELA-reared females display a vaginal pinhole earlier than control-reared females and earlier vaginal opening correlated with heighted acoustic startle response. However, the neuroendocrine mechanisms underlying female-specific pubertal acceleration are unknown. Stimulatory and inhibitory hypothalamic genes are involved in generating the gonadotropin-releasing hormone (GnRH) pulse necessary for pubertal onset. Of these, RF-amide related peptide-3 (Rfrp) and Kisspeptin (Kiss1) are sensitive to stress and are regulated by corticotrophin-releasing factor (Crf). The current study characterizes the development of Rfrp, Kiss1, Crf, and GnRH gene expression in the hypothalamus using qPCR in control and ELA-reared males and females on postnatal days (P)10, P15, P20, and P30 in order to elucidate the underpinnings of pubertal development. We also recorded isolation-induced ultrasonic vocalizations, an indicator of distress in pups, and found altered USV development in ELA-reared pups. We correlated these outcomes with gene expression to determine whether USVs are indicative of underlying neurochemistry. We found that in P20 females, Kiss1, Rfrp, and Gnrh are upregulated by ELA. We then performed in situ hybridization in the hypothalamus to determine colocalized expression of Crf receptor 1 (Crfr1) with Kiss1, Rfrp, or Gnrh to identify the cell-specific role of Crf in the upregulation of these genes after ELA. Together, these experiments reveal a neurobiological link between ELA and female pubertal maturation.

15

MICROGLIA DEPLETION ALTERS THE IMPACT OF EARLY LIFE ADVERSITY ON COGNITIVE BEHAVIORS AND PERINEURONAL NET FORMATION ***Michaela Fanikos, Graduate Student, Northeastern University,*** Kelsea Gildawie, PhD; Rebecca Stamato; Alissa Valentine, MS; Abigail Parakoyi BA; Heather Brenhouse PhD.

The dynamic relationship between the nervous and immune systems plays an integral part in the long-term, sex-dependent impacts of adversity. Microglia are the primary neuroimmune cell and are known for their role in the brain's response to stress as well as their tendency to cause neuronal damage when overactivated. Functional and morphological activation states have been found to differ in male and female animals exposed to early adverse events. Microglia alter neurons and their activity through the release of cytokines, elimination of synapses, and phagocytosis of debris. For example, microglia are known to drive degradation and regulation of perineuronal nets (PNNs). PNNs are specialized structures of the extracellular matrix that preferentially enwrap parvalbumin (PV) expressing interneurons. Furthermore, early-life adversity (ELA) reduces adolescent and adult PV expression in the prefrontal cortex (PFC) which may play a role in the adversity-induced deficits in cognition. We have shown that early adverse experiences induce a long-lasting reduction to PV cell count and PV+ PNN structural integrity in females only. To address if microglial activity in the postnatal period mediates the sex-dependent response to adverse experiences early in life, microglia were transiently depleted during two different time points during ELA: Postnatal day (P)2 or P10. Rats underwent ELA in the form of maternal separation (MS) from P2 to P20 and were left undisturbed from weaning on P21 until behavioral testing on P70. Rats were tested in the spontaneous alternation task to assess working memory. Following this behavioral task, the density and intensity of PV neurons and PNNs in the PFC were quantified. Female rats that underwent MS displayed impaired working memory in the spontaneous alternation task, however this effect was prevented in female rats with P2 microglia depletion. Males did not display changes in working memory following MS or with microglia depletion on P2. These results will elucidate the time-dependent role microglia play in modulating the sex-specific impacts of adverse early experiences on cognitive behavior and PNN formation in the PFC.

16

EARLY LIFE ADVERSITY ALTERS DEVELOPMENT OF CORTICOLIMBIC INNERVATION AND ACOUSTIC STARTLE RESPONSE ***Caitlyn Cody, Graduate Student, Northeastern University,*** Janelle Lardizabal, Lauren Granata MS, Abigail Parakoyi BS, Heather Brenhouse PhD.

Threat evaluation and responsivity are crucial aspects of survival for all animals, and in particular prey animals such as the rodent. Typical development of threat evaluation can be disrupted by early life adversity (ELA), due to the rich postnatal development of the involved circuitry. Prior work from our lab has shown hyper-innervation of gluatamatergic basolateral amygdala (BLA) projections in the prefrontal cortex (PFC) of ELA exposed adolescent rodents. This pathway has been implicated in anxiety related startle potentiation, which is blunted in both ELA exposed human and rodent adults. It remains unknown how these ELA-induced connectivity changes alter the typical potentiation of startle under threatening conditions, and whether the BLA-PFC pathway is directly responsible for the altered startle response. In order to test these questions, we exposed rat pups to an ELA model of maternal separation from post natal day (PND) 2-20. Rats then received an inhibitory DREADD injection in the BLA on PND 26 in order to silence the excitatory projections during the adolescent time period of PND 33-39. Finally, rats were tested in an acoustic startle paradigm under threat in early adulthood to assess the effects of reduced BLA-PFC activity on the previously observed ELA-induced blunted startle response. The acoustic startle paradigm is a two day behavioral assay that first assesses baseline startle response to white noise bursts. Following this initial assessment, rats are tested the following day on the same task, but are first exposed to playback of a 22kHz ultrasonic vocalization (USV). This particular USV signals a potentially threatening environment, and results in an increased startle response when compared to baseline levels in control rats. Here we show that ELA results in a blunted response on the second day of testing, and investigate whether inhibition of this activity rescues this behavior. Density and intensity of BLA axonal boutons in the PFC were then assessed to elucidate how BLA inhibition during adolescence affects innervation later in life. These results shed light on how altered early life environments may affect the development of startle related circuitry within the BLA-PFC pathway, and anxiety responses later in life.

17

THE IMPACTS OF EARLY LIFE ADVERSITY ON ULTRASONIC VOCALIZATION- AND LIGHT-ENHANCED ACOUSTIC STARTLE ***Abigail Parakoyi, Lab Manager, Northeastern University,*** Lauren Granata, MS; Heather Brenhouse, PhD.

Early life experiences strongly influence neuronal maturation and affective processing. Early life adversity (ELA) has been shown to increase risk of anxiety, depression, and cognitive impairments in adolescence and adulthood. For example, ELA alters threat responsiveness, which is an expression of anxiety and its regulation by the prefrontal cortex (PFC). In this study, we used the acoustic startle response in the presence of potential threat as a measure of threat responsiveness. We measured threat-potentiated startle reflex responses in either maternally separated (ELA) or control reared (CON) rats on postnatal day 55 (emerging adulthood). Ultrasonic vocalizations (USV), specifically 22 kHz vocalizations, are naturalistic forms of communication among rats that can be used to indicate the presence of a threat stimulus and warn others of said threat. Additionally, rats are nocturnal and easily preyed upon in nature so aversion to light is a natural threat response. On day 1 rats were placed in the acoustic startle chamber and played randomized bursts of white noise over 20 minutes to get baseline startle measures. Then on day 2 rats were either exposed to playback of 22 kHz USVs prior to the startle paradigm or were exposed to light during the startle paradigm. Male rats showed a heightened baseline startle response than their female counterparts overall. On day 2 we found that in the presence of USVs, male but not female CON rats showed a heightened startle response compared to baseline. However, ELA blunted this enhancement, as males showed a similar startle response to baseline. Preliminary findings suggest that in the presence of light CON rats show heightened startles response, whilst ELA rats show blunted startle responses like USV data. Ultimately, this study can aid in our understanding of how ELA impacts threat responsivity as measured by the startle reflex.

18

EARLY LIFE STRESS DRIVES DIVERGENT NETWORK AND BEHAVIORAL STATES IN ADULTHOOD ***Garrett Scarpa, Graduate Student, Tufts University,*** Grant Weiss, PhD; Jamie Maguire, PhD.

Adverse childhood experiences (ACEs) are major risk factors for psychiatric illnesses, including anxiety and depression; however the precise neural mechanisms mediating this vulnerability remain unclear. We propose that ACEs disrupt the conventional development of the amygdala and prefrontal cortex, two reciprocally-connected brain regions that are involved in stress reactivity and valence processing. Network communication within and between the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA) has been shown to govern affective behavioral states. Research from our laboratory has established a critical role for parvalbumin (PV) interneurons in orchestrating BLA network and behavioral states. The overarching objective of this project is to investigate the impact of early life stress (ELS) on mPFC-BLA network states and determine whether deficits in PV interneuron function may lead to different behavioral outcomes following ELS. Here, we present the first evidence to our knowledge that ELS drives divergent mesoscale network state dynamics, especially with regard to state transitions associated with stress. Our results demonstrate that exposure to a predator odor triggers attenuation of theta and potentiation of gamma oscillations in the basolateral amygdala (BLA) of adult C57BL/6J mice, however the network returns to baseline conditions across both frequencies more rapidly in mice subjected to ELS. Furthermore, these divergent network responses appear to be driven by atypical development and function of fast-spiking interneurons which express parvalbumin (PV). These findings suggest a novel mechanism by which ACEs may increase risk for neuropsychiatric disorders.

19

RAT INFANT ULTRASONIC VOCALIZATION PATTERNS IN AN EARLY LIFE ADVERSITY PARADIGM

Alekhya Rekapalli, Undergraduate, Northeastern University, Isabel Roman; Prachi Thakur; Lauren Granata, MS; Heather Brenhouse, PhD.

Early life adversity is correlated with hindered development of social abilities and an increased risk of psychiatric disorders. Maternal separation (MS) is a widely used rodent model of early life adversity. When pups are separated from their mothers, they produce ultrasonic vocalizations (USVs), which have specific acoustic properties making it possible for their mothers to locate them. This study aims to determine how different characteristics of these vocalizations change as pups develop, and to observe how MS affects USV responses. Standard-reared (Con) and MS-reared pups at p (postnatal day) 10, p15, and p20 were isolated in cages, during which an ultrasonic microphone recorded their calls for 5 minutes. Characteristics of USVs that were studied consisted of frequency, call length, power, bandwidth, and call type sequence entropy to determine developmental shifts. We have shown that in control litters, there is a rise in calls at p15 and a drop in calls at p20, but in MS litters the number of calls stays constant throughout development. Additionally, from p10 to p15, a rise in frequency of the calls was observed for pups in both conditions. However, MS litters displayed higher frequency calls than those of control litters. The data also shows that the entropy of MS USVs at p10 was higher than Con, indicating that their call-type sequences were more random. Our results demonstrate that pups undergoing MS have different USV patterns than Con pups. This is significant because these call patterns could be indicators of social behavior in later life.

20

INVOLVEMENT OF FRONTOPARIETAL NODES IN ONLINE UPDATING OF REACH-TO-GRASP ACTIONS TO PERTURBATIONS OF ARM TRANSPORT

Mariusz Furmanek, PhD, Faculty, University of Rhode Island, Luis F. Schettino, PhD; Madhur Mangalam, PhD; Kyle Lockwood, BA; Sergei V. Adamovich, PhD; Mathew Yarossi, PhD; and Eugene Tunik, PhD.

Humans adeptly adjust their movements to perturbations via integration of multisensory feedback. Contemporary theories of cortical organization of reach-to-grasp actions postulate that hand transport (the reach) and prehension (the grasp) are spatiotemporally coordinated by parallel, dynamic activation of relatively independent frontoparietal pathways. The putative dorsomedial (DM) ‘reach’ pathway consists of the dorsal premotor cortex (PMd) and the superior parietal-occipital cortex (SPOC), while the dorsolateral (DL) ‘grasp’ pathway consists of the ventral premotor cortex (PMv) and the anterior intraparietal sulcus (aIPS). Recent evidence indicates that these assigned roles of the DM and DL pathways may be more interwoven than previously thought. However, this evidence is based primarily on visual perturbations of task goals. The present study tested the differential involvement of frontoparietal nodes (PMv, PMd, aIPS, and SPOC) in online adjustments of reach-to-grasp coordination to mechanical perturbations that disrupted hand transport. Ten healthy right-handed participants (24 ±7.3 years old), after providing informed consent, performed reached-to-grasp movements in a virtual environment towards a rectangular virtual object (dimensions 5.4 × 2.5 × 8.0 cm), located 30 cm from the starting hand position. Mechanical perturbations were applied using a manipulandum attached to the wrist. On a minority of the trials (25%), the manipulandum exerted a 5N continuous force resisting motion along the axis of the reach. Perturbations were applied either 100 ms or 300 ms after movement onset, requiring participants to make online compensatory responses in the transport and grasp. Transcranial magnetic stimulation (TMS) was used to perturb processing, individually, in each of the four nodes (50% of trials) time-locked with the perturbation. Correction magnitude and latency were selected as outcome measures. TMS to PMd and SPOC in the dorsomedial pathway resulted in a reduction of the correction magnitude for late perturbations (3.5 cm/s, $p < 0.05$, 3.4 cm/s, $p < 0.005$, respectively). Conversely, TMS to aIPS decreased correction magnitude to early perturbations (4.1 cm/s, $p < 0.05$). Our results provide evidence that dominance of the DM pathway for processing proprioceptive inputs associated with mechanical perturbations of reach is movement phase dependent. These findings support other recent evidence that strict assignment of the dorsomedial pathway to reach and the dorsolateral pathway to grasp is likely inaccurate. Future work should consider temporal aspects of DM-DL pathways in the coordination of corrective adjustment of reach-to-grasp actions.

21

UNDERSTANDING THE INTERACTION OF CONFLICT-MINIMIZING AND GOAL-SEEKING MOTOR IMPERATIVES IN AUTISM SPECTRUM DISORDER ***Sundararaman Rengaraja, Graduate Student, Northeastern University,*** Jonathan Cannon, PhD; Brendan Baron; Naren Mohan, BE; Leanne Chukoskie, PhD.

According to a classical control-theoretic perspective on self-movement, our brains pilot our bodies to achieve goals, sometimes using open-loop predictive models and sometimes with closed-loop feedback. From this perspective, synchronization of self-motion with a moving target is a simple control problem, where error between body and target is minimized by some combination of responsive and predictive control. However, not all self-movement can be described in terms of goals. A second, more subtle, driver of movement is the reconciliation of multisensory prediction errors. When subjected to a visual/proprioceptive conflict, our bodies respond spontaneously and unconsciously to reduce or resolve it. These two drivers of movement are unified within the theoretical framework of active inference. To study this dynamic interaction of goal-oriented and conflict resolving motor imperatives, we designed a virtual reality task where participants synchronize a cyclic opening and closing hand movement with an opening and closing target hand. We introduce two perturbations: a transient delay of the target hand, and a transient delay of the image of their own hand. We model responses to these perturbations using an active inference model in which the velocities and positions of the oscillating target and the participant's hand are continuously estimated based on two visual streams of information (target hand and own hand images), one proprioceptive stream, and an internal model of the dynamics of the system and the noisiness of each stream. Preliminary results in non-autistic individuals indicate that delays in visual self-motion cause participants to delay their own motion, demonstrating the multisensory-conflict-reducing behavior. In this context, we aim to study interindividual differences in active inference processes. Certain sensorimotor differences reported in autism, including differences in the weighting of proprioceptive relative to visual input and weaker error correction during entrained finger tapping, could follow from reduced sensitivity to sensory/proprioceptive prediction errors in the context of active inference. We are currently recruiting autistic individuals for this task. Our model suggests that reduced sensitivity to sensory/proprioceptive prediction errors should make autistic individuals less responsive to both types of perturbation compared to non-autistic peers (after controlling for visual attention). This result would establish a link among several sensorimotor differences in autism that can be explained in terms of active inference and would help to identify targets for future interventions.

22

PATHWAYS LINKING THE AMYGDALA, HIPPOCAMPUS, AND SUBGENUAL AREA 25 IN THE PRIMATE NUCLEUS REUNIENS. ***Laura Marshall, Graduate Student, Boston University,*** Mary-Kate Joyce, PhD; Shimrani Banik, BA; Jingyi Wang, PhD; Jess Holz, MFA; Danqing Xiao, PhD; Jamie Bunce, PhD; Helen Barbas, PhD.

The primate thalamic Reuniens Nucleus (RE) is the most ventral of the limbic anterior midline nuclei, situated directly above the third ventricle. The RE is distinguished among thalamic nuclei by its bidirectional connections with the hippocampus and medial prefrontal cortex (mPFC). In rats, detailed investigation has demonstrated RE's role in several cognitive functions that require hippocampus-mPFC activity coordination, including spatial working memory, memory consolidation, and strategy shifting. However, in the primate, several foundational questions remain unanswered: What is the chemoarchitecture of RE? Are other limbic structures, such as the amygdala, also connected with RE? To address the chemoarchitecture of the RE, we used immunohistochemistry to label GABA, a marker of inhibitory neurons, and for the calcium-biding proteins parvalbumin (PV), calbindin (CB), and calretinin (CR), all of which label excitatory projection neurons in the thalamus. PV neurons are associated with a core thalamocortical connectional system that terminates in middle layers of cortex. CB and CR are associated with a matrix profile of thalamocortical connections, with terminations in the upper layers 1-3a and layer 6. We found that the RE is primarily populated by CB and CR neurons with sparse PV neurons and processes, classifying it as a matrix-dominant thalamic nucleus. Injections of neural tracers in subgenual cingulate cortical area 25, amygdala, and hippocampus showed robust incoming and outgoing connections with the RE in all three pathways. The hippocampal and posterior area 25 pathways were densest in anterior RE, while the amygdala had a consistent density of connections throughout the anterior to posterior axis of RE. We also used high resolution methods to examine ultrastructural characteristics of incoming connections from the three pathways that form synapses in RE. We observed multiple instances of amygdala axonal boutons forming successive synapses with the same postsynaptic structure. We also observed some hippocampal terminations forming multisynaptic complexes that are comparable to synaptic triads formed by amygdala terminations in the thalamic Mediodorsal Nucleus. In summary, the primate RE shows a specialization by its strong connection with the amygdala. The connections of RE with the hippocampus, area 25, and amygdala positions it to coordinate activity among these regions and is likely an integral node in limbic memory and emotional networks.

23

AN INVESTIGATION OF DIVERSITY IN CHILDHOOD AND ADOLESCENT ANTIDEPRESSANT STUDIES

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Childhood mental illness (e.g., anxiety and depression) is an ongoing public health crisis that has resulted in a notable increase in pharmaceutical interventions, such as the use of antidepressants (e.g., serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) in children. Relative to studies conducted with adult populations, the empirical evidence for the use of antidepressants with child populations is limited. Further, there is also recent evidence highlighting the cultural differences in the utilization and response efficacy of antidepressants in children. The purpose of the present study was to examine the demographic composition of samples used in antidepressant efficacy and tolerability studies with children and adolescents in the last 10 years. A systematic literature review utilizing PubMed and PsycINFO was conducted (March 27, 2021) in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement following predefined criteria: (1) The study examined the efficacy or tolerability of at least one antidepressant (SSRI) among anxiety and depression in both children and adolescent samples and included both children (ages <12) and adolescent (ages 12-18) populations; (2) The study was originally published in English; (3) The study was conducted in the United States; (4) The study used an original or secondary dataset; and (5) The study was published in a peer reviewed journal between 2011 and 2021. Findings are indicative of an overall lack of diversity in studies examining the efficacy and tolerability of antidepressants in youths. Specifically, the lack of diversity was notable among samples in age (e.g., samples were primarily adolescents), race (e.g., were primarily white) and gender (gender was reported only in binary terms). This study fills a gap in the literature by demonstrating an overall lack of diversity in studies that examine the efficacy and tolerability of antidepressants in children and adolescents and underscores the importance of future studies utilizing a more diverse and representative sample. Possible explanations for the lack of inclusion and suggestions on how to better address these disparities are discussed. Limitations included limited generalizability and a single reviewer process. Results indicate a need for more diverse, representative samples when evaluating antidepressant efficacy and tolerability in pediatric samples. Possible explanations for the lack of inclusion and suggestions on how to better address these disparities are discussed.

24

CHARACTERIZING FACIAL DEFORMATION FOR ASSESSING AFFECTIVE COMMUNICATION AND PSYCHONEUROIMMUNOLOGICAL FACTORS IN SPACEFLIGHT ***Daniel Peters, MA Student, MGH,*** Maxwell Schenkel, BA; Bryan White, MA; Jona Cumani, BA; Stijn Thoolen, MD; Quan Zhang, PhD; Guillaume Spielmann, PhD; Gary Strangman, PhD; Vladimir Ivkovic, PhD.

INTRODUCTION: Visible and persistent signs of microgravity-induced cephalad fluid shift are facial edema and deformation. These have the potential to reduce facial expressiveness and thereby capacity to convey non-verbal affective cues. Since facial expression of affective content is a fundamental aspect of social interaction, any changes or limitations in this capacity during spaceflight could exacerbate the negative communication or psychological effects of isolation and confinement, contributing to the risk of behavioral or psychiatric disorders. In addition, sustained exposure to psychological stressors, confinement, and isolation are known to reduce immune competency, promote systemic inflammation, and even reduce physical and psychological functions including facial expressiveness. It is unknown how these factors interact or affect the interpretation of facial expressions. METHODS: Aim 1: Quantify the magnitude and spatial distribution of facial deformation associated with various degrees of head down-tilt (HDT) spaceflight analog. We will generate a database of high-definition (HD) front-facing facial photographs and facial volume heat-maps from n=50 healthy, astronaut-like individuals at the following tilts: +90º, 50º HUT, 0º(supine), -10º HDT, 10º HUT and return to Supine. Aim 2: Investigate the relationship between facial deformation and perceived emotional valence from face photographs at varying degrees of HDT. Aim 3: Investigate the relationship between perceived emotional valence and immune function before and after exposure to a realistic mission stressor of 45-day simulated space missions in Human Exploration Research Analog (HERA). RESULTS: We have developed a novel method to precisely quantify facial edema through the use of 3-dimensional scanning technology, and software. Pilot results with N=4 participants show expected changes under HDT. Test data show a persistent overall increase in facial volume with increase in HDT angle, but not a return to supine baseline. By taking a new scan every 4 minutes (3 per position) we are able to establish preliminary evidence for the trajectory of facial edema relative to time, with pilot data suggesting that the majority of volume shift occurs early relative to each position change. DISCUSSION: Findings from this study will directly contribute to identification of key threats to behavioral health, development of measures for monitoring behavioral health, and understanding interactions between immune, psychological and perceptual factors in behavioral health associated with long duration missions. The currently accomplished work has generated the necessary procedural pipeline for collection and analyses of facial edema data in varying degrees of HUT/HDT. Whereas other techniques used in the past to measure facial edema have largely had to infer total and specific volume change through the use of volume analogs like forehead skin thickness, our approach allows us to measure volume change natively, and in both its regional and global totality. * Supported by NASA grant 80NSSC19K0925.

25

SINGLE-NEURONAL REPRESENTATION OF SEMANTIC SPACE IN ANIMALS AND HUMANS ***Ethan Wong, Undergraduate, Northeastern University,*** Irene Caprara, PhD; Mohsen Jamali, MD PhD; Martina Mustroph, MD PhD; Benjamin Grannan, MD; Arjun R. Khanna, MD; Raymundo Báez-Mendoza, PhD; Ziv M. Williams, MD.

How is semantic information represented across animal species? While most approaches to the evolutionary variability of species highlight neuroanatomical differences, similarities in neuronal encoding are starting to emerge. Whether distinct species such as non-human primates and humans share a common semantic manifold or categorical representations of their environment, however, remains largely unknown. Here, we focused on the primate prefrontal cortex’s capacity to organize everyday experience into different categories to approach this question. We obtained single-neuronal recordings in the prefrontal cortex of Rhesus macaques and human participants while they were given different stimuli in visual or audio format and which could be vectorially categorically classified. Using a combination of single-neuronal and population analyses, we find that many neurons in both humans and monkeys displayed categorical selectivity and that their population activities could be used to decode distinct semantic domains in both species. We also observed however differences in the precise representations of these domains across species and how they were functionally organized. Finally, to verify category-based behavior, we examined the viewing preferences of the primates to different categories in a free viewing choice task, and confirmed that the animals looked first and for longer at images of specific categories than others, e.g., abstract vs. familiar objects. Together, our findings reveal a remarkably detailed cellular representation of semantic information in the prefrontal cortex of monkeys and humans and begin to illuminate the common semantic manifold that is shared across animal species.

26

INHIBITORY CONTROL CONTRIBUTES TO SUCCESSFUL MEMORY-GUIDED PLANNING DURING EARLY CHILDHOOD ***Que Anh Pham, Graduate Student, University of Massachusetts Boston,*** and Tashauna Blankenship, PhD.

Memory-guided planning (MGP) involves sampling from past experiences to generate appropriate plans to achieve a goal or series of goals (Blankenship & Kibbe, 2019, 2022). Critically, when sampling from past experiences one must select the appropriate memory or memories, often when there is other conflicting information, to accurately plan for the goal at hand. Selection of the appropriate memory theoretically relies on inhibitory control (IC), or the ability to suppress conflict. The relation between MGP and IC across early childhood is not well understood. To address this gap in the literature, we examined the relation between IC and MGP in a sample of 2-4-year-old children. 48 children [10 2-year-olds ($M=32.51$, $SD=2.50$, 4 female), 24 3-year-olds ($M=40.67$, $SD=3.40$, 16 female), and 14 4-year-olds ($M=55.09$, $SD=13.20$, 2 female)] completed two IC tasks and one MGP task. The IC tasks included a Day/Night (DN) and Reverse Categorization (RC) task. The DN task consisted of showing children a picture of a sun or moon and having them respond differently based on the condition (congruent, incongruent). In the congruent condition, children responded sun (3-year-olds) or day (4-year-olds) when shown a sun, and moon (3-year-olds) or night (4-year-olds) when shown a moon. In the incongruent condition, children responded moon (3-year-olds) or night (4-year-olds) when shown a sun, and sun (3-year-olds) or day (4-year-olds) when shown a moon. During the RC task children sorted big and little ducks into certain buckets depending on the condition (congruent, incongruent). In the congruent condition, children put the little duck in the little bucket and the big duck in the big bucket. In the incongruent condition, children put the little duck in the big bucket and the big duck into the little bucket. Finally, in the MGP task, children learned that two objects resulted in a specific outcome (blue or green candy dispensing from a machine). During test, children were asked to choose which object to use to retrieve the correct color of candy for an animal character (4 test trials). Critically, the MGP task required children to use memories (object/candy) to complete simple goals (candy for animal). Performance on the MGP task was significantly above chance for 3-year-olds ($M=.78$, $SD=.28$, $t=4.40$, $p<0.001$) and 4-year-olds ($M=.88$, $SD=.19$, $t=7.39$, $p<0.001$), but not for 2-year-olds ($M=.69$, $SD=.35$, $t=1.67$, $p=.13$). A series of ANOVAs showed significant differences between age groups on the DN [$F(2, 45)=11.41$, $p<0.001$] and the RC [$F(2, 45)=7.28$, $p=.002$] tasks, but no significant differences between groups on the MGP task [$F(2, 40)=1.28$, $p=.29$)]. However, age was positively correlated with performance on all tasks, including the MGP task [$r(42)=.31$, $p=.045$]. Finally, a stepwise regression analysis found that together the IC tasks predicted 12% of the variance in MGP performance above and beyond age. Together, these results suggest that, regardless of age, IC plays an important role in MGP during early childhood, perhaps allowing for resolution of conflict during memory retrieval. These data also help to validate online MGP and IC assessments in preschool aged children.

27

MULTIMODAL IMAGING OF ACETYLCHOLINE RELEASE AND HEMODYNAMICS IN THE AWAKE MOUSE BRAIN ***Patrick Doran, Graduate Student, Boston University,*** Martin Thunemann, PhD; Kivilcim KıLıÇ, MD, PhD; Buyin Fu, MD; Natalie Fomin-Thunemann, PhD; Joel Herbert, BA; Anderson Chen, PhD; Jason

Stockmann, PhD; Yuanyuan Jiang, PhD; Bei Zhang, BS; Christian Farrar, PhD; Yulong Li, PhD; Bruce Rosen, MD, PhD; Xin Yu, PhD; Joseph Mandeville, PhD; Sava Sakadzić, PhD; Anna Devor, PhD.

Cholinergic neurons in the basal forebrain project across the cerebral cortex releasing acetylcholine (ACh). ACh is known to modulate "brain states" characterized by different efficiency of information processing. ACh is also a known vasodilator and thus is expected to modulate not only activity of neuronal circuits but also hemodynamics reflected by blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI). The cholinergic system is among the earliest neuronal circuits affected in neurodegeneration including Alzheimer’s disease and other dementias. Therefore, the ability to infer cholinergic activity from BOLD-fMRI in humans would be useful for basic science and clinical applications alike. In mice, ACh release can be measured directly using genetically encoded optical biosensors. In this study, we combined optical imaging of ACh with BOLD-fMRI to study the relationship between cholinergic activity and BOLD-fMRI signals. We expressed genetically encoded red GRABACh sensor (rACh1.4) in the left barrel cortex was induced through intracortical injections of an adeno associated virus. Mice were implanted with large cranial windows to allow optical access to the left hemisphere of the dorsal cortex and PEEK headbars for head fixation within a 9.4T MRI scanner. Mice underwent an extensive training regimen to acclimate them to both head fixation and the sound of the MRI scanner. We engineered an MRI-compatible optical imager to perform fluorescent imaging of ACh inside the scanner. We observed both a BOLD response and ACh release following an airpuff stimulus to the whisker pad. However, the BOLD response preceded ACh release inconsistent with the hypothesis of ACh driving the BOLD signal. Furthermore, we observed intermittent departures between the BOLD signal fluctuations and ACh dynamics during spontaneous ("resting-state") activity. These results suggest that cholinergic control of the resting-state BOLD-fMRI dynamics may vary over time dependent on the local neuronal activity, presence of other neuromodulators, and behavioral context.

28

AUTOMATED FLUORESCENCE IMAGE ANALYSIS TO INVESTIGATE THE ALTERED DEVELOPMENT OF THE GANGLIONIC EMINENCE ALONG SPATIAL AND CELL TYPE DIMENSIONS IN THE ENGRAILED 2-KNOCKOUT MOUSE ***Johan S. Martinez-Fuentes, Graduate Student, Boston University,*** Meaghan Cogswell, PhD; Adrian c. Castelló, PhD; Victor Borrell, PhD; Shelley J. Russek, PhD.

A variety of genetic and environmental factors contribute to autism spectrum disorder (ASD), but exactly how this occurs remains unclear. One hypothesis is that factors may converge to perturb the development and function of the gamma-aminobutyric acid (GABA) system of the brain. The midbrain-hindbrain patterning homeotic transcription factor engrailed 2 (En2) has been associated with ASD in humans, and a mouse model harboring a null mutation of En2 recapitulates behavioral and neuroanatomical abnormalities reminiscent of ASD as well as epilepsy. Interestingly, En2-null mice show reduced GABAergic marker expression in the hippocampus and cortex. How En2 dysfunction leads to neuropathological phenotypes remains unknown. Our recent in vivo work draws attention to the potential role of En2 in radial glial self-renewal and subsequent differentiation, implicating the birth of GABAergic interneurons and spiny projection neurons. To aid investigation into the role of En2 in the development of forebrain GABAergic neurons, we are assessing the cell and molecular alterations that may occur at the ganglionic eminence (GE, age E13), the major birthplace of inhibitory neurons, in En2-null mice compared to wild-type (WT) controls. We hypothesize that, in line with our in vivo work, loss of EN2 function leads to a decrease in the apical radial glia population but a relative increase in the proportion of postmitotic neurons, at least at the lateral GE region. Here, we use sensitive multiplexed fluorescence in situ hybridization with high-resolution fluorescence microscopy to track spatial distributions of WT En2 transcript, and to track transcriptionally defined subtypes of progenitor cells based on overlapping expression of Gsx2 and Ascl1 at GE regions (i.e., medial, lateral, caudal GE) across WT and En2-null conditions. To this end, we apply MATLAB automated image analysis to quantify transcript signal in thousands of individual cells along spatial and cell type dimensions. Data show that En2 is regionally widespread cross the LGE (~70% cells express at least one En2 transcript), but sparse in its density (~96% of En2+ cells express <5 transcripts). Interestingly, ongoing results at the LGE suggest enrichment of high-expressing En2+ cells in the ventral subregion, potentially indicating vulnerability of cells from this area to progenitor dysfunction in the En2-null condition. Taken together, widespread but sparse presence of En2 suggests this factor may play a role in cells arising from the GE, raising questions as to how En2 dysfunction along GABAergic maturation trajectories may contribute to etiology of ASD and other developmental disorders of brain function.

29

HIGH OXYGEN METABOLISM IN LAYER I REVEALED WITH TWO-PHOTON PHOSPHORESCENCE LIFETIME MICROSCOPY IN AWAKE MICE UNDER REST AND STIMULATION ***Natalie Fomin-Thunemann, Graduate Student, Boston University,*** P. MÄCHLER (Phd, MD); M. THUNEMANN (Phd); M. J. SAETRA (Phd); M. DESJARDINS (Phd); K. KILIÇ (Phd, MD); T. VAYISOGLU (Phd, MD); P. DORAN (BS); J. JIANG (BS); E.MARTIN (BS); I. SENCAN (Phd); B. LI (Phd); P. SAISAN (Phd); Q. CHENG (MD); K. L. WELDY (BS); D. A. BOAS (Phd); R. B. BUXTON (Phd); G. T. EINEVOLL (Phd); A. M. DALE (Phd); S. Sakadžić (Phd); A. DEVOR (Phd).

Quantifying oxygen availability and metabolism in cortical tissue offers a crucial physiological parameter in health and disease. According to a common notion, cortical layer IV has a higher oxygen consumption than other layers due to higher mitochondrial density. We tested this hypothesis using direct measurements of the partialpressure of O2 (pO2) around diving arterioles across cortical layers obtained with 2-photon phosphorescence lifetime microscopy (2PLM) in awake mice. We estimated CMRO2 from pO2 gradients around diving arterioles using a modified Krogh model of O2 diffusion from a cylinder. Our results show that, contrary to the commonnotion, the baseline CMRO2 in layer I was higher than that in other layers. In addition, we observed the same effect under stimulation. Stimulus-induced CMRO2 increase outlasted the stimulus duration, likely indicating higher metabolic demands of an active brain state. We hypothesize that the higher CMRO2 in layer I could beexplained, at least in part, by ascending inputs targeting apical pyramidal dendrites. In our study, the pO2 probe Oxyphor 2P was microinjected into the cortical tissue1-2 hours before the measurements. To control for potential adverse effects of this procedure on cortical physiology, experiments are underway using alternative methods of the probe delivery. Our results question the use of mitochondrial density to quantify oxidative metabolism. Understanding the laminar profile of CMRO2 is important for interpretation of high resolution Blood Oxygen Level Dependent (BOLD) functional Magnetic Resonance Imaging (fMRI) studies.

30

FAST, VOLUMETRIC 2-PHOTON IMAGING OF PIAL ARTERIOLAR DYNAMICS AND SURROUNDING NEURONAL ACTIVITY ***Kıvılcım Kılıç, MD, PhD, Staff***, John T. Giblin, BS; Anderson I. Chen, PhD; John X. Jiang, BS; Seong-Wook Park, PhD; Martin Thunemann, PhD; David Boas, PhD; and Anna Devor, PhD.

Pial cortical arterioles integrate local neuronal activity with the intrinsic arteriolar vasomotion as well as subcortical and ascending inputs. Previously, we hypothesized that this integration could produce dynamic patterns of coherent oscillations in the arteriolar diameter effectively parcellating the cortical mantle potentially offering a biophysical explanation for 'functional connectivity networks' observed with fMRI (1). In mice, 2-photon microscopy with an axially extended Bessel focus is perfectly suited for addressing this hypothesis providing large-scale, simultaneous measurements of the arteriolar diameter (2) and neuronal activity. Combined with high-speed galvanometer scanners, this technology allows continuous monitoring of dilation along hundreds of microns of arteriolar length simultaneous with measurements of surrounding neuronal activity. We used awake GCaMP6s transgenic mice with 'crystal skull' optical windows (3). Vasculature was labeled Texas Red dextran. Data were acquired by scanning a Bessel focus with 1.0 NA and 100-μm axial FWHM. ~350x100x100 μm3 volumes with ~1.35 μm resolution in xy were acquired at ~10Hz. With Bessel 2-photon imaging, the fluorescence signal arising from the pial vascular lumen is proportional to the vessel diameter 2. GCaMP transients reflecting brief epoch of increased neuronal activity preceded arteriolar dilation events. With our temporal resolution of ~100 ms, the GCaMP signal increased simultaneously across the imaged field of view. Despite this synchronous onset of neuronal activity, some locations along the arteriole were leading some other locations by up to ~200 ms. In addition, we observed slow fluctuations in the arteriolar diameter (< 1Hz) that were independent from the GCaMP signal. Experiments are underway to determine the underlying mechanisms for this vasodilation dynamics. 1 Machler, P. et al. Curr Opin Biomed Eng 18 (2021). 2 Fan, J. L. et al. Nat Commun 11, 6020 (2020). 3 Kim, T. H. et al. Cell Rep 17, 3385-3394 (2016).

31

UNDERSTANDING THE BIOPHYSICS OF DEEP BRAIN STIMULATION ***Sukhneet Bhogal, Undergraduate, Boston University,*** Cara Ravasio, Krishnakanth Kondabolu, PhD, Sam Zhou, Rebecca Mount, Sukhneet Bhogal, Xue Han, PhD.

Deep brain stimulation (DBS) is a surgically invasive therapy that delivers electrical impulses to specific regions of the brain. Electrodes are implanted in the region of interest and send electrical impulses through a neurostimulator (1). Previous studies have shown remarkable neuroprotective effects in patients with a variety of neurological conditions such as Parkinson's disease, epilepsy, dystonia, Alzheimer's disease, and more (2). Although DBS has improved quality of life for many patients, its therapeutic mechanisms remain widely unknown because electrical interference from the stimulator makes it difficult to acquire cellular level electrophysiological recordings. However, new cutting-edge single cell imaging techniques (3) have made it possible to measure the real time effects of DBS and illuminate its underlying therapeutic biological mechanisms. For this project, we are using this single cell imaging technology in working mouse models to perform experiments that investigate the sustained effects of electrical stimulation and how neurons in different brain regions respond to this stimulation. Specifically, we are performing chronic electrical stimulation in the hippocampal and motor cortical regions of the mouse's brain at different frequencies and use calcium imaging to monitor the underlying neurological activity.

32

POSITRON EMISSION TOMOGRAPHY (PET) BRAIN IMAGING WITH THE [11C]PBR28 RADIOLIGAND TO STUDY NEUROIMMUNE ACTIVATION IN 'LONG-COVID' OR PASC (POST-ACUTE SEQUELAE OF COVID-19) ***Hannah Bues, Clinical Research Coordinator, MGH,*** Minhae Kim, BA; Zeynab Alshelh, PhD; Deena Saadi, BA; Ludovica Brusaferri, PhD; Darin Dougherty, MD; Marco Loggia, PhD; Michael VanElzakker, PhD.

INTRO It has been known for decades that, following acute illness, viral and other infections can have long term consequences, described with labels such as chronic fatigue syndrome/myalgic encephalomyelitis (ME/CFS), post-SARS, post-Ebola, or post-Zika syndromes. For scientists studying this phenomenon, it is not surprising that the novel SARS-CoV-2 virus, driving the ongoing COVID-19 pandemic, is also driving a similar post-acute syndrome, referred to as "Long-COVID" or PASC (post-acute sequelae of COVID-19). Like other iterations of infection-associated chronic illness, PASC involves a constellation of chronic symptoms including debilitating fatigue, cognitive problems, post-exertional malaise, widespread pain, orthostatic intolerance and autonomic dysfunction. According to the CDC, approximately 20% of patients infected with the SARS-CoV-2 virus, or approximately 7% of total US-American adults, are currently still having symptoms of PASC. While it is unlikely that a single cause drives all PASC manifestations (Proal & VanElzakker 2021), we hypothesize that the unique properties of neuroglia, the broad class of innate immune cells within the central nervous system, may contribute especially to subjective symptoms of PASC such as fatigue and difficulty concentrating. Many PASC symptoms overlap with the subjective and behavioral aspect of the innate immune system's "sickness response." We have designed a functional neuroimaging study to test the hypothesis that individuals with PASC show relatively increased ongoing neuroglial activation versus matched controls. METHODS We recruited individuals with WHO-defined PASC (i.e., ongoing symptoms more than 6 weeks following acute COVID-19 onset), using recruitment materials selecting for fatigue and cognitive problems. Participants were scanned with a dual PET-MRI (positron emission tomography - magnetic resonance imaging) neuroimaging device, capable of acquiring both PET and MRI in the same session at the same time. We used the PET radioligand [11C]PBR28, which binds to the translocator protein (TSPO), an outer mitochondrial membrane protein upregulated in innate immune cells during activated states. Our group has previously used these methods to study conditions such as amyotrophic lateral sclerosis (ALS), fibromyalgia, Gulf War Illness, and arthritis pain. Our brain-dedicated PET-MRI scanner is located in Building 149 of the Charlestown Navy Yard campus of Massachusetts General Hospital, and is a high-resolution 3-Tesla (3T) 32-channel whole-body MRI fitted with a high-resolution BrainPET head camera insert. Dual PET-MRI technology allows for complementary hybrid imaging whereby the strengths of each modality can be exploited in the same participant in one imaging session. PET has biological sensitivity unique to in-vivo imaging, and MRI at 3T field strength can achieve a spatial resolution of 1mm^3. MRI-quality spatial resolution allows us to, at an individual level, make reasonable predictions about the location of relevant functional nuclei. With that spatial information, one can superimpose PET images onto high-resolution structural MRI from the same session to identify precisely where [11C]PBR28 uptake is occurring. RESULTS We present here preliminary results from an ongoing study. In a case-control between-groups analysis, data from individuals with PASC were compared to normative data using existing scans from age, sex, and TSPO genotype matched healthy controls, scanned by the same acquisition parameters. Standardized uptake values normalized by occipital cortex signal (SUVR) and distribution volume (VT) were computed from the [11C]PBR28 data. We conducted both whole-brain voxelwise and a priori region of interest (ROI) based analyses, with ROIs based in the “sickness response” animal and human neuroimmunology literature. Our primary ROI in the dorsal anterior cingulate cortex (dACC) or anterior midcingulate cortex (aMCC), a central hub in the dorsal cognitive control network that has been shown by meta-analysis to be uniquely activated by peripheral inflammatory challenges. Secondary ROIs include the caudate nucleus within the striatum of the basal ganglia, and the dorsal brainstem, both of which have also been shown by meta-analysis to be activated by peripheral inflammation. Caudate is particularly associated with subjective fatigue, and dorsal brainstem is the site of the nucleus of the solitary tract (NTS), the location of the majority of cell bodies from afferent vagus nerve cytokine-detecting chemoreceptor terminals. CONCLUSION The current data are part of an ongoing PASC neuroimaging study intended to elucidate the mechanisms driving PASC symptoms. Other data collected include: -Performance on a cognitive control task during functional MRI (fMRI) acquisition -Magnetic resonance spectroscopy (MRS) of the three a priori ROIs via single-voxel, slice, and whole-brain acquisition sequences -Objective sleep quality index (SQI) measured during the two pre-scan nights via take-home SleepImage wearable device -Self-reported symptom and health-related measures -Metagenomic assays on peripheral blood -Measurement of amyloid/fibrin “microclots” in peripheral blood -Oral microbiome and inflammatory proteins measured in saliva samples -Spontaneous neutrophil motility assay performed on “live” fresh blood.

33

OBSERVATIONAL STRESS IMPAIRS ENDOGENOUS NEUROSTEROID SIGNALING IN THE BLA ***Aiden Evans-Strong, Research Technician, Tufts University,*** Najah Walton, BS; Abby Roper, BS, MA; Tiffany Donaldson, PhD; Jamie Maguire, PhD.

Behavioral and physiological responses to stress can be socially transmitted, which is thought to be an adaptive way to communicate a threat without direct exposure. However, witnessing a traumatic event has been shown to lead to the development of post-traumatic stress disorder (PTSD). Previous research has implicated deficits in 5α-reduced neurosteroids in the development of PTSD. To better understand the role that 5α-reduced neurosteroids play in the development of PTSD associated with witnessing a traumatic event, we used an observational fear model in which an observer mouse witnesses a demonstrator mouse experience a series of footshocks. Results from these studies demonstrate increased freezing in observer mice compared to naive controls upon contextual recall. This suggested to us that neural circuits involved in fear learning, such as the basolateral amygdala (BLA), may be involved in observational fear. We quantified transcript levels of 5α-reductase type 1 and 2 (Srd5a1&2), the rate-limiting enzymes involved in neurosteroidogenesis, and the GABAA receptor δ subunit (Gabrd), the primary site of action for 5a-reduced neurosteroids, and demonstrated that expression of Srd5a2 and Gabrd is reduced in both demonstrator and observer mice compared to naive controls. Notably, the decrease in observer expression was comparable to that of demonstrators. We then administered a synthetic neurosteroid analog (SGE-516) which significantly decreases freezing upon recall compared to vehicle mice. Conversely, we administered Finasteride, a 5α-reductase inhibitor, which significantly increased freezing in mice during the recall session. Taken together, this data demonstrates a role for endogenous neurosteroidogenesis in the BLA in the behavioral outcomes of observational fear and suggests that targeting endogenous neurosteroidogenesis may be a useful therapeutic intervention for PTSD, including cases resulting from witnessing a trauma.

34

MOTOR FACILITATION IS FORCE AND FORCE RATE DEPENDENT ***Spencer Jacobs-Skolik, Staff, Northeastern University,*** Nathaniel Pinkes, BS, MPH; Spencer Jacobs-Skolik, BS; Mathew Yarossi, PhD, Vaishnavi Sharma, BS; Erica Kemmerling, PhD; Eugene Tunik, PT, PhD; Oscar Soto, MD, PhD.

Abnormal voluntary activation of motor neurons is a hallmark of numerous neurological diseases, including amyotrophic lateral sclerosis (ALS). Voluntary activation is associated with an increase in the excitability (facilitation) of the cortical and spinal motoneurons participating in the task. Transcranial magnetic stimulation provides a non-invasive means to probe facilitation of motor outputs, however most attempts to date have done so only under static isometric contractions, likely missing effects of activation dynamics. Our goal was to characterize the relationship between facilitation of corticospinal drive (measured as the amplitude of the TMS-induced motor evoked twitch, MET) and two key features of isometric force production: the instantaneous force level and the rate of change in force level. Eleven healthy young individuals participated following IRB approved informed consent. Participants were seated in front of a LCD with each arm-hand supported by an armrest. The right hand gripped a standard sphygmomanometer bulb connected to a pressure transducer sampled at 2000Hz. Participants traced 4 ramp-and-hold shaped pressure trajectories (Fig. 1, left). TMS to the contralateral motor cortex was triggered at 5, 10, 20, and 50% of MVC for each ramp condition. Participants completed 10 trials per ramp velocity - trigger threshold pair, pseudorandomized across 4 blocks. Pressure velocity (dP/dt) at the time of stimulation was calculated as the mean velocity in a 100 ms window prior to TMS. MET amplitude was quantified from the twitch in response to TMS. For each pressure level, a linear regression was fit to determine the slope of the relationship between MET amplitude and dP/dt at the time of stimulation (force-dependent facilitation, FdF). FdF was found to be strongest at lower force levels and decays as the force level increases (Fig. 1, right). This measure may provide a 'stress test' to corticospinal facilitation that may serve as a sensitive way to measure failure of facilitation in neurological diseases such as ALS.

35

INSULAR-PREFRONTAL CIRCUIT DRIVING COMPASSIONATE SOCIAL BEHAVIOR ***Renee Cauchon, Undergraduate, MGH,*** S. William Li, BS, MS4 MD-PhD Candidate; Pauline Gabrieli, BA, MS; Moeko Suzuki, BS; Omer Zeliger, BS; Jack Demaree, BS; Renee Cauchon, BS Candidate; Nicole Occidental, BS; Hamzeh Al-Hajjiri, BS Candidate; Ziv Williams, MD.

Compassionate behavior, or the ability to help others in need, is a cornerstone of prosocial interaction in mammals and humans. To benefit others, it is necessary for individuals not only to perceive the internal states or emotions of others but also to take appropriate actions. For example, one must identify the distress of another and perform specific actions that will relieve their discomfort. Yet, how mammalian neurons precisely link social-specific information with such complex adaptive behavior has been a major challenge to understand. Here, we developed a place-preference assay that allowed mice to directly control in real-time the experience of a nearby conspecific while also allowing the animal's own actions to be dissociated from the other's identity and experiences. Behaviorally, we found that wild-type male mice consistently chose to reduce the aversive experience of familiar but not unfamiliar partners, actions that were not observed when visual and olfactory cues were blocked. Their decisions were uncorrelated with hierarchical rank, baseline sociability or vocalizations; together suggesting that the mice displayed consistent compassionate behavior towards familiar partners. By recording from neurons in the anterior insular (AI), we identified cells that encoded task relevant information, including the social identity of the animal's partners and their specific experience. Cells in the dorsal anterior cingulate cortex (dACC), by contrast, preferentially encoded information about the act of helping their partners, displaying changes in activity prior to making their decisions. Further, whereas information about the experience of others could be predominantly decoded from AI activity, information about the animal's prosocial actions could be predominantly decoded from dACC activity; demonstrating a partitioning of information within the insular-prefrontal circuit. Finally, chemogenetic excitation of AI-to-dACC projectors but not dACC-to-AI projections increased helping behavior when the animals were paired with unfamiliar partners suggesting that the AI transmitted social-specific information to the dACC. Inhibitions of both dACC to AI as well as AI to dACC projectors, on the other hand, decreased helping behavior with familiar partners - further suggesting that the dACC controlled compassionate decisions based on information from the AI. Taken together, these findings identify a putative insular-prefrontal circuit for driving compassionate behavior and a mechanism that could allow insular neurons to instruct social-specific actions through prefrontal control

36

NEURONAL KINASE LMTK3 MODIFIES KCC2 CHLORIDE EXTRUSION FUNCTION VIA PP1-MEDIATED DEPHOSPHORYLATION MECHANISM ***Noell Cho, Graduate Student, Tufts University,*** Georgina Kontou, PhD; Joshua Smalley, PhD; Nicholas Brandon, PhD; Giorgios Giamas, PhD; Paul Davies, PhD; Stephen J. Moss, PhD.

In the adult mammalian CNS, fast hyperpolarizing postsynaptic inhibition is mediated by GABAA receptors. Deficits in GABAergic inhibitory transmission is implicated in the pathophysiology of epilepsy, anxiety, mood disorders, schizophrenia, and autism spectrum disorders. The establishment and maintenance of proper hyperpolarizing GABA signaling is dependent on the expression and activity of KCC2, a neuron-specific K+/Cl- cotransporter. KCC2 is dynamically regulated by phosphorylation of individual serine and threonine residues within the intracellular C-terminus. Using affinity purification coupled to LC-MS/MS, our lab has previously identified numerous proteins that comprise the KCC2 proteome on the mouse plasma membrane. Lemur tyrosine kinase 3 (LMTK3), a brain specific transmembrane serine/threonine-protein kinase, is highly enriched within KCC2-containing complexes (Smalley et al., 2020). Given the consequences of KCC2 dysfunction, it is of great interest to identify targets that can modulate KCC2 levels and transporter activity. Our current study focuses on evaluating the role of LMTK3 in regulating KCC2-mediated GABA signaling. Using biochemical experiments, immunocytochemistry, and immunohistochemistry, we have characterized the interaction between KCC2 and LMTK3 in the brain. To investigate the functional impact of LMTK3 and KCC2 in dentate granule neurons, we have utilized a whole-cell patch-clamp Cl- loading electrophysiological assay to measure the reversal potential of GABAAR-mediated currents (EGABA) that reports KCC2 activity and Cl- extrusion capacity under specific chloride loads. Our observations show a significant hyperpolarizing shift of EGABA in dentate granule cells of 8-week-old LMTK3-/- mice. Furthermore, our immunoblotting experiments demonstrate a PP1-dependent dephosphorylation of S940-KCC2, complementing the functional downregulation of KCC2 directed by LMTK3. Our findings suggest that LMTK3 is a novel kinase regulator of inhibitory neurotransmission and we hope further investigation of the phosphorylation balance among key players that mediate GABAergic inhibition will provide a better understanding of how these are able to be modulated and used for novel therapies.

37

BASOLATERAL AMYGDALA ASTROCYTES ARE ENGAGED BY THE ACQUISITION AND EXPRESSION OF A CONTEXTUAL FEAR MEMORY ***Rebecca Suthard, Graduate Student, Boston University,*** Ryan Senne, BA; Michelle Buzharsky; Rebecca Cole, BA; Mia Diep; Angela Pyo, BA; Steve Ramirez, PhD.

Astrocytes are key cellular regulators that are actively involved in cognition and behavior. The field of neuroscience has focused primarily on the role that neurons play in many complex brain processes, but recently there have been a plethora of studies elucidating the role that glial cells play in the circuits involved in learning and memory, including in the amygdala. Here we sought to characterize the real-time calcium activity of basolateral amygdala (BLA) astrocytes across the acquisition, recall and extinction of a contextual fear memory. To accomplish this goal, we utilized the genetically-encoded calcium indicator, GCaMP6f, in astrocytes within the BLA to record calcium dynamics across fear learning using in vivo fiber photometry. Mice were subjected to contextual fear conditioning, recall and three extinction sessions to dissociate contextual- vs. shock-driven activity. We found that astrocytes display robust, high amplitude, short duration calcium events in response to 1.5mA foot shock during fear acquisition, while the un-shocked group displayed little astrocyte activity in the absence of the aversive stimulus. Additionally, astrocytic calcium activity in the shock group remained remarkably elevated and presented unique event characteristics across days in comparison to un-shocked control animals. Finally, astrocytes responded to the initiation and termination of freezing bouts during contextual recall, but this behavior-locked pattern of activity did not persist throughout the extinction sessions. Our ongoing work will further investigate the role of complementary canonical fear learning 'hubs', by recording of both neurons and astrocytes simultaneously with fiber photometry in ventral CA1 of the hippocampus (vCA1) during fear conditioning, natural recall, and optogenetic reactivation of a fear memory 'engram' in dorsal dentate gyrus of the hippocampus (dDG). Together, these experiments will provide a more comprehensive understanding of the unique contributions of neurons and astrocytes to the acquisition and maintenance of learned fear.

38

ENCODING, MODULATING, AND DECODING ***Robert Allen Brown, Hobbyist, RAB Technologies***

The fundamental way of dealing with large amounts of information is to use the process of encoding, modulating, and decoding. This EMD process is shown dramatically by the dispersion of Sun light by a prism that encodes Sun light into the seven colors of the rainbow, which can be modulated by selectively blocking one or more of the seven colors with slats, and decoding (combining) the remaining colors by a second prism, creating an single hue from an almost infinite number of different hues. This EMD process is used universally where a large amount of information needs to be organized, such as the Dewey Decimal System to classify and shelve millions of books in our libraries, the stocking of warehouses and store shelves, even organizing our household tools, and representing the miles traveled by a car by an odometer. These EMD systems rely upon a hierarchy of the input variables from lesser to greater significance. Billions of photons are encoded by the prism into the seven colors of the rainbow ranking from lower to higher frequencies of light, and millions of miles are encoded (represented) by just six figures (numbers) on a tiny odometer, each figure representing 1 mile, 10 miles, 100 mile, 1,000, mile 10,000, and 100,000 using a base 10 encoder. I show how a binary (base 2), ternary (base 3), or quaternary (base 4) are far superior encoder and decoder systems compared to a base 10 system. I show how a specific combination of these few encoded (intermediate) variables can be modulated (selected), and decoded (recombined) to produce any unique desired output from the original input. The brain is faced with the task of making sense of the firing of billions of neurons. In my poster I show from my book, Machines That Learn, Oxford University Press, 1994, how the brain can use encoding, modulating, and decoding to learn what it can and cannot do in its environment. DNA uses a base 4 system. Finding a system of base 2, base 3 or base 4 in the brain will transform neuroscience the way that DNA transformed biology.

39

CELLULAR AND STRIATUM-WIDE REPRESENTATIONS OF MOVEMENT DURING VISUALLY GUIDED INSTRUMENTAL BEHAVIOR ***Gabriela Rodriguez, Graduate Student, Boston University,*** R Feldman; T Otchy, PhD; D Boas, PhD; MW Howe, PhD.

The striatum integrates convergent sensory, motor and cognitive information to regulate appropriate instrumental actions. Activity of striatum projection neurons correlates with movement initiation, kinematics, and vigor and manipulations have region-specific effects on different aspects of movement. Moreover, the direct and indirect pathway SPNs (dSPNs and iSPNs), are believed to make unique contributions to movement control. However, it is unknown how dynamics of these populations support stimulus guided control of instrumental behaviors and how this may vary across the three-dimensional volume of the striatum. To capture regional variations in cell-type specific cue and movement related population signaling in SPNs, we have implemented a multi-fiber photometry technique that allows us to simultaneously monitor cell-type specific calcium signals from multiple locations across the striatum volume in head-fixed mice performing a two-choice instrumental task. To measure coordination of dSPN and iSPN ensembles at the cellular level, we have performed two-photon microscopy of identified SPNs in the dorsal striatum. Preliminary data indicates modulation of SPNs at the cellular and population levels to cue- and movement-related task variables. At the population level, we have observed cell-type specific differences in movement representations across regions: some regions show a preference for lateralized movement initiation while others more strongly represent continuous lateralized movement during task execution. At the cellular level, we have observed a dynamic recruitment of cell populations that vary across days with both cell types showing tuning to the task contingencies. Together, these initial findings provide insight into how striatal output supports cue-guided control of movement.

40

STRIATAL CHOLINERGIC INTERNEURONS MODULATE PERFORMANCE OF A LEARNED VOCAL MOTOR SKILL ***Douglas K. Reilly, Postdoctoral Fellow, Tufts University,*** and Mimi H. Kao, PhD

Striatal cholinergic signaling is critical for motor learning and control. Imbalances in basal ganglia (BG) neurotransmitter levels have been implicated in neurodegenerative and neuropsychiatric disorders, including Parkinson's disease, Huntington's disease, and Tourette syndrome. Prior work has suggested that the balance between dopaminergic (DA) and acetylcholine (ACh) signaling in the striatum is crucial for movement control: loss of DA in the BG in Parkinson's disease is accompanied by increased ACh. ACh can modulate DA release in the BG as well as the response of striatal spiny neurons to DA. Here, we use the vocal learning zebra finch to investigate the role of cholinergic signaling in regulating a complex, learned motor skill. Male zebra finches possess a cortical-basal ganglia circuit specialized for song learning and plasticity. Previous work has shown that lesions of the song basal ganglia (sBG) prevent stabilization of song sequence in juvenile birds and drive changes in song in adult birds, including stuttering. Here, we selectively ablated cholinergic interneurons in the sBG using the immunotoxin anti-choline acetyltransferase saporin (ChAT-SAP). Injections of ChAT-SAP reduced cholinergic interneurons by ~25% (compared to control birds injected with IgG-SAP) and drove changes in song sequence, such as novel syllables, syllable repetitions, and increased sequence variability. These studies begin to shed light on how striatal cholinergic signaling modulates performance of a complex learned motor skill. Ongoing work aims to understand whether loss of ACh signaling affects variability in syllable structure and/or context-dependent modulation of song.

41

THE EFFECTS OF LASER INTERSTITIAL THERMAL THERAPY ON THE CAPILLARY PHYSIOLOGY OF SECONDARY BRAIN TUMORS ***Shruti P. Gajjar, Undergraduate, Boston University,*** Timothy J. Carroll, Ph.D.; and Peter C. Warnke, M.D.

MRI-guided Laser Interstitial Thermal Therapy (LITT) for secondary brain tumors is a rising non-invasive, low-risk treatment option compared to traditional craniotomies, with studies exhibiting significantly longer progression-free survival (PFS). However, unlike previous studies using PFS and overall survival (OS) to assess the efficacy of LITT, our study directly quantifies its effects on the capillary bed of the tumor region and its success in reducing vasogenic edema, or peritumoral fluid build-up leading to Intracranial Pressure (ICP) in patients. Tumor-induced angiogenesis causes immature, leaky vessels in the capillary bed and increases permeability, promoting tumor growth and vasogenic edema due to disruption of the Blood-Brain Barrier (BBB). Permeability changes can be used as biomarkers for treatment response. We hypothesize that LITT will reduce permeability in the peritumoral region, decrease vasogenic edema, and increase relative cerebral blood flow (rCBF) by closing the BBB. This study has two specific aims: 1) to establish the physical response of capillaries in the tumor region to LITT and 2) to study chemotherapeutic drug delivery via pharmacokinetic modeling. We analyzed permeability statistics before and after LITT in a consecutive series of 5 patients by calculating regional K1 (blood-to-tissue transfer constant), k2 (tissue-to-blood efflux), Vp (tissue plasma vascular space), and Ve (tumor extracellular space) using a two-compartment Patlak model following a Gadolinium-based MRI contrast-agent. To determine if the expected increase in regional cerebral blood flow (rCBF) occurred after restoring the BBB with LITT, we conducted perfusion studies using a standard deconvolution process of the arterial input function. Preliminary region-of-interest (ROI) analysis and contralateral brain matter studies in patients have shown a decrease in K1, representing decreased tumor permeability post-surgery. Data suggests that LITT is sealing endothelial junctions of the disrupted BBB and shows great potential for further research. With defined relationships between LITT, rCBF, and permeability, we better understand the vasculature of the tumor region post-LITT and verify pharmacokinetic modeling as a viable tumor MRI-analysis technique. This knowledge could optimize chemotherapy and radiation strategies, which likewise hold a vascular dynamic in the tumor region. In future work, we strive to use quantified changes in permeability as a medical biomarker.

42

THE OLFACTORY BULB DRIVES DEFAULT MODE NETWORK-LIKE ACTIVITY IN BASAL FOREBRAIN OF MICE ***Jinyoung Choi, Postdoctoral Fellow, Harvard Medical School,***

The neural mechanisms which support consciousness and our engagement with the external world remain poorly understood. Such processes necessitate the precise cooperation of specialized functional networks, which must dynamically coordinate their activity and interactions as a function of task performance and cognitive demand. One such functional network that has garnered significant interest over the last few decades is the default mode network (DMN), a large-scale brain circuit that is activated during quiet wakefulness and deactivated during goal-directed tasks which evoke cognitive load. Abnormalities in DMN functional connectivity and activation appear across many neuropsychiatric disorders, including schizophrenia. Recent evidence suggests the basal forebrain is functionally and structurally important for regulation of DMN activity. Here, we attempt to test and extend this hypothesis using multisite local field potential (LFP) recording techniques. Adult mice (n = 4) were implanted with LFP electrodes targeting up six distinct brain regions: several associated with DMN (Basal Forebrain, Prefrontal Cortex, Posterior Parietal Cortex) and several not (Primary Visual Cortex, CA1 and Olfactory Bulb). Spontaneous LFP activity was recorded from freely behaving animals in a home cage, to promote DMN-like activity, and a novel environment. In our experimental work, we have been able to recapitulate prior findings showing pronounced gamma activity in both the basal forebrain and prefrontal cortex during quiet wakefulness in the home cage and suppression of this activity during active exploration of a novel environment. Surprisingly, DMN-like activity was also observed in the olfactory bulb. As expected, this effect was not observed in visual cortex or CA1. However, the posterior parietal cortex, which has been identified as a likely-DMN node, also did not exhibit DMN-like activity. Finally, granger causality analysis of our data shows that gamma activity in the olfactory bulb drives gamma activity in the BF. This finding suggests the functional importance of respiratory-entrained neural activity in the modulation of DMN neural network activity. Overall, this research provides valuable insight into the brain regions that comprise functional networks such as DMN, and their properties. The mechanisms which regulate their function can provide novel targets for therapeutic interventions for cognitive deficits associated with a number of neurological and psychiatric disorders.

43

THE EFFECTS OF FILOREXANT, A DUAL OREXIN RECEPTOR ANTAGONIST, ON EVOKED PREFRONTAL CORTICAL AND HIPPOCAMPAL GAMMA OSCILLATIONS IN A MODEL OF NMDA RECEPTOR HYPOFUNCTION ***Eden Maness, Postdoctoral Fellow, VA Boston Healthcare System,*** David Aguilar, PhD; Joshua Burk, PhD; James McNally, PhD; Robert Strecker, PhD.

Gamma frequency oscillations (30 to 100 Hz, centered around 40 Hz) are associated with enhanced wakefulness, sensory processing, and cognition. Evoked oscillatory gamma activity is impaired in individuals with schizophrenia, highlighting dysfunctional gamma-generating cells in cortical circuitry that are thought to underlie not only the psychosis associated with schizophrenia, but the cognitive deficits as well. Our recent findings (Maness doctoral thesis, 2022) have shown that sub-sedative doses of the dual orexin receptor antagonist filorexant are sufficient to improve attentional impairments produced by the psychomimetic NMDA receptor antagonist MK-801. The aim of the present experiment was to determine if these attention enhancing qualities of filorexant could stem from the rescue of abnormalities in evoked gamma oscillatory activity in the prefrontal cortex and hippocampus. To test this, mice (N = 5) were surgically implanted with local field potential electrodes targeting the medial prefrontal cortex and dorsal hippocampus, administered filorexant (0, 10, 30, or 60 mg/kg, ip) and/or MK-801 (0, 0.3, 0.5, or 1 mg/kg, ip). The mice were then exposed to repeated 40 Hz trains of auditory stimuli. MK-801 alone suppressed both phase synchrony and narrowband gamma power (35 to 45 Hz) during 40 Hz auditory steady state stimulation in both the prefrontal cortex and hippocampus. By itself, filorexant also reduced hippocampal, but not prefrontal cortical, gamma power during the 40 Hz auditory stimulus. When co-administered with 0.5 mg/kg of MK-801, none of the concentrations of filorexant were able to normalize deficient gamma entrainment. In conclusion, dual orexin receptor antagonism may improve attentional behavioral outcomes in an NMDA receptor hypofunction model of schizophrenia through mechanisms not relevant to the restoration of evoked gamma activity in these attention-associated brain areas.

44

GUT MAGIC: DO GUT MICROBIOTA UNDERLIE PSILOCYBIN-INDUCED ANTIDEPRESSANT EFFECTS?

Catherine Bostian, Graduate Student, MGH, JoAnna Pollonais, MSc; Vladimir Ivkovic, PhD.

Motivation: Major depressive disorder (MDD) is a serious public health concern; roughly one-third of patients do not achieve remission, even with therapy and as many as four different antidepressants—often with deleterious side effects. New, efficacious treatments are needed and psilocybin, a psychedelic and phytochemical, shows promise. Studies demonstrate psilocybin-induced long-term antidepressant effects with clinically significant response rates as high as 71% four weeks post-intervention. Despite mounting literature on psilocybin's antidepressant efficacy, the underlying mechanisms remain unknown. Psilocybin's effects on the rat gut microbiome have been observed, but to date, no study has investigated its impact on gut microbiota in humans, the potential association of microbiome changes with changes in functional cortical activity, or potential underlying mechanisms. Only a few studies have looked into synergistic interactions between psilocybin and naturally existing precursors. Approach: We will use a double-blind, placebo-controlled, within-subject crossover design in a population with MDD. Administration of psilocybin adheres to the Fadiman protocol, with 3x weekly 0.7g psilocybin microdoses administered every week for five weeks. Antidepressant effects are operationalized as changes in scores of the short version of the Depression Anxiety Stress Scales (DASS-21). Changes in gut microbiome composition will be assessed using whole-genome shotgun sequencing and analysis from fecal samples taken at baseline, and at the end of each week. Emotional Faces Attention Task (EFAT) will be used to assess depression symptom severity. Prefrontal cortical functional activity during EFAT will be monitored via cortical hemodynamic changes (oxygenation, perfusion) using the NINscan brain and physiologic monitoring system. We will explore two potential synergistic interactions: (1) psilocybin- and gut microbiota-induced effects on kynurenine pathway metabolites, and (2) psilocybin's antimicrobial/anti-quorum sensing properties. These will be analyzed from plasma and fecal sample extracts, respectively. Results: We are currently conducting pilot data collection to assess interoperability of data collection methods. Implications: If successful, the study will yield a novel research paradigm for assessing the effects of psychedelics on gut microbiome composition, its antidepressant effects, underlying mechanisms, and the associated changes in functional cortical activity. These findings hold the potential to improve therapeutic management of MDD and related psychiatric conditions.

45

OXYCODONE SELF-ADMINISTRATION DURING PREGNANCY: DO NEONATAL ULTRASONIC VOCALIZATIONS PREDICT ADULT RISK OF SUBSTANCE USE DISORDER ***Chantal Aaron, Graduate Student, Tufts University,*** Kelsea Gildawie, PhD, Jillian F. Celatka, Kerri E. Budge, Sara B. Isgate, Katie R. Fleming, Fair Vassoler, PhD., Elizabeth M. Byrnes, PhD.

The use and abuse of opioids has increased dramatically over the past decade, resulting in a fivefold increase in the number of infants experiencing neonatal opioid withdrawal syndrome (NOWS). To date, the factors influencing NOWS severity as well as the relationship between NOWS and long-term neurodevelopmental effects remain unknown. We previously reported alterations in ultrasonic vocalizations (USVs) in oxycodone-exposed pups and disrupted maternal behavior in rat dams following daily 1h oxycodone self-administration (SA) during pregnancy. In the current study we used a long-access SA model (6h daily) as well as cross-fostering to determine the relationship between prenatal oxycodone intake and USV number/quality in that absence of alterations in maternal care. In addition, we examined whether maternal intake level and/or USV parameters are associated with increased risk of substance use disorders in adult offspring. Female Sprague-Dawley rats were surgically implanted with a jugular catheter and trained to self-administer oxycodone in daily operant conditioning sessions (2h/day, 5 days/week for 2 weeks, 6h/day 5 days/week for 1 week; fixed ratio (FR1) schedule; 0.1 mg/kg/infusion). Once females were pregnant, they had daily access (7 days/week) to operant chambers and were allowed to self-administer oxycodone for 6h/day. On postnatal day 1 (PND1) litters were culled to 4 females and 4 males, and cross-fostered to time-mated drug naïve donor mothers. Pup USV, body weight and activity levels were analyzed on PND3, 6, 9, and 12. As adults, male and female offspring were trained on either oxycodone or cocaine SA with progressive ratio schedules used to assess motivated responding. Results indicate alterations in USVs are intake dependent during early postnatal development and preliminary data suggest effects on oxycodone but not cocaine SA in adult offspring. Analyses in progress will determine whether USVs and/or intake correlate with adult outcomes. This work is supported by the National Institute on Drug Abuse R01 DA049531 and Cummings School of Veterinary Medicine Office of R Besearch and Graduate Education.

46

NEONATAL OPIOID WITHDRAWAL SYNDROME (NOWS) MODEL TRAITS IN FVB SUBSTRAINS AND SPECTROTEMPORAL CHARACTERIZATION OF ULTRASONIC VOCALIZATION PROFILES USING DEEPSQUEAK MACHINE LEARNING SOFTWARE ***Kelly K. Wingfield, Graduate Student,Boston University,*** Kayla T. Richardson (BS), Teodora Misic, Mia B. Rubman, Nalia Abney, Kaahini Jain, Emily J. Yao (BS), Jacob A. Beierle (PhD), Camron D. Bryant (PhD).

Concomitant with the opioid epidemic, there has been a rise in pregnant women diagnosed with opioid use disorder and cases of infants born with neonatal opioid withdrawal syndrome (NOWS). NOWS refers to symptoms following cessation of prenatal opioid exposure that comprise low body weight, impaired thermoregulation, and hyperirritability. Multiple factors contribute to NOWS severity; however, the genetic factors are unknown. Our long-term goal is to use mouse models to identify genetic variants that contribute to NOWS severity, with the hope of translating aspects of these findings to humans. As a first step, we phenotyped genetically similar inbred substrains of FVB/N mice to identify genetic differences in NOWS model traits, including spontaneous hyperalgesia, hypothermia, locomotor agitation, and hyperirritability. In our opioid exposure regimen, neonatal pups were injected twice daily from postnatal day 1 (P1) to P15 with morphine (10mg/kg, s.c.) or saline. This early life exposure model represents the approximate third trimester in humans regarding developmental timing and this time period is both necessary and sufficient for inducing NOWS in humans and NOWS model traits in mice. Importantly, this exposure model avoids maternal exposure that could affect maternal care and it also permits precise control of morphine dosing across pups. We assessed several phenotypes during spontaneous morphine withdrawal on P7 and P14, including ultrasonic vocalizations (USVs) and concomitant locomotor activity, followed by nociceptive testing (hot plate and tail-flick latency assays). Morphine pups of all four substrains displayed lower body weights and hypothermia and showed thermal hyperalgesia on the hot plate and tail withdrawal assays. Interestingly, on P14, but not P7, morphine pups emitted more USVs than saline pups, consistent with the excessive crying in human infants born with NOWS. In mouse pups, USVs indicate emotional states and are emitted in response to thermal change, distress, and maternal separation. USVs are classified into different syllable types based on frequency changes and duration. We explored the composition of USV syllables to determine if certain syllables were associated with the aversive affective state of opioid withdrawal. We also looked for divergence among FVB substrains in USV composition that would provide evidence for genetic differences in the severity of the affective opioid withdrawal state. We implemented the machine learning software, DeepSqueak, to characterize USVs based on previously known syllables. During spontaneous withdrawal on P14, we observed a significant increase in the percentage of complex three (C3) syllables across all four substrains; however, there were notable substrain and sex differences in USV composition. Of the four substrains tested on P14, female NJ morphine pups emitted significantly more USVs and a greater proportion of C3 syllables than male morphine pups. To confirm that the increase in C3 syllables is a withdrawal phenotype that generalized to other genetic backgrounds, we classified P14 USVs from outbred CFW mice. Like FVB mice, morphine CFW pups also emitted a significantly greater proportion of C3 syllables compared to saline pups. To further investigate the effect of withdrawal on USV composition, we administered the opioid receptor antagonist, naloxone (4mg/kg, s.c.), to induce an aversive state in morphine naïve FVB pups on P9. Again, we observed a significant increase in the proportion of C3 syllables in naloxone-treated pups. On P15, we administered a maintenance dose of morphine (10mg/kg) or saline to FVB mice to determine if morphine could relieve withdrawal and thus restore the USV profile to a saline-like profile. Morphine induced a significant reduction only in the proportion of C3 syllables emitted by morphine pups toward a saline control-like level. Together these data suggest implicate C3 syllables as a unique marker for an emotional distress signal associated with the opioid withdrawal state. On P16, 16 h after the final injections, brainstem tissue from the FVB/NJ substrain was collected from pups for RNA sequencing. We found a significant upregulation of genes related to addiction, such as the µ- and k-opioid receptor (Oprm1 and Oprk1, respectively) and a downregulation of genes associated with myelination (Mbp and Plp1, respectively). NJ morphine pups also showed an upregulation of the dopamine transporter, Slc6a3 and interestingly, this effect was entirely driven by the females. These data highlight sex and substrain differences in physiological and emotional withdrawal-associated phenotypes and provide insight into the biological mechanisms contributing to differences in NOWS symptom severity. Future experiments include quantitative trait locus (QTL) mapping to identity potential candidate genes underlying differences in observed withdrawal-related phenotypes between the two most phenotypically divergent FVB substrains.

47

IMPACT OF NEONATAL OPIOID WITHDRAWAL SYNDROME ON SLEEP ARCHITECTURE IN MALE AND FEMALE MICE ***Benjamin R. Williams, Lab Manager and Research Technician, Boston University,*** Mackenzie Gamble, BS; Navsharan Singh, MS, MPH; Ryan W. Logan, PhD.

Despite rising rates of opioid misuse, addiction, and overdose deaths, prescription opioids persist as the gold standard treatment for severe and chronic pain, even in pregnant persons. Unlike some drug classes, opioids have been shown to cross the placenta. Thus, long-term use of opioids during pregnancy may induce fetal development of physical dependence and, eventually, neonatal opioid withdrawal syndrome. While long-term opioid use is associated with numerous side effects, abrupt cessation of opioid use may induce withdrawal symptoms, including hallucinations, aches and pains, and insomnia. Withdrawal-associated insomnia is of particular concern as perturbed sleep quality is a known risk factor for relapse, among other health concerns. Surmounting clinical evidence indicates that newborns with neonatal opioid withdrawal syndrome also experience disrupted sleep patterns. However, the exact impact of opioid withdrawal on newborn sleep architecture remains unknown. Using Swiss Webster (CFW) mice, we completed twice daily fentanyl injections from P1 to P21, the developmental equivalent of the third trimester of gestation in humans. Immediately after cessation of fentanyl administration, mice experiencing withdrawal underwent non-invasive sleep-wake analysis for 8 days. Current work aims to determine how early-postnatal fentanyl administration impacts endogenous opioid receptor and clock gene expression. Our preliminary findings show that prepubescent mice treated with fentanyl during the early-postnatal phase of development exhibit elevated rapid eye movement sleep compared to saline treated controls across all 8 days of recording. However, the proportion of wake and non-rapid eye movement sleep varied significantly by day, indicating that neonatal opioid withdrawal symptoms differentially modulate non-rapid eye movement sleep and wake state during acute and chronic withdrawal periods.

48

NEURAL MECHANISMS OF FEEDING DYSREGULATION FOLLOWING PRENATAL OPIOID EXPOSURE IN MALE AND FEMALE RATS ***Kelsea Gildawie, PhD, Postdoctoral Fellow, Tufts University,*** Sara Isgate, BA; Kerri Budge, BS; LuJuana Jackson, MS; Fair Vassoler, PhD; Elizabeth Yen, MD; Elizabeth Byrnes, PhD.

Opioid use disorder among women of reproductive age has escalated dramatically in recent years, increasing the likelihood of fetal drug exposure and subsequent development of neonatal opioid withdrawal syndrome (NOWS). While medication-assisted treatment (MAT), e.g., buprenorphine or methadone, provides treatment for opioid use disorder, over 50% of infants born to mothers on MAT experience hyperphagia, an early withdrawal sign that is associated with more severe withdrawal course and varies by sex. Here, adult Sprague Dawley females were implanted with osmotic minipumps filled with methadone (10 mg/kg/day) or buprenorphine (1 mg/kg/day) dissolved in dH2O or saline control (2.5 µl/hour for 28 days) and mated one week later. On postnatal day (PND)1, pups were cross-fostered with drug-naïve dams. On PND2, PND7 and PND12, pups were tested for hyperphagia via assessment of postnatal feeding behavior. Following feeding sessions, brain tissue was harvested and cryosectioned. Recent human work implicates alterations in the expression of dopamine receptor type 2 (DRD2), neuropeptide Y2 receptor (NPY2R), and proopiomelanocortin (POMC) in NOWS-related hyperphagia. Therefore, RNAscope in situ hybridization was performed to visualize gene expression in the ventral striatum (reward) and arcuate nucleus of the hypothalamus (feeding). In adulthood (PND70), remaining subjects began sucrose pellet self-administration to assess the motivational strength of palatable food in MAT-exposed animals vs. controls. We found that maternal MAT results in reduced offspring body weight at PND1, as well as sex-dependent developmental and long-term feeding dysregulation – where males experienced increased neonatal feeding and enhanced food-motivated behavior – that may be driven by changes in the expression of DRD2, NPY2R, and POMC in the brain. These data provide insight into the mechanisms driving MAT-induced changes in neural modulation of reward- and feeding-related behaviors.

49

SEX SPECIFIC RECRUITMENT OF THE BASOLATERAL AMYGDALA COMPLEX AFTER HABITUATION TO NOVEL FOOD AND FEEDING CONTEXT ***Zoe Irving, Graduate Student, Boston College,*** Eliza Greiner, MA; Gorica Petrovich, PhD.

Novel foods and novel environments both impact consumption, but their interaction is poorly understood, especially how this interaction varies across habituation and by sex. Our prior work showed that rats consume less of a novel, palatable food when fed in a novel environment, and female rats habituated slower than males to eating in a novel context. We also established that amygdala nuclei were distinctly recruited upon exposure to novelty, with Fos induction in the anterior basomedial nucleus (BMAa) remaining high after habitation. Additional analyses showed that the anterior basolateral (BLAa) and lateral (LA) nuclei were recruited in a sex-specific manner after habituation to novelty. Here, we examined the posterior basomedial (BMAp) and the posterior basolateral (BLAp) nuclei, and evaluated correlations across amygdala nuclei. Male and female Long Evans rats (n=8/group) were food deprived for 20h prior to consumption of novel, palatable Test Diet pellets in either their home cage or a novel context for 4 habituation sessions and 1 final test. Rats were perfused 90 minutes after the start of the test, and brain tissue was processed for analysis of Fos induction. During the final test, rats tested in the novel context consumed significantly less than those tested in the home cage ($F(1,28)=16.75$, $p=0.02$), and females ate less than males ($F(1,28)=8.68$, $p=0.08$). Fos induction in the BMAp differed by context, with more induction in the novel context than the home cage ($F(1,22)=34.21$, $p=0.001$), but did not differ by sex. In the BLAp, Fos induction differed by both sex ($F(1,22)=12.38$, $p=0.06$) and context ($F(1,22)=13.17$, $p=0.055$), with more induction in females and rats tested in the novel context. Males tested in the novel context showed strong correlations in amygdala Fos induction. BMAp Fos induction was correlated with the BMAa ($p=0.058$) and the BLAa ($p=0.008$), but not with the BLAp, while the BLAp was correlated with the LA ($p=0.06$). Fos induction in home cage males was not consistently correlated across amygdala nuclei, except for a moderate correlation ($p=0.07$) between the BMAp and BLAp. For females tested in the novel context, Fos induction in the BMAp and BLAp were highly correlated ($p=0.009$), as well as the

BMAp with the BMAa (p=0.063), and the BLAp with the BLAa (p=0.04), LA (p=0.05), and the BMAa (p=0.053). For females tested in their home cage, Fos induction in the BMAp and BLAp were not correlated, but BLAp Fos induction was correlated with the BLAa (p=0.01) and the BMAa (p=0.02). Collectively, these results suggest that the BLAp and the BMAp are part of a larger sex and context specific network of amygdala nuclei which control consumption after habituation to novelty.

50

HEPATIC ZHX2 OVERXPRESSION REDUCES ACUTE OXYCODONE-INDUCED LOCOMOTION AND ENHANCES STATE-DEPENDENT LEARNING/RECALL OF OXYCODONE REWARD IN BALB/CJ FEMALE MICE ***Will Lynch, Graduate Student, Boston University,*** Ida Kazerani, Gabriel A. Saavedra, Ava Farnan, Binh-Minh Nguyen, Camron D. Bryant.

Opioid Use Disorder (OUD) maintains epidemic proportions in the U.S., with current treatments limited to opioid substitution therapy. OUD has an estimated variability of 50%. Furthermore, sensitivity to the subjective and physiological responses to opioids also has a genetic component that could influence addiction liability to opioid drugs. We recently used an F2 cross and quantitative trait locus (QTL) mapping to identify Zhx2 as a candidate gene underlying increased oxycodone (OXY) metabolite brain concentration in BALB/cJ (J) females vs. BALB/cByJ females (By) following systemic administration of OXY (1.25 mg/kg, i.p.). The metabolite, oxymorphone (OMOR), is more potent and efficacious than OXY and thus could contribute to the enhanced state-dependent learning and recall of OXY-induced conditioned place preference (CPP) that is also greater in J vs. By females. Due to a mouse retroviral insertion (MERV) within intron 1, Zhx2 expression is dramatically reduced in J vs. By mice. Thus, in this study, we sought to restore Zhx2 levels in J females via AAV-mediated overexpression (OE) in the liver to determine whether this manipulation would reduce OXY-induced behaviors to a level previously observed in By females. We retro-orbitally injected adenoassociated virus (AAV) using a construct containing cloned Zhx2 DNA that targeted its expression to the liver. There was no significant difference in initial distance traveled or time spent on each side of the CPP chamber following Zhx2 OE. However, Zhx2 OE mice showed reduced acute OXY-induced locomotion (distance, 1.25 mg/kg, i.p.) when confined to the OXY-paired side, while showing no significant difference when injected with saline (SAL, i.p.) and confined to SAL-paired side. When testing for state-dependent OXY-CPP (open access) following a subsequent OXY injection (1.25 mg/kg, i.p.), there was no difference in total distance, although Zhx2 OE mice showed increased distance traveled in, and enhanced preference for, the OXY-paired side. In sum, Zhx2 hepatic OE reduced OXY acute locomotion, yet enhanced state-dependent learning/recall of OXY-CPP in J females, providing evidence for gene validation of Zhx2 as a quantitative trait gene underlying OXY behavior. Further characterization of the liver and brain tissue from these mice as well as germline gene editing are underway to provide further evidence for validation and to elucidate mechanism linking Zhx2 with OXY metabolism and behavior.

51

CELL-TYPE SPECIFIC TRANSCRIPTIONAL SIGNATURES ASSOCIATED WITH OPIOID USE DISORDER USING SINGLE NUCLEI RNA-SEQ OF HUMAN POSTMORTEM BRAIN ***Ryan Logan, Associate Professor, Boston University,*** Madelyn H. Ray PhD, BaDoi Phan BS, Madeline Kuppe BS, Marianne L. Seney PhD, Jill R. Glausier PhD, Allison Tipton BS, Shelley J. Russek PhD, Andreas Pfenning PhD, David A. Lewis MD.

Background: In the United States, rates of opioid use disorder (OUD) and deaths from overdose are unprecedented. Despite the enormous public health impact of OUD, we have a limited understanding of the changes in the brain in patients with OUD. Few studies have directly examined the cellular and molecular changes in the human brain associated with OUD. A recent study from our group utilized bulk RNA sequencing in human postmortem brain in the dorsolateral prefrontal cortex and nucleus accumbens in OUD and control subjects. This study identified significant alterations in transcripts enriched for neuroinflammatory and extracellular matrix signaling in the brains of subjects with OUD. Additionally, cell type specific enrichment of microglia markers in demonstrates a potential primary role for microglia in OUD. Collectively, this study suggests that OUD may induce the upregulation of neuroinflammatory markers, particularly in microglia. However, a limitation to using bulk RNA sequencing is the lack of cellular resolution. To address this limitation, we are utlizing single nucleus RNA sequencing in human postmortem brain. Human neuroimaging studies investigating OUD have implicated functional changes in the dorsal striatum, an area integral in reward processing, habitual drug-seeking, craving, and relapse. The dorsal striatum is comprised of the caudate nucleus and putamen. In the current study, we conducted single nuclei RNA sequencing on human postmortem caudate nucleus and putamen in subjects diagnosed with OUD and comparison control subjects in order to identify cell-type specific transcriptional changes associated with opioid use disorder. This study is the first of its kind to identify key changes in transcriptional signatures associated with opioid use disorder at the single nucleus level in human postmortem dorsal striatum. Methods: In the current study, we conducted single nuclei RNA sequencing on postmortem brain tissue from male and female subjects diagnosed with opioid use disorder (N=6; 3 females and 3 males; average age: 41.17) or comparison subjects (N=6; 3 females and 3 males; average age: 44.67). For each subject, both the caudate and putamen were processed. Nuclei were isolated from 24 fresh frozen post-mortem human brain samples. Briefly, ~50mg of tissue was dounce homogenized with 1,000µl nuclei lysis buffer containing dapi using a glass douncer with 9 pestle strokes. Homogenate was filtered using a 40um cell filter. Samples were sorted on a BD FACS Aria II Flow Cytometer at the Boston University Flow Cytometry Core using Fluorescent Activated Cell Sorting (FACS) for dapi. Nuclei were sorted at a flow rate of 1.5-2.0 with gating criteria set to hierarchically select whole, single nuclei. For each sample, approximately 150,000 nuclei were collected into a tube containing 11.40µl PBS with 0.04% BSA. Samples were evaluated for nuclei concentration and viability on a hemocytometer. 7,000 nuclei were targeted using the 10x single cell dual index 3' gene expression protocol to prepare dual index next generation sequencing libraries. Finally, libraries are pooled and sequenced on the Next-seq 2000 (illumina) at the Boston University Single Cell Sequencing Core. Sequencing targeted 7,000 nuclei per sample and 50,000 reads per nuclei. Following completion of sequencing, data was pre-processed. Pre-processing included aligning the reads (STARsolo), ambient mRNA detection & correction, empty droplet and doublet filtering, and QC analysis. Next, data was labeled and verified using a dorsal striatum macaque dataset (He & Kleymen et al., 2021). Finally, full analysis was conducted using analyses for differential expression, DNA damage signatures, and differential abundance. Results: We identified transcriptional alterations associated with OUD in specific cell types of the striatum, including significant shifts in the expression of dopamine receptor subtypes in GABAergic medium spiny neurons. When examining differentially expressed genes (DEGs) by cell types comparing OUD and control subjects, DEGs were found in the following cell types: astrocytes, endothelial, microglia, oligodendrocytes, D1-matrix, D1-striosome, D1/D2 hybrid, D2-matrix, D2-striosome, interneuron. Next, we analyzed DNA damage signatures. DNA damage signature analyses revealed increased DNA damage specific to OUD subjects compared to control subjects (p=0.002). When looking at cell type specific DNA damage, mural cells have a higher proportion of DNA damage (p=0.025) in OUD subjects. Additionally, looking at average DNA damage scores across cell types revealed increased DNA damage in OUD subjects in microglia (p=0.004), endothelial (p=0.006), oligodendrocytes (p=0.021), and interneurons (p=0.023). Additional exploratory approaches are currently underway to reveal cell-specific alterations in gene expression and compare different cell types across striatal subregions and sex. Conclusions: Our results are the first to demonstrate significant alterations in gene expression across different cell types of the striatum in the human brain associated with OUD using single nuclei RNA-seq. Additionally, our results suggest that OUD is associated with higher rates of DNA damage, particularly in microglia subtypes. Uncovering molecular mechanisms of opioid use will propel the identification of new therapeutic targets and the development of successful treatment strategies for OUD.

52

IMPLICATION OF 2-ARACHIDONOYLGLYCEROL IN NON-REM SLEEP REMEDIATION DURING WITHDRAWAL FOLLOWING CHRONIC FENTANYL EXPOSURE IN MICE ***Sophia Miracle, Graduate Student, Boston University,*** Mackenzie C. Gamble, BS; Ryan W. Logan, Ph.D.

Background Fentanyl use has become one of the leading causes of opioid induced deaths in the past decade. For individuals who are exposed to fentanyl, they experience detrimental side effects, including sleep disturbances, during withdrawal periods. These disruptions in humans are known to appear at withdrawal day 4 (WD4) and last for months, resulting in a high relapse rate to attenuate the symptoms.

To this point, much has been explored about the effects of fentanyl use in terms of opioid use disorder (OUD), but more attention must be focused on the withdrawal phenotypes and neural mechanisms to prevent relapse, the development of tolerance, and overdose. Investigating the underlying mechanisms of these insomnia-like sleep disturbances could pose as a novel target for therapeutic interventions to lessen fentanyl withdrawal symptoms. Recent studies evaluating chronic fentanyl use on sleep patterns in mouse models have shown that non-REM (NREM) sleep is significantly attenuated during fentanyl withdrawal but returns to the individual's baseline when fentanyl was reintroduced. The endocannabinoid (eCB) system, specifically the eCB 2-arachidonoylglycerol (2-AG), has been theorized to innervate other behavioral systems, including sleep and reward, making it a possible candidate for pharmacological intervention, in the intention of alleviating fentanyl withdrawal induced sleep disturbances. Objective To begin to investigate the possible functionality of 2-AG in the sleep in the context of fentanyl withdrawal in male and female mice. Methods Utilizing a chronic fentanyl exposure paradigm in a mouse model (4 male, 4 female), we established opioid dependence with the administration of fentanyl twice a day (morning and evening) for 7 days and recorded their sleep and wake activity via Piezo Sleep System for the entirety of the experiment. Once dependence is fixed, 2-AG was introduced once a day for the first 3 days of withdrawal for half of the mice (2 males, 2 females), and WD4 was used as a washout period. Results From this study, we have preliminary data showing that mice who received 2-AG compared to the control showed increased NREM sleep in their night phase during withdrawal, but is most prominent on WD4, suggesting that 2-AG is somehow maintaining baseline/homeostatic sleep durations during withdrawal in a phase specific manner as withdrawal progresses. We also see signs of possible sex differences at this stage, illuding to the possible sensitivity of females to eCB infusions compared to males. Conclusions Studies are currently underway to further investigate and signify these data trends. If this trend continues across cohorts, 2-AG could be a viable agent to utilize in the efforts to reduce sleep disturbances in individuals experiencing fentanyl withdrawal, and further, to decrease the percentage of opioid users that relapse.

53

IMPACT OF HUNGER STATE ON PALATABLE FOOD-CUE ASSOCIATIVE LEARNING AND CONSUMPTION IN MALES AND FEMALES ***Rebecca Shteyn, Graduate Student, Boston College,*** Danielle S. Lafferty, PhD; Gorica D. Petrovich PhD.

Physiological hunger state and food palatability could impact food consumption either jointly or independently through homeostatic and hedonic mechanisms. Both also influence food-seeking and learning about cues for food. Here we examined how males and females learn about and consume palatable food (PF; Test Diet pellets) under sated and hungry conditions. Male and female adult Sprague Dawley rats were either food restricted (85% ad libitum body weight) or had ad libitum access to regular chow (n=8 per group). Rats learned cue-food associations across 8 Pavlovian conditioning sessions, followed by cue-only presentations for 4 extinction sessions in a different context. They were then tested for renewal of conditioned responding (time spent at the food cup) to the food cue in the conditioning compared to extinction context, counterbalanced. Before learning and after renewal testing, rats were tested for consumption of PF and chow in their home cage (1hr test/day per food; counterbalanced). Hungry rats ate more PF and chow than sated rats regardless of sex ($p<.001$). Sated females ate more palatable food than sated males during each test ($p=.003$, $p=.006$). Hungry rats exhibited higher conditioned responding during early conditioning (sessions 1-3; $p<.005$) and had a higher learning rate than sated rats (higher rate of change from the first half of session 1 compared to second half of session 2; $p=.001$). The rate of learning during early conditioning was also marginally higher for females ($p=.053$). For the remaining 5 training sessions, all groups responded similarly. As expected, during extinction, all groups decreased their conditioned responding. All groups had significantly higher conditioned responding to the food cue in the acquisition compared to extinction contexts, confirming the renewal effect. Importantly, the amount of palatable food eaten during the initial consumption test was positively correlated with subsequent cue-food learning during early conditioning, in terms of both conditioned responding and learning rate ($p<.05$, $p<.001$). Overall, these results found that hungry rats of both sexes were more motivated to consume food and acquired cue-food responding faster initially. However, both sexes were able to learn, extinguish and renew responding without hunger. Sated females were more motivated to consume palatable food than sated males, and regardless of hunger state, females exhibited higher initial rates of learning. These findings suggest several sex-specific differences concerning food motivation. Future research of the neural substrates in both sexes is needed in order to identify the drivers of hedonic food motivation without hunger.

54

UNDERSTANDING PERIPHERAL OPIOID TOLERANCE: INVOLVEMENT OF KERATINOCYTES AND PDGFRB SIGNALING ***Stephanie Puig, Assistant Professor, Boston University,*** L. Posa, Ph.D.; S.A. Miracle, BA; A.K. McDonald; Z. Freyberg, M.D., Ph.D.; K.M. Albers,Ph.D.; R.W. Logan, Ph.D.

Chronic systemic prescription opioid use leads to misuse, addiction, and overdoses death, via central mechanisms. Topical and peripheral application of low doses of opioids are safer alternatives, as they provide efficient local analgesia with limited central penetration. However, peripheral opioid tolerance develops rapidly via cellular mechanisms that remain unknown. Recent work identified new mechanisms for centrally mediated tolerance, involving mu-opioid receptors (MOR) mediated spinal release of platelet-derived growth factor B (PDGF-B), activating platelet-derived growth factor receptor beta (PDGFRβ). Previous reports hypothesized that central and peripheral tolerance occur by different mechanisms. However, in preliminary studies, we found that imatinib can block peripheral morphine tolerance, suggesting similar mechanisms centrally and at the periphery. We also found that, MOR and PDGF-B are expressed in skin keratinocytes, and PDGFRβ is expressed in peripheral sensory neurons (PSNs), which are known to convey tolerance. Prior studies showed that selective optogenetic stimulation of keratinocytes in mice can elicit PSNs activation which may be dependent on release of keratinocytes-derived factors. Therefore, we hypothesized that PDGF-B/PDGFRβ signaling is critical for peripheral morphine tolerance and that keratinocytes are a component of the peripheral circuitry that mediates tolerance. Using a pharmacological approach in mice, we found that intraplantar (i.pl.) co-administration of morphine with a PDGF-B scavenger or with a selective monoclonal antibody targeting PDGFRβ, prevented peripheral tolerance. In parallel, we found that repeated optogenetic activation of keratinocytes in in opioid naïve mice induced peripheral morphine tolerance that could be reversed with imatinib. Repeated i.pl. administration of PDGF-B was also sufficient to induce peripheral tolerance to morphine in opioid naïve mice. Finally, in situ hybridization showed that MOR and PDGF-B are co-expressed in keratinocytes, and PDGF-B expression is increased after repeated peripheral morphine injections, suggesting that PDGF-B could be released from opioid activated keratinocytes. Together, these data show that activation of keratinocytes is sufficient to induce peripheral morphine tolerance in a PDGFRβ signaling dependent manner, and that PDGF-B ligand is required to mediate this phenotype. Our data shows that FDA-approved PDGFRβ inhibitors could be repurposed to treat chronic pain by preventing peripheral tolerance and reducing the risk of opioid addiction, overdoses and death.

56

REGULATION OF AMPA RECEPTOR ACETYLATION IN HOMEOSTATIC SYNAPTIC PLASTICITY ***Lucy Petersen, Graduate Student, Boston University,*** and Heng-Ye Man, MD, PhD.

Homeostatic synaptic plasticity (HSP) plays a crucial role for the maintenance of neural functional stability. HSP is expressed via changes in the amount of AMPA receptors (AMPARs) at the post-synaptic domain, however, the molecular mechanisms underlying AMPAR relocation during HSP remain less clear. We have previously discovered that AMPARs are subject to acetylation on GluA1 C-terminal lysine residues, which competes with ubiquitination and increases GluA1 synaptic retention. Here, we show that GluA1 acetylation is increased after chronic activity deprivation both in vitro in primary neurons and in vivo in the visual cortex. We also find that this change in acetylation is mediated by increased synaptic expression of the acetyltransferase p300, and that this increased acetylation is necessary for the expression of HSP.

THE EFFECTS OF KETAMINE AND 60 HZ TONE & LIGHT ENTRAINMENT ON PERINEURONAL NETS IN THE VISUAL & AUDITORY CORTICES OF MICE ***Victoria Lombardi, Undergraduate, VA Boston Healthcare System,*** Christopher Dennehy, Megan MacIver, Eden Maness, PhD, James T. McKenna, and James McNally, PhD.

Critical period neuroplasticity is extremely important to the normal growth and development of the brain. Perineuronal nets (PNNs) are extracellular matrix structures composed of proteoglycans that coat fast-spiking parvalbumin (PV) interneurons in the visual, motor, and somatosensory cortices to provide stabilization of synapses. The presence of PNNs limits neuroplasticity and temporary reductions in PNNs are thought to allow changes in cortical synaptic arborizations. Anesthetic doses of ketamine have been recently shown to degrade PNNs and induce neuroplasticity in mice (Venturino et al., 2021); thiseffect may underlie the therapeutic effects of ketamine on refractory depression in humans. These authors also found that PNNs were decreased in mice exposed to 60 Hz light entrainment a manipulation that also restored juvenile-like neuroplasticity. Here, we compared the effects of lower, sub-anesthetic doses, of ketamine (30mg/kg, 3 doses 3 days apart) with 60 Hz light and tone entrainment on -PNNs colocalized with PV+ cortical interneurons in the visual and auditory cortices. Double labeled cells(PV+ and PNN+) in the experimental groups were compared to untreated controls. We found that neither ketamine or 60 Hz light and tone entrainment resulted in a decrease in PNNs in either the visual or auditory cortices compared to controls. Ongoing experiments using higher doses of ketamine (3 doses of 100mg/kg every 3 days) are designed to more closely reproduce the methods of Venturino and collaborators (2021).
References. Venturino A, et al. with Siegert S. (2021) Microglia enable mature perineuronal nets disassembly upon anesthetic ketamine exposure or 60-Hz light entrainment in the healthy brain. Cell Rep. 36(1).