# 14th Annual Neuroscience, Behavior, and Health Forum (2024)

# ABSTRACTS

## Keynote Address

### Translating brain mechanisms of fear to understanding PTSD and trauma-related disorders

Kerry Ressler, Ph.D.

Stress responses and threat processing are central to understanding debilitating disorders such as Anxiety and Posttraumatic stress disorder (PTSD). Recent progress has been made in understanding the neural circuits underlying threat-or fear-memory formation that complements a decades-old appreciation of the neurobiology of fear, involving hub structures such as the amygdala. I will review evidence from our lab and others for genetic, neurobiological, and neural circuit mechanisms that underlie fear processing in preclinical neuroscience model systems, and how these findings can be used to help understand Posttraumatic Stress Disorder (PTSD) and other trauma-related disorders in human patients. I will also discuss future approaches to pharmacotherapy and other treatments for PTSD that have been developed via a bench to bedside translational models.

# PLATFORM TALKS

## Developing and optimizing electrochemical biosensors for mental health disorders

Yangguang Ou

Department of Chemistry, University of Vermont

According to the National Institute of Mental Health, more than 1 in 5 U.S. adults suffer from some form of mental health disorder. Current pharmacological treatments for brain disorders is based on the premise of chemical imbalance in the brain. While some patients have found relief from these treatments, not everyone has benefited from them. This is due to the critical gap in available direct evidence for neurochemical alterations underlying brain disorders. Our lab develops and optimizes electrochemical biosensors to directly measure these neurochemical changes. Our base platform is fast scan cyclic voltammetry at carbon fiber microelectrodes. We modify the surface of the electrodes to be selective for different biomarkers of interest. For example, we have anodically attached 1,4-diethynylbenzene for selective quantitation of Cu(II) ions, which are implicated in neurodegeneration. We have also modified the electrodes with molecular imprinted polymers for selective quantitation of Met-enkephalin, an endogenous neuropeptide implicated in PTSD.

**Modeling somatic mutations in non-coding receptor tyrosine kinases in intractable epilepsy.**

Marcus Weinman, Trevor Wolf, Kathryn Laprade, Pranav Mathkar, Emily Dean, Matthew Weston, Matija Snuderl, James Stafford

Epilepsy is a neurological disorder that affects 1% of the US and global populations. Epidemiological data indicate that many epilepsy cases are idiopathic with no known cause. The focus of epilepsy research in recent years has focused on genetic changes in neurodevelopment. Somatic copy number gains in two noncoding enhancer regions upstream of EGFR and the promoter region of PDGFRA in intractable epilepsy. These phenomena lead to aberrant receptor tyrosine kinase (RTK) expression in neurons that do not ordinarily express these genes. Recent data in glioma research also indicates that enhancer copy number gains can epigenetically potentiate oncogenes, including EGFR. However, it is unknown how these somatic mutations promote epilepsy, and furthermore, whether inappropriate RTK expression in neurons leads to electrophysiological and neuropathological indicators of epilepsy. To bridge this gap in knowledge, we have created a tractable model to explore the consequences of inappropriate RTK expression in murine primary cortex neurons (PCNs) via EGFR/PDGFRA-bearing and enhancer-activating lentiviral constructs. We will use this model to (1) understand the molecular and electrophysiological features of RTK+ neurons in how they alter neuronal character and lead to epileptogenesis, (2) treat RTK+ neurons with a receptor tyrosine kinase inhibitor to reverse aberrant phenotypes, and (3) understand the mechanism(s) by which these enhancers epigenetically activate Egfr expression. By combining epigenetic, molecular, and electrophysiological approaches, the proposed study aims to provide insight into the vast consequences of RTK expression seen in human epilepsy patients and lay the foundation for eventually treating these patients with tyrosine kinase inhibitors.

**Effect of chronic neuroendocrine stress mechanisms on urinary bladder function**

Jenan Husain, Gerald Herrera, Anna Pace, Benedek Erdos

Department of Pharmacology, University of Vermont, Burlington, VT

Chronic stress can lead to urinary bladder dysfunction and pain either through actions within the central nervous system or by mechanisms that are locally induced in the bladder. Prolonged upregulation of brain-derived neurotrophic factor (BDNF) in the paraventricular nucleus of the hypothalamus (PVN) has been shown to chronically elevate activities of the sympathetic nervous system and hypothalamus-pituitary-adrenal axis. Taking advantage of this mechanism, we created a model of chronic neuroendocrine stress by subjecting male Sprague Dawley rats to bilateral PVN injections of viral vectors expressing either BDNF or GFP (for control). Bladder activity was assessed 10 weeks post-injections by monitoring the number of urinary voids and average urinary void volume along with water and food intake over a 48-hour period ($N = 6$ per group). To further characterize changes in the urinary bladder, bladder weights were recorded (GFP: $N = 5$; BDNF: $N = 6$) and in vitro bladder strip experiments were conducted ($N = 2$ per group) 14 weeks after viral vector injections. We hypothesized that model-induced chronic neuroendocrine stress would impair bladder function.

BDNF overexpression in the hypothalamus led to significantly lower daily urine output even though water intake remained unaffected, suggesting that BDNF-treated animals showed a significantly higher non-urine-related fluid loss (also known as insensible fluid loss) compared to GFP controls (daily urine output = GFP: 33.85±4.94, BDNF: 14.82±2.18, $p < 0.01$; insensible fluid loss = GFP: 7.71±1.68, BDNF: 20.60±1.39, $p < 0.001$). Interestingly, despite a lower total daily urine volume, BDNF-treated animals had a significantly higher number of urinary voids (GFP: 7.50±1.42, BDNF: 15.38±2.76; GFP vs. BDNF: $p < 0.001$) and lower average void volume (GFP: 1.19±0.15, BDNF: 0.27±0.07; GFP vs. BDNF: $p < 0.01$), thus emulating the overactive bladder phenotype, a common complication in humans with chronic stress. However, *in vitro* bladder strip experiments revealed no significant differences in contractility between the experimental groups as tested with high K+ concentration, electric field stimulation and carbachol-induced stimulation of smooth muscle muscarinic receptors. In addition, urinary bladder weights were also unaffected by BDNF overexpression.

In summary, we demonstrated that prolonged hypothalamic BDNF overexpression, a model of chronic neuroendocrine stress, leads to significant alterations in bladder function such that BDNF-treated rats exhibited a higher number of urinary voids and lower average void volume compared with GFP controls. The lack of differences in *in vitro* bladder preparations suggests that this overactive bladder phenotype may be caused by changes in central as opposed to peripheral mechanisms. These findings will help elucidate pathways through which hypothalamic stress-related mechanisms may contribute to the development of the overactive bladder syndrome.

**Bed nucleus of the stria terminalis (BNST) PAC1 receptor neuron projections and chemogenetic modulation**

Mahafuza Aktar, Ava Cardarelli, Victor May and Sayamwong E. Hammack

Pituitary adenylate cyclase activating polypeptide (PACAP) is a highly conserved neuropeptide playing essential roles in numerous physiological functions. We and others have implicated central PACAP neurocircuits in mechanisms by which stressor exposure increases anxiety. PACAP binds to several receptor subtypes, including PAC1, VPAC1 and VPAC2, to activate several signaling cascades that can alter neuronal excitability as well as enhance indices of neuroplasticity. We have demonstrated that PACAP release in the bed nucleus of the stria terminalis (BNST) is critical for many of the behavioral and physiological consequences of stressor exposure, and much of our prior work has suggested that these effects of BNST PACAP anxiogenic behavior depend on the activation of PAC1 receptors. Here we use chemogenetic approaches in PAC1-ires-Cre mice, in which Cre recombinase expression is downstream of the PAC1 receptor gene promoter to specifically stimulate or inhibit the activity of PAC1 expressing cells in the BNST. Using a designer receptor exclusively activated by designer drugs (DREADD) strategy and the PAC1-Cre mice, we injected a viral vector to elicit cre-dependent expression of the excitatory hM3Dq, inhibitory hM4Di gene, or a control reporter gene, in BNST PAC1-expressing neurons. After recovery, we administered systemic injections of clozapine N-oxide (CNO) and assessed anxiety-like behavior on an elevated plus maze. CNO significantly changed open-arm exploration without reducing total locomotor activity, suggesting that the modulation of PAC1 expressing neurons in the BNST can change anxiety like behavior, and consistent with our prior work, suggesting a key role for BNST PACAP receptor activation in anxiety and stress responding. We also examined BNST PAC1 receptor mCherry reporter expression to circuit map its downstream targets. We observed robust PAC1 expressing neurons in BNST, including the anterior, posterior and ventral regions of the BNST. We also observed BNST PAC1 receptor mCherry fiber expression in the nucleus accumbens (NAc), paraventricular nucleus of thalamus (PVT), lateral habenula (LHb), lateral hypothalamus (LH) and substantia nigra (SNR), consistent with other reports demonstrating that these brain regions receive BNST PAC1 projections. These observations help to clarify the neural circuits involved in anxiety like behavior.

**Age, Sex and Alzheimer's disease: A longitudinal study of 3xTg-AD mice reveals sex-specific disease trajectories and inflammatory responses mirrored in postmortem brains from Alzheimer's patients**

Alicia J. Barber, Carmen L. del Genio, Anna Beth Swain, Elizabeth M. Pizzi, Sarah C. Watson, Vedant N. Tapiavala, George J. Zanazzi, Arti B. Gaur

Background
Aging and sex are major risk factors for developing late-onset Alzheimer's disease. Compared to men, women are not only nearly twice as likely to develop Alzheimer's, but they also experience worse neuropathological burden and cognitive decline despite living longer with the disease. It remains unclear how and when sex differences in biological aging emerge and contribute to Alzheimer's disease pathogenesis. We hypothesized that these differences lead to distinct pathological and molecular Alzheimer's disease signatures in males and females, which could be harnessed for therapeutic and biomarker development.
Methods
We aged male and female, 3xTg-AD and B6129 (WT) control mice across their respective lifespans while longitudinally collecting brain, liver, spleen, and plasma samples (n=3-8 mice per sex, strain, and age group). We performed histological analyses on all tissues and assessed neuropathological hallmarks of Alzheimer's disease, markers of hepatic inflammation, as well as splenic mass and morphology. Additionally, we measured concentrations of cytokines, chemokines, and growth factors in the plasma. We conducted RNA sequencing (RNA-Seq) analysis on bulk brain tissue and examined differentially expressed genes (DEGs) between 3xTg-AD and WT samples and across ages in each sex. We also examined DEGs between clinical Alzheimer's and control parahippocampal gyrus brain tissue samples from the Mount Sinai Brain Bank (MSBB) study in each sex.
Results
3xTg-AD females significantly outlived 3xTg-AD males and exhibited progressive Alzheimer's neuropathology, while 3xTg-AD males demonstrated progressive hepatic inflammation, splenomegaly, circulating inflammatory proteins, and next to no Alzheimer's neuropathological hallmarks. Instead, 3xTg-AD males experienced an accelerated upregulation of immune-related gene expression in the brain relative to females, further suggesting distinct inflammatory disease trajectories between the sexes. Clinical investigations revealed that 3xTg-AD brain aging phenotypes are not an artifact of the animal model, and individuals with Alzheimer's disease develop similar sex-specific alterations in canonical pathways related to neuronal signaling and immune function. Interestingly, we observed greater upregulation of complement-related gene expression, and lipopolysaccharide (LPS) was predicted as the top upstream regulator of DEGs in diseased males of both species.
Conclusions
Our data demonstrate that chronic inflammation and complement activation are associated with increased mortality, revealing that age-related changes in immune response act as a primary driver of sex differences in Alzheimer's disease trajectories. We propose a model of disease pathogenesis in 3xTg-AD males in which aging and transgene-driven disease progression trigger an inflammatory response, mimicking the effects of LPS stimulation despite the absence of infection.

**LOX-1 inhibition selectively prevents aortic but not cerebral stiffness in a model of preeclampsia**

Jennifer L. Anderson, Sarah M. Tremble, Jayden A. McGreer, Abigayle V. Tainter-Gilbert, Marilyn J. Cipolla

**Introduction:**
Preeclampsia (PE) causes widespread vascular dysfunction. PE patients have large artery stiffness associated with increased pulse wave velocity and microvasculature damage, particularly in the brain. PE is also linked with increased oxidative stress and endothelial dysfunction. PE patients have increased oxidized low-density lipoprotein (oxLDL) that binds to the LOX-1 (lectin-like oxLDL-1) receptor, a primary means of increased oxidative stress. Here, we assessed the impact of LOX-1 inhibition on large artery (aorta) stiffness and cerebrovascular structure.
**Methods:**
Adult female Sprague Dawley healthy pregnant (LP+IgG), experimental preeclamptic (ePE+IgG, high-cholesterol diet d7-19) or ePE LOX-1 inhibited (ePE+LOX1i) rats were compared. Treatment (LOX1i or IgG) was delivered via osmotic mini pump from gestational day 12 to 19 (euthanasia). Pup and placenta weights were recorded. Isolated and pressurized third-order posterior cerebral arteries (PCAs) were studied under passive conditions and diameters, wall thickness, and distensibility determined. Thoracic aortas were fixed for histologic metrics of stiffness. Metrics of oxidative stress - Plasma 3-nitrotyrosine (3-NT), PAI-1 (Plasminogen Activator Inhibitor-1) and oxidized LDL (oxLDL) were measured via ELISA. A Kruskal-Wallis test with Dunn's posthoc analysis was used to compare data. Data are expressed as mean±SEM.
**Results:**
There were no differences in pup numbers, resorptions, or weights. Placentas were smaller in ePE+IgG compared to LP+IgG (0.365 ± 0.013 g vs. 0.420 ± 0.010 g, $p$=0.03) but not ePE+LOX1i (0.381 ± 0.187 g, $p$>0.99). Plasma oxLDL was greater in both ePE groups (LP+IgG 7458 ± 258 vs. ePE+IgG 10758 ± 672 pg/mL, $p$<0.01 and ePE+LOX1i, 10635 ± 479 pg/mL, $p$<0.01). While ePE animals demonstrated a trending increase in plasma 3-NT, this was not significant (LP+IgG 1521 ± 271 nM vs. ePE+IgG 2262 ± 448 nM, $p$=0.055, and ePE+LOX1i 2444 ± 614 nM, $p$=0.11). There was no difference in plasma PAI-1. ePE+IgG showed lower aortic elastin content than ePE+LOX1i (81.93 ± 0.78 versus 86.32 ± 1.21, $p$=0.04), but not LP+IgG (84.58 ± 1.97, $p$=0.47). Elastin lamina sinuosity trended lower in ePE+IgG than LP+IgG (4.48 ± 0.30% vs. 5.46 ± 0.33%, $p$=0.11) but not ePE+LOX1i (5.00 ± 0.35%, $p$=0.48). There were no differences in aortic medial thickness or elastin lamina counts. There were no differences in PCA passive lumen diameter or distensibility.
**Conclusion:**
LOX-1 inhibition in ePE selectively prevented aortic stiffness without decreasing oxidative stress, oxLDL, or PCA structural changes. This suggests a selective, regionally dependent effect of LOX-1 receptor inhibition with oxidative stress as the underlying mechanism. As large artery stiffness is associated with cardiovascular disease, understanding and preventing vascular dysfunction in PE women may lead to better overall cardiovascular health.

**Assessing strengths and well-being in primary care for adolescents with mental health and substance use concerns**

Deepinder Singh, Heidi K. Schumacher, Christine A. Pellegrino, Breena W. Holmes, Rachel L. Garfield, Valerie S. Harder

The Larner College of Medicine at the University of Vermont, Vermont Child Health Improvement Program

As the COVID-19 pandemic highlighted gaps in meeting adolescent behavioral health needs, primary care providers (PCPs) were a locus for interventions to address adolescent mental health and substance use concerns. Strengths-based approaches may support PCP promotion of positive behavioral health in adolescents, but competing priorities or other factors may inhibit their use. We analyzed health record review data from 31 primary care practices to assess utilization of strengths-based approaches during the health supervision visit (HSV) for adolescents with and without behavioral health concerns. We found that most had strengths identified (78%) or well-being topics addressed (83%). However, adolescents screening positive for depression were 40% less likely to have strengths identified, while those screening positive for anxiety or substance use were 89% and 163%, respectively, more likely to have well-being topics addressed. PCPs may need support for integrating strength-based approaches when managing adolescents screening positive for depression.

**Multivariate approaches to elucidating the effects of child adversity on neurodevelopment, behavior, and health**

Alexis Brieant

University of Vermont

Adverse experiences are heterogeneous and vary widely across contexts and individuals. Multidimensional and multimodal approaches are necessary to capture this complexity and the related neurobiological and mental health consequences across childhood and adolescence. The present study included 7,115 youth from the Adolescent Brain Cognitive Development (ABCD) Study, a racially and economically diverse sample from 21 different sites across the USA. At baseline (9-10 years old), we used exploratory factor analysis to characterize 10 robust dimensions of adversity which captured factors such as caregiver psychopathology, socioeconomic disadvantage lack of neighborhood safety, and caregiver lack of support. We then used a Bayesian multivariate multilevel model to examine associations between these factors and psychopathology and cognitive functioning. Higher levels of caregiver psychopathology and physical trauma exposure were associated with higher internalizing and externalizing problems, but not with cognitive functioning. Higher levels of socioeconomic disadvantage/lack of neighborhood safety were associated with lower cognitive flexibility and inhibitory control. Delineating the specific associations among adversity and developmental outcomes is a critical step in identifying how youth with different types of adversity exposure may be at risk for certain types of outcomes.

**Habit learning and goal-directed behavior in rats and people**

Eric A Thrailkill

Department of Psychological Science, University of Vermont

Behavior tends to occur in “chains” of responses performed in a sequence leading to a reinforcing outcome. For example, someone that normally smokes cigarettes while driving in their car must also procure cigarettes from a store. In the laboratory, we have studied behavior chains with rats by arranging a first response (R1; e.g., a lever press) that, in the presence of a discriminative stimulus (S1; e.g., tone), turns S1 off and turns on a second stimulus (S2; e.g., click-train) that signals the opportunity to make a second response (R2; e.g., a chain pull) that turns off S2 and earns a food-pellet outcome. In a behavior chain, R2 may function as a “goal” for R1, and extinction might effectively “devalue” R2. Behaviors in a chain may transition from being goal-directed to being habitual. Two experiments sought to study habits and goal-directed actions in behavior chains with both our rat method and a novel chain task developed for human participants. In Experiment 1, two groups of rats received either minimal or extended training on our chain task. Half the rats in each training group then received extinction of R2 or exposure to the chamber in the absence of response manipulanda and discriminative stimuli. After extinction, all rats were tested with S1 with the response manipulanda present. After brief training, R1 was weakened after extinction of R2. This is analogous to a reinforcer devaluation effect, a hallmark of goal-directed behavior. In contrast, after extended training, R2 extinction did not weaken R1, which suggests habit formation. In Experiment 2, we used a computer task to study behavior chains in humans. Participants were assigned to complete either minimal or extended training on our chain task. Half the participants in each training group then received extinction of R2 or extinction of an irrelevant R3 response which had not been trained as part of a chain. After extinction, all participants received a test with S1. After brief training, R1 was weakened after extinction of R2. The reinforcer devaluation effect was observed with humans suggesting goal-directed behavior. Importantly, after extended training, R2 extinction did not weaken R1, which suggests habit formation. The results suggest parallel evidence for goal-direction and habit learning in rat and human instrumental conditioning procedures. This line of research integrates our understanding of instrumental behavior in a novel way and may help address practical difficulties in the study of habits in humans.

**Initial efficacy of a novel prolonged exposure therapy protocol for improving therapy session attendance and PTSD symptoms among buprenorphine- or methadone-maintained adults with PTSD**

Kelly R. Peck[a,b*], Jillian Giannini[a,b], Gary J. Badger[c], Rebecca Cole[a], & Stacey C. Sigmona,[b]

[a]Department of Psychiatry, University of Vermont
[b]Department of Psychology, University of Vermont,
[c]Department of Medical Biostatistics, University of Vermont

Although prolonged exposure (PE) therapy is a first-line treatment for posttraumatic stress disorder (PTSD), little is known about the effects of PE in individuals with co-occurring opioid use disorder (OUD). Furthermore, its efficacy is commonly undermined by poor attendance. This randomized clinical trial is part of a programmatic effort to develop a novel PE therapy protocol for improving PE attendance and PTSD symptoms among patients receiving medications for OUD (MOUD) with a concurrent diagnosis of PTSD. Fifty-two buprenorphine- or methadone-maintained adults with PTSD were randomized to receive either: (a) continued MOUD treatment as usual (TAU; n=17), (b) Prolonged Exposure therapy (PE; n=17), or (c) PE with financial incentives delivered contingent upon PE session attendance (PE+; n=18). Participants were permitted to complete PE sessions via telemedicine or in-person. Primary outcomes included PE session attendance and PTSD symptom severity. PE+ participants attended more therapy sessions compared to PE participants (88% vs. 33%; $p<.001$), regardless of modality. All three experimental groups achieved significant reductions in PTSD symptoms between intake and study week 12 ($p<.001$). However, PE+ participants were more likely to achieve diagnostic remission (83%) at week 12 and no longer met criteria for PTSD compared to TAU (40%) and PE (40%) participants ($p<.05$). These findings provide support for the efficacy of PE+ for improving PE attendance and PTSD symptoms in individuals with co-occurring PTSD and OUD. These promising results justify a larger scale randomized clinical trial to more rigorously evaluate the efficacy of a telemedicine-based approach for delivering PE to MOUD patients.

**RNA-directed therapy for C9ORF72-linked ALS using zinc finger nucleases**

Kathryn H. Morelli[1], Qishan Liang[2], Maya L. Gosztyla[2], Daniel R. Scoles[3], Sharan Paul[3], Gene W. Yeo[2]

1 Department of Neurological Sciences, University of Vermont
2 Department of Cellular and Molecular Medicine, University of California San Diego
3 Department of Neurology, University of Utah

A GGGGCC hexanucleotide repeat expansion (G4C2EXP) in the first intron of the C9ORF72 gene is the most common known cause of familial and sporadic ALS (C9-ALS) and has been linked to RNA-mediated pathogenesis due to the formation of toxic RNA foci from both sense G4C2EXP and antisense (C4G2EXP) transcripts. We engineered an RNA-targeting zinc finger nuclease fusion system (Z1-PIN) that targets both forms of mutant RNA in vitro and eliminates each, simultaneously, within C9-ALS patient-derived iPSC-derived spinal cord organoids, with minimal transcriptomic off-target effects. We also demonstrate that delivery of Z1-PIN via an adeno-associated viral vector to the central nervous system of a transgenic mouse model of C9-ALS alleviates signs of neurodegeneration without gross adverse effects. Our data provides proof of principle that a member of this new class of zinc-finger RNA-targeting effectors can potentially treat C9-ALS, with implications for other neurodegenerative diseases such as frontotemporal dementia

**Sema6A/PlexinA2 bidirectional signaling is essential for proper development and maintenance of the zebrafish retina**

Alicia Ebert, Caroline Dumas, Riley St. Clair, Sarah Emerson, Bryan Ballif

The Department of Biology, University of Vermont, Burlington VT, 05405

The eye is a complex organ that develops from a field of early progenitors into a laminar structure with many different neuronal subtypes. The Plexin family of transmembrane receptors and their Semaphorin ligands are well known axon guidance molecules. We have shown that loss of either PlxnA2 or Sema6A leads to zebrafish with smaller, more disorganized retinas. Recently, it has been demonstrated that this signaling pair can signal bidirectionally, with Sema6A and PlxnA2 acting as both the ligand and the receptor. We previously uncovered important receptor-proximal signaling events required for traditional "forward signaling" through PlxnA2 leading to proper retinal progenitor proliferation and downstream transcriptional regulation. We now have identified a role for "reverse signaling" through Sema6A in retinal development and maintenance. Together these results have led to a better understanding of the unique and collective contributions of these bidirectional ligand-receptor pairs in zebrafish eye development.

## DATA BLITZ

### ABC renewal after extinction and counterconditioning in male and female rats.

Samantha K. Moriarty, Hannah L. Schoenberg, Neil E. Winterbauer, Sayamwong E. Hammack, Donna J. Toufexis, & Travis P. Todd

Renewal occurs when an extinguished CS is tested outside the extinction context. There is some evidence that renewal might not be as robust in female rats. We examined renewal in male and female rats in an ABC fear renewal paradigm where rats were conditioned to a tone paired with foot-shock in Context A, the tone was extinguished in Context B, and renewal was tested in either the extinction Context B or a Context C where the tone had never been experienced. As a follow up experiment, we examined potential sex differences in counterconditioning, a preparation similar to renewal, however, instead of the tone being presented alone in Context B, it is presented with delivery of sucrose pellets. In both experiments, we observed fear renewal in male and female rats and no significant difference in magnitude of renewal based on sex, suggesting that renewal of fear may not differ between sexes.

**Behavioral evaluation of brain EC-specific Piezo1 gain of function in mice.**

Mohammad Elmahdy

University of Vermont

**Estradiol influenced functional connectivity in postmenopausal women.**

Abigail Testo

University of Vermont

Functional connectivity is a type of brain connectivity that assesses the similarity of signals arising from anatomically distinct brain regions. Previous studies have found that estrogens play a role in functional connectivity in the brain, however, little research has been done regarding how functional connectivity changes following the decline in estrogen levels that occurs following the menopausal transition. (Jacobs et. al., 2017). The purpose of this study was to examine the relationship between estradiol and functional connectivity in postmenopausal women. Estradiol level was found to enhance parahippocampal gyrus anterior division left functional connectivity during ROI-to-ROI regression analysis $F(3,84)=5.88$; p-FDR=0.009. Estradiol enhanced functional connectivity between the parahippocampal gyrus anterior division left and the precuneus ($t(86)=3.10$, p-uncorrected=0.003, p-FDR=0.011) as well as the parahippocampal gyrus anterior division left and parahippocapal gyrus posterior division right ($t(86)=3.05$ p-uncorrected=0.003 p-FDR 0.011).

**Electrical control of melatonin production for implantable cell-based therapies using 3D pinealocyte cultures**

Abigail J. Myers[1], Kathryn A. Laprade[1], Toheeb Adegoke[1], Rusul Mustafa[1], Zifang Zhao[3], Nofar Hemed[2], Alberto Salleo[2], Dion Khodagholy[3], David Punihaole[1],Yangguang Ou[1],James M. Stafford[1]

[1]The University of Vermont, [2]Stanford University, [3]Columbia University

Recent advancements in research surrounding implantable medical devices have revealed the efficacy of cell-based biofactories for the detection and treatment of a diversity of human diseases and disorders. Careful control over the factors that regulate the production and release of therapeutic components from these devices is necessary for patient safety and treatment success. Here, we characterize the regulation of melatonin secretion from primary pinealocytes and incorporate cellular production units into a biocompatible device designed to treat circadian rhythm misalignment. While pinealocytes classically produce melatonin in response to noradrenergic signaling from sympathetic postsynaptic fibers, the expression of voltage-gated $Ca^{2+}$ channels within the membrane of these cells suggests the possibility of an electrically controlled production process. The present results provide support for this hypothesis, demonstrating melatonin production in pinealocytes electrically stimulated in a monolayer and in 3D heterotypic structures. Furthermore, our pinealocyte cultures maintain melatonin production capacities when electrically stimulated in an implanted device. We hope to optimize our stimulation protocol to gain precise control of cellular melatonin secretion, allowing for timed delivery of the therapeutics necessary to realign the biological clock.

**Fabrication and optimization of a molecularly imprinted carbon fiber microelectrode for selective detection of met-enkephalin**

Nikki Villarini

University of Vermont

The mechanistic role of endogenous opioid peptides in various physiological processes is still being debated due to the absence of a selective technique capable of measuring ambient endogenous opioid peptides, like met-enkephalin (Met-Enk), on a rapid time scale. Fast scan cyclic voltammetry (FSCV) at carbon fiber microelectrodes (CFM) is a viable technique for measuring in vivo Met-Enk as it permits rapid measurements and eliminates inflammation caused by more invasive techniques with larger probe sizes. However, traditional FSCV is limited in selectivity due to the highly adsorptive nature of the activated carbon surface. In this work, we leverage molecularly imprinted polymer technology on CFMs to increase selectivity of the electrode for Met-Enk. Specifically, we identify the critical ratio of the monomers to the template molecule (Met-Enk) for Met-Enk selectivity and assess the sensor's FSCV response to both MetEnk and the hexapeptide fragment Angiotensin II (3-8). Our long-term goal is to utilize the optimized sensors in implantable in vivo applications.

# POSTERS

## Poster 1

### Elucidating the ionic mechanism of burst-firing and its implications in Pten knockout neurons

Asan F. Abdulkareem, Bryan Luikart

Geisel School of Medicine, Dartmouth College

Pten is tumor suppressor and regulatory antagonist of PI3K/Akt/mTOR pathway that is involved in cellular differentiation and growth. Loss of function mutation in Pten is one of the most common genetic aberrations associated with autism spectrum disorder (ASD), which is an increasingly diagnosed neurodevelopmental disorder. Pten depleted neurons have shown an increase in soma size, dendritic arborization, migration, and increased hyperexcitation. Knocking out Pten in granule neurons of dentate gyrus in mice has resulted in an increased burst-firing phenotype as well as a smaller fast AHP (after hyperpolarization) of the action potentials. While burst-firing accomplishes numerous functions in the brain such as emotional regulation, release of neurotransmitters and other peptides, abnormal bursting has been detected in various neuropathies. Through genetic manipulation and pharmacological targeted inhibition, we evaluate changes in the ionic activities and determine possible signaling pathways involved in burst-firing. Therefore, identifying the underlying cause of neuronal hyperexcitability in these neurons. Findings from genetic inhibition of downstream signaling intermediates of Pten indicated the possibility of Akt/mTORC2 involvement in producing excessive burst-firing rather than mTORC1. This is based on the decrease in bursting probability seen in Pten/Akt/Akt3 triple knockout and Pten/Rictor (mTORC2) double knockout neurons compared to Pten/Raptor (mTORC1) double knockout where there is no significant change in the burst probability. Also, electrophysiological evaluations suggest changes in multiple channels indicated by their corresponding altered current. This was shown by a decrease in BK (large conductance calcium-dependent potassium channel) currents as well as changes in currents of both low voltage-gated and high voltage-gated calcium channel. Altogether, our data thus far suggest an ionic dysregulation governed by players downstream of Pten in the formation of burst-firing that needs to be further investigated.

**Poster 2**

## AKT is necessary for dentate granule neuron hypertrophy and excitatory synaptogenesis caused by Pten knockout

Mackenzi L. Prina[1,3], Andrew R. Goyette[1,3], Kamran Tariq[1] , Asan F. Abdulkareem[1] , Wei Wang[1] , Meijie Li[1] , Lucky Drucker[1] , Helena Seo[1] , Nicole M. Desmet[1] , Bridget Shafit-Zagardo[2] , Bryan W. Luikart[1]

Mutations to phosphatase and tensin homolog deleted on chromosome 10 (PTEN) are a known contributor to Autism Spectrum Disorder (ASD), macrocephaly, and epilepsy. PTEN negatively regulates the mTOR signaling pathway. Pten knockout (KO) mouse models exhibit neuronal hypertrophy, hyperexcitability, seizures, and ASD-like behaviors. Using transgenic mouse lines and retroviral-mediated genetic alterations, we can analyze pathway outputs in response to the manipulation of various genes. In doing so, we have identified Akt1 and Akt3 as the specific downstream signaling intermediates mediating the robust neuronal hypertrophy caused by Pten loss. When Pten, Akt1, and Akt3 are all lost, soma area, spine density, migration, hyperexcitability, and dendritic overgrowth are completely rescued to wildtype levels. Upon overexpression of either AKT1 or AKT3 in a Pten/Akt1/Akt3 KO mouse, neuronal hypertrophy returns to Pten KO levels. This work indicates that AKT is an essential intermediary regulating Pten loss-driven neuronal hypertrophy. Understanding the interactions of the downstream effectors within the mTOR pathway and how these go awry in patients with ASD, macrocephaly, and epilepsy will broaden the knowledge of these disease pathologies and identifies potential therapeutic targets.

**Poster 3**

## The effect of hyperglycemia on the axonal cytoskeleton, and rescue by Pten knockdown

J. Reglewski*; H. Rose*; S. Evans; J. Hong, M.D.**
*These authors contributed equally, and will both attend the forum to present a joint poster
**Principal investigator

Up to 50% of adults with diabetes will develop diabetic neuropathy (DN) in their lifetime, resulting in pain, loss of sensation, and decreased quality of life. The most common type of DN, peripheral sensory neuropathy, is believed to be induced by extracellular conditions, including hyperglycemia, dyslipidemia, and ischemic changes. However, peripheral sensory neurons (PSNs) damaged by diabetes show impaired regenerative ability even in healthy extracellular environments, implying that diabetes induces lasting intracellular changes that prevent regeneration. Despite the epidemiological scope of this problem, the mechanisms regulating neuronal regeneration are not well understood. One promising gene of interest is phosphatase and tensin homolog (Pten), a tumor suppressor gene that regulates many growth-related cellular processes. Past work from our lab has shown Pten-KO increases microtubule (MT) polymerization velocity in a variety of neuronal subtypes, and work from other labs has shown that Pten is upregulated in PSNs in mouse models of diabetes. Here, we use a primary dorsal root ganglion (DRG) culture system to study the effects of diabetes on the axonal cytoskeleton and overall neuronal growth. First, we culture DRG PSNs from healthy adult mice in a hyperglycemic environment. We show that high extracellular glucose levels decrease MT polymerization rates in an apparently dose-dependent manner, and Pten-KO rescues these effects. Second, we describe a mouse model of Type II diabetes generated using a high-fat diet and streptozotocin injections, which induce metabolic syndrome and destroy insulin production, respectively. This mouse model has impaired glucose metabolism, and male mice develop a more severe disease phenotype than females, reflective of human disease presentation. We show that this mouse model can then be used to study DN through both in vivo behavioral and in vitro cellular-level measures. Overall, our data supports the hypothesis that extracellular conditions found in diabetes (ie. hyperglycemia) disrupt the axonal cytoskeleton, possibly contributing to impaired regeneration, and that our mouse model can be used to further elucidate the mechanisms underlying this disruption. Most importantly, we highlight the cytoskeleton as a novel target for future development of regenerative DN treatments, which has the potential to improve the lives of millions of patients.

**Poster 4**

## Reversing cognitive deficits in the PTEN knockout model of ASD: From behavior to in vivo electrophysiological recording

Noah H. Elste, Dylan H. Marchand, Jeremy M. Barry

Department of Neurological Sciences, University of Vermont

Despite the prevalence of ASD, the relationship between its underlying genetics and the expression of its varied cognitive deficits and behavioral phenotypes remain vexing and complex for clinicians and basic scientists (Srivastava et al., 2014). A candidate gene mutation in some ASD cases is the Phosphatase and Tensin Homolog (PTEN) gene, which codes for the PTEN phosphatase (Busch et al., 2019). When mutated or absent in neurons, cell overgrowth and hyperexcitability occur (Getz et al., 2022). This can lead to changes in how cells receive input from other brain regions and their responses to this input (Carracedo and Pandolfi., 2008). In these experiments, PTEN loss and its effects on cognition are assessed through the knockout of PTEN in a fraction of dentate gyrus granule cells in model mice. These are cells in the hippocampus which play a critical role in regulating cortical input from the entorhinal cortex. We have found that PTEN loss ultimately leads to a specific cognitive deficit in the association of objects with novel spatial locations during performance of a spatial accuracy task. High density silicon neural probes are also used in a head-fixed apparatus to show functional differences in the local field potentials and action potential properties of hippocampal cells. Several mice with PTEN ko demonstrate interictal epileptiform discharges, an EEG signature often seen in epilepsy. We hypothesize that these discharges stem from the hyperexcitability of ko DG granule cells. We found that these discharges were significantly attenuated through the silencing of PTEN ko neurons usings DREADDs. Further research will help specify how cellular and morphological changes can create a specific behavioral phenotype, and gain insights into the relationship between ASD, epilepsy, and their reciprocal relationship with cognitive function.

**Poster 5**

## Optogenetic control in the hilus of KCNT1 mice

D. Marchand, N. Elste, W. Tobin, M. Mahoney, & J. Barry

Department of Neurological Sciences, University of Vermont

Mutations of the Kcnt1 Na+ gated K+ slack channel are associated with frontal lobe epilepsies, and are a candidate for the genesis of these types of seizures. Kcnt1-Y796H mice have been shown to be good models for frontal lobe epilepsies with a reduction in excitability of non-fast spiking L2/3 interneurons in the M2 region of the frontal lobe. SST+ HIPP interneurons in the hilus are another common area of seizure genesis. These SST+ cells gate cortical inputs which throughput to the outer and medial molecular layers, making them ideal targets for intervention. Using optogenetics to drive theta frequencies in these SST+ has prevented dentate spikes, the disruption of which is associated with interictal epileptiform discharge, as well as revealed physiological limits to SST+ HIPP interneurons continuous rate of firing. These findings could be significant for understanding the role of SST+ HIPP interneurons in seizure genesis, as well as treating frontal lobe epilepsies.

**Poster 6**

## E342V-Tau: Characterization of a missense mutation implicated in frontotemporal dementia

Liam Clancy

University of Vermont

Tau, a microtubule associated protein (MAP), is primarily expressed within the axons of neurons and contributes to important cellular functions such as regulating microtubule dynamics, modulating axonal transport, and acting as a signaling molecule. One component of neurodegeneration, which includes disorders such as Alzheimer's Disease and Frontotemporal Dementia (FTD), is the formation of neurofibrillary tangles composed of Tau. Soluble Tau aggregates likely form from reduced microtubule binding interaction via hyperphosphorylation or point mutations in the Tau gene. Many pathological mutations are located within the C-terminal region of Tau, which contain the microtubule binding repeat motifs. Missense mutations within this region of Tau typically decrease microtubule binding affinity and promote aggregation. E342V-Tau, however, has been shown to increase tubulin polymerization, a behavior antithetical to the canonical view of how C-terminal mutations operate. We therefore hypothesized that E342V-Tau does not decrease its binding affinity for the microtubule when compared with the 4RL-Tau wild-type isoform. We present evidence that E342V-Tau and 4RL-Tau bind the microtubule in a similar manner, elucidating a potential distinct pathogenic mechanism for this mutation. Further characterization of E342V-Tau includes studying the binding behavior of single Tau molecules and its effects on kinesin-1 motility.

**Poster 7**

**Behavioral evaluation of brain EC-specifc Piezo1 gain of function mice**

Mohammad Elmahdy

University of Vermont

**Poster 8**

**Cholesterol depletion reduces Piezo1 activity of brain capillary endothelial cells**

Xin Rui Lim

University of Vermont

**Poster 9**

## Investigating mitochondrial transfer and targeting peroxide metabolism in diffuse midline glioma

Margaret Trout, Alqassam Abuarqoub, Trevor Wolf, Brian Cunniff, John Salogiannis, James Stafford

Diffuse midline glioma (DMG) is a devastating pediatric brain cancer that typically occurs in children and currently has a two-year survival rate of less than 10%. Therefore, efforts are underway to better understand this cancer and find more effective treatment options. Our current investigations center around the energy metabolism of these cancers, specifically through mitochondrial transfer and targeting the mitochondrial antioxidant enzyme PRX3. Towards the first aim, intact mitochondria have been shown to transfer between several cell types and evidence has suggested that this transfer may have important effects on the cells' metabolism and their response to chemotherapy in brain tumors. As such, we set out to investigate the possibility of mitochondrial transfer from neurons and glia to DMG, using live confocal microscopy and flow cytometry. Second, we build on work showing that the mitochondrial vulnerabilities in DMG, particularly ROS production, leading to a reliance on antioxidant enzymes such as peroxiredoxin 3 (PRX3). Encouraged by evidence that DMG are highly sensitive to the PRX3 inhibitor Thiostrepton, we are further seeking to understand this mechanism as drug target that might synergize with ONC201, a drug with great promise in clinical trials of DMG. Overall, we seek to identify better treatment options for DMG, specifically through investigating the role of the mitochondria and peroxide metabolism.

**Poster 10**

## Exploring medication-induced immunomodulation in early-stage parkinson's disease

Petra Bachanová[1], Steven Pike[1,2,3], Lucas Salas[1,3]

1 Dartmouth College, Integrative Neuroscience
2 Dartmouth Health, Department of Neurology
3 Geisel School of Medicine at Dartmouth, Department of Epidemiology

Parkinson's disease (PD) is often characterized by motor and cognitive deficits as well as immune dysregulation. Recent studies have highlighted differences in the immune cell profiles of PD patients, indicating a shift towards a more inflammatory phenotype as the disease progresses, with some suggesting a causal relationship between inflammation and disease onset. Inflammatory response is complex and can be influenced by many lifestyle and health factors, such as patient comorbidities and medications. This study aims to investigate the interplay between medication usage and immune cell profile changes in early-stage PD patients to better understand the immune dysregulation in this patient population and how they may affect the immune dynamics of their disease progression.
The Parkinson's Progression Markers Initiative (PPMI) database was chosen for its comprehensive data on medications in early-stage PD (PD medication-naïve) and DNA methylation data that enable interrogation of peripheral blood immune profiles. Participants included 83 Healthy Controls (HC, median age 61.9, 34% female), 175 Prodromal Patients (Prod, median age 63.2, 48% female), and 298 PD Patients (median age 63.2, 39% female). Only 15 PD patients were on L-DOPA during their baseline visit and were excluded from the analysis. 4572 unique medications (containing misspellings and generic and brand names) were clustered into 88 medication groups using a semiautomated approach, combining string-similarity-based Levenstein distance and a Large Language Model Data Analysis using the ChatGPT API. A manual curation of the groups followed to ensure accuracy. Once reclassified, we processed the data to identify trends in broader medication cohorts and calculated the Charlston comorbidity index to evaluate associations with participant mortality risk. Immune cell proportions were deconvolved using DNA methylation data using constrained projection/quadratic programming and a reference to 12 cell types as previously published by our group.
Preliminary results indicate variations in immune cell profiles associated with different medication groups, such as the increase in CD4 memory T cells in PD patients on antidepressants in contrast to those PD patients not taking antidepressants. This change is not apparent in HC and Prod on antidepressants compared to those naïve for this medication. Understanding the immunomodulatory potential of different medications is crucial for delivering personalized medicine to early PD patients and those at risk of the disease.
Keywords: Parkinson's disease, immune cell profiling, medication categorization, cohort analysis, immunomodulation.

**Poster 11**

## Modeling neuro-immune interactions in the anterior eye

Arielle Sclar[1, 2, 3], Anais Rhoades[3], Myles Ford[3], Pamela C. Rosato[3], Alexander G.J. Skorput[1, 2]

[1] Dartmouth College, Hanover, NH, Integrative Neuroscience at Dartmouth
[2] Dartmouth-Hitchcock Medical Center, Lebanon, NH, Department of Neurology
[3] Dartmouth College, Geisel School of Medicine, Department of Microbiology & Immunology

Dry Eye Disease (DED) is a disease of the anterior eye that is characterized by chronic pain and inflammation at the ocular surface. Patients with DED experience decreased quality of life due to their symptoms, as well as deficits in their visual acuity. This disease is thought to involve dysregulation of the adaptive immune system. Thus, understanding how the adaptive immune system interacts with pain sensing neurons (nociceptors) in the anterior eye is crucial to our understanding of this chronic pain disease. Many patients with DED have altered populations of resident memory T cells ($T_{RM}$) at their ocular surface. Our lab uses the clinically relevant herpes simplex virus-1 via ocular infection to produce a population of $T_{RM}$ at the ocular surface in mice. With this population of cells, we can model $T_{RM}$-nociceptor interactions in the context of DED.

**Poster 12**

## The role of Abl-dependent reverse signaling in zebrafish retinal development

Collin M. MacLeod, Caroline M. Dumas, Gillian G. Berglund, Bryan A. Ballif, and Alicia M. Ebert

The development of the nervous system is a complicated and intricate process, in which many signaling systems exist in harmony. One such system is semaphorin-plexin signaling. Semaphorins (sema) and plexins (plxn) are protein families necessary for a broad spectrum of development including but not limited to the central nervous system, the heart, and bone. We have previously identified Sema6A and PlxnA2 to be necessary for eye size, eye field cohesion, and retinal lamination. Upon Sema6A and PlxnA2 binding, bidirectional signaling events revolving around Sema6A and PlxnA2 are referred to as reverse and forward signaling, respectively. Using zebrafish and cultured cells as models, we have previously characterized important features of forward signaling through PlxnA2, however, reverse signaling via Sema6A remains largely uncharted. However, we have found that knocking down zebrafish sema6A and rescuing with a mouse mRNA encoding for a truncated version of the protein lacking the intracellular domain led to acellular regions in the retina while eye size was unaffected. To date, the characterized features of reverse signaling are dependent on the tyrosine kinase, Abl. We have identified Abl-dependent phosphorylation of the Sema6A intracellular domain by mass spectrometry. Here we explore the functional relevance of Abl-mediated reverse signaling on retinal integrity. We also describe the identification and characterization of Abl-dependent Sema6A binding partners. Together these data provide insight Sema6a reverse signaling.

**Poster 13**

## Molecular mechanisms of early eyefield cohesion in the developing zebrafish

Taylor Bean, Alicia Ebert, & Bryan Ballif

Department of Biology, University of Vermont

Semaphorin6A (Sema6A) is a transmembrane protein that plays important roles in development, including aspects of cell migration, adhesion, proliferation, and differentiation (Alto & Terman, 2017). Plexins are the main functional receptors of Semaphorins. When a Semaphorin binds to Plexin this initiates forward signaling through the intracellular domain of Plexin, which has been widely studied and shown to have a necessary role in zebrafish eye development (Ebert et al., 2014). Published data from our lab has shown that Sema6A functions to maintain cohesion in the eye field of zebrafish, however it is unknown which downstream signaling pathway(s) Sema6A/PlexinA2 are using to regulate eye cohesion (St. Clair et al., 2018 & 2019). Cultured eyefield explants show loss of integrity which is prevented by adding Sema6A. Using pharmacological inhibitors and conditioned media, we have identified the necessity of specific signaling pathways involved in the maintenance of eyefield cohesion.

**Poster 14**

## Insulin receptor signaling in sugar-sensing gustatory neurons impacts 'sweet' sensitivity

Christian Arntsen[1], Molly Stanley[1]

1 University of Vermont, Department of Biology, Burlington, VT

Hunger can directly modify the sensitivity of food-sensing chemosensory cells as a way to encourage food consumption when nutrients are low. Previous work in Drosophila melanogaster demonstrates that gustatory receptor neurons (GRNs) are directly impacted by food deprivation in a way that increases the detection and consumption of sugars. Dopamine plays a role in this modulation, but it is currently unknown if hunger/satiety hormones, such as insulin, also impact taste sensitivity. Using the single-cell transcriptomics database Fly Cell Atlas, we find that the insulin receptor (InR) is expressed in more than half of the cells identified as sugar-sensing GRNs based on expression of Gustatory Receptor 64f (GR64f), a sugar receptor. Therefore, we manipulated InR signaling specifically in sugar-sensing GRNs by using GR64f-Gal4 to drive expression of UAS-InR[DN], UAS-InR[CA], or UAS-InR[WT], to induce inactive, overactive, or wildtype signaling, respectively. We then performed in vivo calcium imaging to quantify sucrose-induced taste responses and behavioral assays to investigate the impact on 'sweet' sensing and sugar preferences in flies that were either sated (fed) or food-deprived for one day (starved). With starvation, 'wildtype' flies showed significantly higher sucrose sensitivity in the proboscis extension assay, increased preference for sucrose in the two-choice feeding assay, and increased calcium activity in sugar-sensing GRNs, as expected. InR[DN] expression led to subtle increases in sucrose sensitivity and calcium responses in the fed state compared to controls. Conversely, overactive InR signaling led to subtle decreases in sucrose sensitivity and calcium responses in the starved state. Overall, we conclude that InR signaling in sugar-sensing GRNs impacts 'sweet' sensitivity in a state-dependent manner.

**Poster 15**

## Amino acids activate parallel chemosensory pathways in Drosophila melanogaster

Grace Davis, Kayla Audette, Jessica Cerniglia, Molly Stanley

Mammals and insects alike depend on foodstuffs as an essential source of proteins and their constitutive amino acids, several of which cannot be synthesized by the organism. Amino acid consumption by D. melanogaster plays a crucial role in a variety of behaviors that impact the animal's survival and fitness, including feeding, mating, and egg laying. Underlying neural circuits that dictate such behaviors begin with the activation of distinct subsets of gustatory receptor neurons (GRNs) in the fly labellum, which subsequently transmit taste information to the brain for its integration. However, the mechanisms by which GRNs detect amino acid ligands to elicit characteristic behavioral responses are not fully characterized. The present study employs behavioral paradigms that evaluate the external and internal mechanisms of tryptone taste detection to describe the roles of the five distinct GRN classes within the labellum in amino acid sensing. We show that tryptone, a mixture of amino acids, reliably induces the proboscis extension response (PER) in female flies, and PER to tryptone is dependent on hunger and biological sex. We implicate multiple groups of GRNs in the detection and intake of amino acids through chronic and acute neuronal silencing methods, and use PER to tryptone and binary choice assays to demonstrate the individual contributions of three GRN populations. Furthermore, we describe the effects of ionotropic co-receptors IR76b and IR25a, which are widely expressed throughout the labellum, on tryptone PER and preference in binary choice assays. Future aims of this project will evaluate the involvement of water- and salt-detecting GRNs of the labellum in amino acid detection and characterize the full repertoire of gustatory and olfactory receptors involved in this process.

**Poster 16**

## Hysteresis plots highlight cross-frequency coupling in EEG analysis of narrative comprehension

Katie Ekstrom Grenon

University of Vermont

Characterizing the dynamic relationships between oscillatory frequency bands is increasingly prioritized in brain research. Visual representations of complex data can expand the potential for intuitive and meaningful interpretations. Hysteresis plots are often used in the fields of physics and engineering, and capture change over time in dynamically interacting variables. Because they represent and highlight the lag between an influential force and responsive changes, these plots are especially appropriate for visualizing the interactions hypothesized in cross frequency coupling; time-lag is considered intrinsic to the mechanism that couples neuronal cell assemblies.

In this time-frequency EEG study, we use hysteresis plots to illustrate the dynamic coupling of oscillatory frequencies during visual narrative comprehension. To explore how frequency bands interact over the course of narrative processing, we plotted hysteresis curves of power over time in several different frequency-band pairings. Our application of hysteresis visualizations to the cognitive process of story comprehension yields insight into the poorly understood relationship between alpha and low-beta suppression, as well as highlighting the contrast between lower-frequency couplings and those slower rhythms that influence gamma band activity.

**Poster 17**

## Modal cognition in rodents: Can rats reason about non-actual possibilities?

Catherine Holland, Eleonore Duvelle, Tyler Amell-Angst, Qasim Qureshi, Jacob Schumate, Matt van der Meer, Jonathan Phillips

Psychological Brain Sciences Department & Cognitive Sciences Department, Dartmouth College

Abstract: Modal cognition, the capacity to reason about necessities, possibilities, and impossibilities, has long been considered unique to humans. Recent studies on mental time travel, hippocampal replay, and animal counterfactual thought challenge this notion, suggesting a more widespread presence of this cognitive skill in the animal kingdom. This project investigates whether rats, in particular, exhibit modal cognition by exploring their ability to reason about possibilities in a specially designed Modal Maze. The maze features a symmetrical design, multiple decision locations, and an automated reward + door system which allows us to analyze the rats' adaptive strategies when faced with blocked possibilities. Our first pilot showed that rats successfully navigate the maze, demonstrating adeptness in finding the largest reward, exploiting it, and showcasing hierarchical reward preferences. Despite these successes, Pilot 2 revealed challenges in rats' ability to associate auditory cues with non-available blocked possibilities. This struggle, however, does not negate their capacity to reason about non-actual possibilities but highlights the intricate nature of associating auditory cues with potential outcomes (or non-outcomes). This study contributes to the ongoing discourse on the extent of cognitive abilities across species and investigates the potential role of hippocampal replay as a neural instantiation of modal thought in rats.

**Poster 18**

# Assessing fear deficits following shockwave-induced traumatic brain injury in mice: pitfalls and areas for improvement

Kathryn Bates, B.S.[1,2], Olivia MacLean, B.S.[2] , Dakota Ma, B.S.[2] , Krista DiSano, PhD[2-4] , Travis Todd, PhD[5] , Wilder Doucette, MD, PhD[2,6] , Paul Holtzheimer, MD[2,4,6,7] , & Crystal Noller, PhD[2,4,6,7] .

1 Integrative Neuroscience at Dartmouth graduate program, Dartmouth College, Hanover, NH
2 White River Junction VA Healthcare System, White River Junction, VT
3 Department of Neurology, Geisel School of Medicine at Dartmouth, Hanover, NH
4 Department of Veterans Affairs, National Center for Posttraumatic Stress Disorder, White River Junction, VT
5 Department of Psychological Science, University of Vermont, Burlington, VT
6 Department of Psychiatry, Geisel School of Medicine at Dartmouth, Hanover, NH
7 Department of Surgery, Geisel School of Medicine at Dartmouth, Hanover, NH

Traumatic brain injury (TBI) is a significant problem worldwide, especially for military personnel. Individuals affected by TBI are at higher risk for developing neuropsychiatric complications, including elevated and persistent fear responses, which are difficult to treat clinically. Animal models present an opportunity to develop treatments targeting underlying mechanisms, however, the temporal development of these deficits have not been well described. Our laboratory is currently evaluating behavioral consequences of shockwave-induced, blast TBI, using an experimental rodent model that recapitulates primary injuries sustained by exposure to an improvised explosive device in combat. The current study was designed to assess the temporal development of heightened fear following TBI. Adult male mice were subjected to experimental TBI or sham injury and allowed to recover. Subsequently, a 7-day fear conditioning assay was performed at either one week (acute phase) or six weeks (chronic phase) post-injury. Briefly, the assay consisted of fear conditioning (day 1) followed by contextual and generalized fear assessment (days 2 and 3, respectively) and fear extinction (days 4-7). Despite using a published assay, we did not detect measurable differences in fear responses between injury groups at either timepoint. Opportunities to refine the fear induction component of the assay and subsequent analytical approaches are discussed and invited.

**Poster 19**

## ABA and AAB fear renewal in male and female rats

Samantha K. Moriarty, Hannah L. Schoenberg, Neil E. Winterbauer, Sayamwong E. Hammack, Donna J. Toufexis, & Travis P. Todd

An extinguished response to a CS can return when experienced outside of the extinction context, a phenomenon known as renewal. It is not clear whether male and female rats renew similarly across conditions. A series of experiments investigated how male and female rats renew across ABA and AAB fear renewal preparations. In addition, a third group of ovariectomized female rats probed the role of female cycling hormones in renewal. All three groups renewed approximately equivalently in all preparations suggesting no sex difference or role for cycling hormones in fear renewal.

**Poster 20**

**A context-dependent cue-evoked neural code in the retrosplenial cortex**

Han Yin Cheng, Linghua Li, & Travis P. Todd

University of Vermont

The retrosplenial cortex, a dorsally located cortical structure in rats, receives inputs from sensory cortices including visual and auditory cortex, and has been implicated in a range of spatial and cognitive processes. Particularly, the role of the retrosplenial cortex in visual encoding and visually guided spatial behaviors is well-documented. In contrast, less is known about the role of the retrosplenial cortex in auditory as well as multi-sensory encoding. Here, we sought to further understand sensory processing in the retrosplenial cortex by recording single units in freely moving rats as they experienced three auditory (noise, high and low frequency tone) cues and a visual cue. These cues were presented in two operant chambers that served as distinct contexts; chambers differed with respect to odor, tactile, color and geometric cues. Consistent with the inputs retrosplenial cortex receives from both visual and auditory cortex, we observed cue-evoked responses to both visual and auditory cues. However, we did not observe any multi-sensory cued responses in retrosplenial neurons, suggesting parallel streams of information processing for visual and individual auditory cues. Surprisingly, cue-evoked responses also did not translate across contexts, indicating modulation of cue-evoked neural responses by context. Thus, our preliminary data suggests a context-dependent cue-evoked neural code in the retrosplenial cortex.

**Poster 21**

## Time as a contextual cue in operant conditioning

Emery W. Harlan, Eric A. Thrailkill, Mark E. Bouton, & John T. Green

Department of Psychological Science and Neuroscience Graduate Program, University of Vermont

Many types of background stimuli can serve as contextual cues for Pavlovian and operant conditioning (Bouton, 2019). For example, physical contexts (e.g., olfactory, visual, auditory, and tactile sensations) as well as internal states (e.g., hunger, stress, drugs) can modulate whether other stimuli predict important events. Similarly, animals can learn to use the intervals between events to modulate the meaning of other stimuli. The present experiments extended prior research (e.g., Bouton & García-Gutiérrez, 2006; Bouton & Hendrix, 2011) to assess the modulation of discriminated operant responding by a "temporal context" created by differing interval lengths. Rats were trained to respond (i.e., press a lever for food pellets) when a 10 second tone was presented after a long interval (4-min) (4+), but not to respond when a short interval (1-min) preceded the tone (1-). In experiment 1, Group 1 received the standard discrimination procedure (4+/1-), and Group 2 received a pseudo-discrimination procedure (4±/1±), where responding was reinforced on half of the trials for both interval lengths. On training days, rats were also exposed to a second (untrained) physical context (i.e., a second operant chamber with different features compared to the training context). During test sessions, when responding was not reinforced, rats that received the discrimination procedure responded more on trials preceded by a 4-min interval compared to trials preceded by a 1-min interval, while rats that received the pseudo-discrimination procedure responded in equal amounts to both trial types. When testing rats in the untrained context, we saw a significant reduction in responding overall, suggesting at least some control of responding by the physical context, in addition to the interval duration. In experiment 2, we trained rats with the standard (4+/1-) discrimination procedure, and also exposed them to the second, untrained, physical context with food pellets non-contingently dispensed at random intervals throughout the session. During the test session, rats exhibited more responding than in experiment 1, but had similar levels of responding to both the 4-min and 1-min cues (i.e., the temporal discrimination was lost) in the untrained context. Lastly, prior work from our lab has shown that a region of the rodent medial prefrontal cortex, the prelimbic cortex (PL), is important for contextual modulation of operant conditioning (Thomas et al., 2020, 2023a, 2023b; Trask et al., 2017). In experiment 3, after temporal discrimination training and prior to test sessions in the training context, we pharmacologically inactivated the PL cortex using the GABA receptor agonists baclofen and muscimol. Responding was reduced on both trial types, and the temporal discrimination was absent. Overall, the data suggest that temporal cues can serve as contextual stimuli in operant conditioning, along with the physical context, and that the PL cortex may play a role in mediating the effects of those cues.

**Poster 22**

## Adolescent gender diversity and substance use exposure: A mediating role of peer victimization

Annabel Diestel; Sarahjane L Dube; Sarah Rodrigues; Zoe Hulce; Arielle Cohn; Leigh-Anne Cioffredi; Dawn Bounds; Hugh Garavan; Alexandra Potter

Gender diverse adolescents are at higher risk for earlier substance use. This may be partially due to risk factors associated with minority stress. The present study aims to examine the relationships between gender diversity, substance use, and peer victimization at age 12-13 yrs.

**Method:**

The Adolescent Brain Cognitive Development Study 3-Year Follow-Up (data release 5.0) was used (n = 10,248; 52.5% female; ages 12-13 yrs). Multilevel mixed-effects logistic regression was used to model ever having a puff of marijuana and ever having an alcoholic beverage. Gender diversity was a 5 pt scale of felt-gender such that higher scores indicated more diversity. Three scales of peer victimization ranging from 3-15 were added to assess any mediating effect. Covariates included age (months), pubertal stage, and household income. Subjects were nested within families to account for siblings.

**Results:**

We found that, after controlling for covariates, each increase in gender diversity was associated with 1.66 times higher odds ($p=.003$) of having tried a puff of marijuana. However, after including peer victimization in the model, gender diversity was only associated with 1.47 times the odds ($p=.033$). Similarly, an increase in gender diversity was associated with a 1.79 times higher odds ($p<.001$) of having had an alcoholic beverage, which dropped to 1.59 times ($p=.005$) after including peer victimization scores in the models.

**Conclusion:**

We found that diversity of felt-gender in a large community sample, was associated with having tried marijuana or an alcoholic beverage by age 12-13 years old. Importantly, this study provides evidence to suggest that some of the disparities in substance use seen in gender diverse youth may be partially explained by other risk factors, such as being the victims of peer aggression.

**Poster 23**

## Exploring the impact of LSD on cognitive flexibility

Anna Lyndaker[1*], Petra Bachanova[1*], Mizuki Tojo[1], Matthew Ciolkowski[1], Johnathan Rodgers Gochicoa[1], Matthew Company[1], Moriah McGuier[1], Lucas Dwiel[2], Wilder Doucette[1,2]

1 Integrative Neuroscience at Dartmouth Graduate Program, Dartmouth College
2 Psychiatry, Dartmouth-Hitchcock Medical Center

Introduction: Recent reports on psychedelic-assisted therapies suggest promising treatment outcomes for a broad spectrum of psychiatric conditions, including depression, substance use disorders, and obsessive-compulsive disorder. A transdiagnostic feature of these disorders is diminished cognitive flexibility, or the ability to shift cognitive processes to adapt to changing environmental conditions. Patients with depression have shown enhanced cognitive flexibility as well as decreased depression scores after psychedelic therapy. Therefore, disrupting fixed mental and behavioral states could contribute to psychedelic's therapeutic efficacy. Currently, there are few studies focusing on the effects of a single psychedelic drug exposure on cognitive flexibility. This study explores how acute exposure to lysergic acid diethylamide (LSD), a canonical serotonergic psychedelic, affects cognitive flexibility as measured by reversal in a delayed alternation task.
Methods: Two cohorts of male (n = 12) and female (n = 8) Sprague Dawley rats were trained to perform a delayed alternation task. The rats were trained to alternate between two simultaneously presented levers with a 2 second delay interval in which levers were retracted. The reversal was initiated after five sessions (100 trials per session) of successful alternations (accuracy ≥ 90%). For the intervention, a saline or no injection control vs. LSD intraperitoneal injection was given one hour before the task (total LSD: male n = 5, female n = 4; Control: male n = 7, female n = 4). Successful reversal was defined as reaching 7 out of 10 correct responses on a continuous sliding trial basis. The number of trials, win-stay-lose-shift strategies, and accuracy was compared between LSD and control animals using Mann-Whitney U tests. These comparisons were made at three stages: until first reversal, on the day of first reversal, and three days post first reversal.
Results: Preliminary analyses reveal that the number of trials until first reversal was lower ($p = 0.0295$) in LSD rats (mean = 110, 95% CI [42, 179]) relative to controls (mean = 162, 95% CI [117, 208]). There were no other group differences in performance.
Conclusions: We found that rats treated with LSD needed fewer trials to successfully complete the initial reversal relative to controls, suggesting that a single dose of LSD boosted the capacity to learn a novel behavioral strategy. However, our study revealed no long-term benefits, as performance following first reversal was comparable between groups. Overall, the potential to facilitate new cognitive-behavioral patterns with psychedelics is an exciting therapeutic approach and should be investigated further.

**Poster 24**

## Effects of N,N-dimethyltryptamine on fear extinction and alcohol consumption

Caleb J. George-Hinnant, Kathryn A. Laprade, Cate Szpila, James M. Stafford

Mental illnesses including but not limited to anxiety, depression, and post-traumatic stress disorder (PTSD) are among the most significant contributing conditions leading to disability worldwide and rising suicide rates. Indolamine psychoplastogens such as psilocybin, lysergic acid diethylamide and N-N-dimethyltryptamine (DMT) have garnered interest in the treatment of mental illness disorders as the current standard of care for PTSD and depressive disorders typically requires several weeks to provide benefit. DMT, the hallucinogenic compound in Ayahuasca tea, has also accumulated attention and may provide meaningful effects in treatment resistant populations at psychoactive doses or sub-psychoactive doses in a shorter treatment period. DMT remains less studied in its therapeutic potential and has unique potential in efficacy given its rapid onset of action and short duration. In our studies, we aim to investigate both the acute and lasting effects of intraperitoneal DMT on fear extinction and alcohol consumption in C57BL/6J mice. In our first studies, mice were examined for stereotyped behavior of hallucinogenic effects of DMT in a neutral setting. Preliminary results suggest a U-type dose dependent effect on mobility state of mice. Once we established effective dosing with minimal locomotor effects, we tested the impact of DMT on alcohol relapse behavior in a binge drinking paradigm (Drinking in the Dark). In our third group of studies we evaluated whether DMT mediated an increase in synaptic plasticity catalyzed fear extinction and expression. Interestingly, DMT administration immediately prior to retrieval leads to decreased fear expression with lasting impacts on subsequent tests suggesting an extinction enhancement. It is worth noting that this effect appears limited to certain features of cued and contextual components of the session. Future studies will investigate a chronic low-dosing paradigm to link sub-hallucinogenic mediation of synaptic plasticity to changes in fear extinction, socialization, and anxiety.

**Poster 25**

## Housing effect on alcohol consumption in a binge drinking model following foot shock stress

Kathryn A. Laprade, James M. Stafford

Alcohol use disorder (AUD) has wide reaching impacts, both in the United States and globally. Annually, AUD accounts for over 2 million deaths in the United States and is the leading disease risk among those ages 25 to 49. The onset of the COVID-19 pandemic brought about traumatic instances of stress, mandated self-isolation, and unique alcohol-purchasing options. Overall, the United States witnessed increased alcohol sales and consumption early in the pandemic, concurrent with many stay-at-home mandates. Literature suggests that impactful instances of stress as well as social isolation may have led to these early increases in alcohol consumption. A primary goal of this project was to evaluate the ability of social isolation to modulate alcohol consumption following an adverse event. To accomplish this, I employed a well-characterized, repeated foot shock (adverse event) combined with chronic single or paired housing conditions to model social isolation. The interaction between housing condition and foot shock on ethanol consumption was explored using an established binge-drinking model, Drinking in the Dark (DID). I hypothesized that the combination of isolation and experience of repeated foot shock would lead to the highest levels of alcohol consumption as compared to paired and control groups. During DID, an effect of shock was seen in female mice only, with shocked mice consuming significantly more alcohol than not shocked. This sex effect remained through the final day of the DID procedure with no significant effect of housing. Though results from this model did not mimic the rise in drinking levels as seen correlated with social isolation during the COVID-19 pandemic, it has bridged a current gap in combination adverse event and social isolation animal models. Results from this study also surfaced some interesting sex differences that could use further studying.

**Poster 26**

## Acute stress facilitates habitual behavior in female rats

Russell Dougherty[a], Eric A. Thrailkill[a,b,c], Zaidan Mohammed[a], Sarah VonDoepp[a], Ella Hilton-VanOsdall[a], Sam Charette[a], Sarah Van Horn[a], Adrianna Quirk[a], Adina Kraus[a], and Donna J. Toufexis[a]

[a]Department of Psychological Science, University of Vermont, 2 Colchester Ave, Burlington VT05405
[b]Department of Psychiatry, The Robert Larner, M.D. College of Medicine, University of Vermont, 1 South Prospect Street, MS 446AR6, Burlington, VT 05401
[c]Vermont Center on Behavior and Health, University of Vermont, 1 South Prospect Street, MS 482, Burlington, VT 05401

Instrumental behavior can reflect the influence of goal-directed and habitual systems. Contemporary research suggests that stress may facilitate control by the habitual system under conditions where the behavior would otherwise reflect goal-directed control. However, it is unclear how stress modulates the influence of these systems on instrumental responding to achieve this effect, particularly in females. Here, we examined whether acute restraint stress experienced before acquisition training (Experiment 1), or prior to the test of expression (Experiment 2) would influence goal-directed and habitual control of instrumental responding in female rats. In both experiments, rats acquired an instrumental nose-poke response for a sucrose reward. This was followed by a reinforcer devaluation phase in which half the rats in Stressed and Non-Stressed conditions received pairings of the sucrose pellet with illness induced by lithium chloride until they rejected the pellet when offered. The remaining rats received a control treatment consisting of pellets and illness on separate days (Unpaired). Control by goal-directed and habitual systems was evaluated in a subsequent nonreinforced test of nose poking. The results of Experiment 1 indicated that the Non-Stressed Paired group reduced nose-poking compared to the Unpaired controls, identifying the response as goal-directed, whereas the Stressed Paired and Unpaired groups made a similar number of nose pokes identifying the response as habitual despite the same amount of training. Results from Experiment 2 indicated habitual control of nose-poke responding was present when stress was experienced just prior to the test. Collectively, these data suggest that stress may facilitate habitual control by altering the relative influence of goal-directed and habitual processes underpinning instrumental behavior. These results may be clinically relevant for understanding the contributions of stress to dysregulated instrumental behavior in compulsive pathologies.

**Poster 27**

# PAC1 receptor antagonism in the medial habenula rescues anhedonic effect of chronic stress in rats

Nicholas R. Fontaine[1], Mahafuza Aktar[1], Melissa Boucher[1], Khalil AbedRabbo[1,2,] William Black[1], Aimee Mahler[1], Victor May[2] & Sayamwong E. Hammack[1]

Affiliations:
1. Department of Psychological Science, University of Vermont, 2 Colchester Avenue, Burlington, VT, 05405, United States
2. Department of Neurological Sciences, University of Vermont Larner College of Medicine, 149 Beaumont Avenue, Burlington, VT, 05405, United States.

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Previously, we have demonstrated that the activation of the central pituitary adenylate cyclase activating polypeptide (PACAP) systems plays a crucial role in mediating the effects of exposure to stressors. Additionally, chronic stress leads to a significant increase in PACAP expression in the bed nucleus of the stria terminalis (BNST). We have argued that this increase is vital for the behavioral and physiological consequences resulting from exposure to stressors. By using PACAP-ires-Cre mice with Cre-dependent reporter expression, we have identified a PACAP projection from the BNST to the medial habenula (MHb) and have also confirmed the presence of PAC1 receptors in MHb neurons. Anhedonia, which refers to the inability to experience pleasure from typically enjoyable activities, is a common symptom of depression. It is also a behavioral consequence of chronic stress in rodents and may be influenced by MHb function. We have previously shown PACAP infusions to the MHb to be sufficient to induce anhedonia as measured by the sucrose preference test—and that such behavior is PAC1-dependent. Here we investigated whether PACAP antagonism would block the effects of chronic stress. To do so, male rats were subjected to a two-week chronic variate stress paradigm and received bilateral MHb infusions of PAC1 antagonist PACAP(6-38) prior to sucrose preference testing. We found that PAC1 antagonism rescued the anhedonic effects of stress and restored sucrose preference to levels comparable those in no-stress controls. This, along with our previous findings, suggests that the binding of PACAP to PAC1 receptors in the MHb is both necessary and sufficient to induce anhedonia in male rats.

**Poster 28**

## PAC1 expressing neurons in the bed nucleus of the stria terminalis (BNST) and their projections

Mahafuza Aktar, Victor May and Sayamwong E. Hammack

University of Vermont

Pituitary adenylate cyclase activating polypeptide (PACAP) is a highly conserved neuropeptide playing essential roles in numerous physiological functions, and we and others have implicated central PACAP neurocircuits in mechanisms by which stressor exposure increases anxiety. PACAP binds to several receptor subtypes, including PAC1, VPAC1 and VPAC2, to activate several signaling cascades that can alter neuronal excitability as well as enhance indices of neuroplasticity. We have demonstrated that PACAP release in the bed nucleus of the stria terminalis (BNST) is critical for many of the behavioral and physiological consequences of stressor exposure, and much of our prior work has suggested that these effects of BNST PACAP anxiogenic behavior depend on the activation of PAC1 receptors. Moreover, we have shown that chemogenetic inhibition of BNST PAC1-expressing neurons reduces anxiety-related behaviors in mice. Here, we use PAC1-ires-Cre mice to examine the projection targets of BNST PAC1 neurons. We infused AAV2-EF1a-DIO-mCherry reporter vector into the BNST region of PAC1-ires-Cre mice to identify BNST PAC1expressing neurons, and examined mCherry expression in several downstream targets of the BNST. We observed robust PAC1 expressing neurons in BNST. PAC1 neurons were observed in anterior, posterior and ventral regions of the BNST. We also observed mCherry expression in the nucleus accambens (NAc), paraventricular nucleus of thalamus (PVT), lateral habenula (LHb), lateral hypothalamus (LH) and substantia nigra (SNR), consistent with other reports demonstrating that these brain regions receive BNST projections. These observations help to clarify the neural circuits involved in anxiety like behavior.

**Poster 29**

# Modulation of emotion perception via amygdala stimulation in humans

Camilo Castelbloanco Riveros

Dartmouth College

**Poster 30**

## Assessing behavior and HPA-axis related changes in a SPS model for PTSD in rats

Moriah McGuier, Anna Lyndaker, Lucas Dwiel, Matthew Company, Elise Bragg, Paul Holtzheimer, Wilder Doucette

Background: Understanding the factors that contribute to the development and maintenance of the chronic effects of post-traumatic stress disorder (PTSD) remains an ongoing challenge. The hypothalamic-pituitary-adrenal (HPA) axis and circulating glucocorticoids (i.e., cortisol, corticosterone) are believed to play a role in the response to stress and PTSD development. The single prolonged stress (SPS) model for PTSD in rats has been previously used to investigate behavioral and HPA-related changes acutely following trauma-exposure. This preliminary work aims to investigate anxiety-like and social behaviors following SPS at multiple timepoints, including paired serum collection for corticosterone assessment. We hypothesized that SPS exposed rats would have increased anxiety-like and social-avoidance behavior and blunted corticosterone levels one-week following SPS. Through the understanding of behavior and corticosterone changes, we plan to later explore the efficacy of MDMA-assisted therapy on PTSD-like symptoms in this preclinical model. Methods: 12 male Sprague-Dawley rats were exposed to the SPS model with 12 paired controls. SPS involves exposure to three acute stressors (e.g. restraint, forced swim, loss of consciousness via diethyl ether) followed by a quiescent week. Serum samples from multiple timepoints were collected to assess corticosterone changes via ELISA. Behavioral effects of SPS were examined at 1 and 2 weeks post-SPS, including open field (OF) and a social motivation (SM) task. We utilized video analysis and automated behavioral scoring to assess multiple behavioral metrics which were statistically tested using 2-way Anova. Results: During OF (700 lumens center), we did not detect anxiety or locomotion related group differences. During the SM task, SPS rats had a significantly higher % of time immobile ($p=0.016$) but no other group differences were detected in other metrics (i.e., percent time interacting with novel rat or novel object, frequency, or number of alternations). Preliminary data (N=6 for each group) for corticosterone levels at baseline, 1 week and 2 weeks following SPS exposure did not show significant group differences in corticosterone post-SPS ($p>0.05$). Conclusions: Preliminary results from behavioral metrics in OF and SM tasks did not reveal substantial anxiety-like or social-avoidance group differences. General locomotion and exploratory behavior in OF were low for both groups, which we theorize was related to the 700-lumen center light, and future OF will be tested at 400-lumen center to reduce floor effects. SPS-exposed animals did have an increased level of immobility during the SM task, which may be linked to low motivation to explore a novel rat or novel object following trauma exposure. The limited behavioral effects following SPS, and an initial lack of a corticosterone signal suggest that optimization of the SPS model may be required.

**Poster 31**

## Effects of enteric bacterial tryptophan on anxiety-related behavior, serotonin chemistry, and neuronal activity in the brain

Lily Russo-Savage[1], Theresa B. Legan[1], Emilia Norberg[2], Margaret Vizzard[1], Matthew J. Wargo[2], Sayamwong Hammack[3], Gary M. Mawe[1], Brigitte Lavoie[1]

Department of Neurological Sciences[1], Department of Microbiology and Molecular Genetics[2], and Department of Psychology[3], University of Vermont, Burlington, VT, USA;

The microbiota-gut-brain axis is a rapidly emerging area of focus that has led to important implications of influences that gut health and functioning can have on the brain. Serotonin (5-hydroxytryptamine, or 5-HT) is a versatile signaling molecule that acts as a neurotransmitter in the brain amongst regions that regulate anxiety. Tryptophan (Trp), the precursor to 5-HT, is an essential amino acid that can be obtained through the diet as well as through bacterial synthesis in the gut. However, there is a gap in research determining if levels of 5-HT in the brain can be affected by Trp-producing bacteria in the gastrointestinal tract. The Mawe-Lavoie lab has previously demonstrated that short-term oral administration of the Trp synthesizing bacteria *Bacillus (B.) subtilis* has dramatic effects on colonic motility and 5-HT signaling as well as circulating Trp levels. Preliminary data from our lab has suggested similar treatment with B. subtilis decreases levels of molecules associated with 5-HT signaling in the hippocampus and brainstem. Finally, preliminary data from our lab has demonstrated significant increases in cFos immunoreactivity in the amygdala, and paraventricular nucleus of the thalamus (PVN) after short-term treatment with B. subtilis and restraint stress. These results have led to further questions how long-term treatment with B. subtilis will affect the 5-HT signaling in the same areas of interest as well as the effects on downstream anxiety behaviors.

**Poster 32**

## Tryptophan-synthesizing bacteria enhance colonic serotonin signaling and gastrointestinal motility

Theresa B. Legan[1], Lily Russo-Savage[1], Brigitte Lavoie[1], Emilia Norberg[1], Stephanie Tack[1], Thomas A. Tompkins[2], Matthew J. Wargo[3], Gary M. Mawe[1]

Department of Neurological Sciences[1] and Department of Microbiology and Molecular Genetics[3] University of Vermont, Burlington, VT, USA; Lallemand Bio Ingredients, Montreal, QC, Canada[2]

An emerging strategy to treat symptoms of gastrointestinal (GI) dysmotility utilizes the administration of isolated bacteria. However, the underlying mechanisms of action of these bacterial agents are not well established. Here, we elucidate a novel strategy to promote serotonin (5-HT) signaling and intestinal motility by exploiting the biochemical capability of specific bacteria to produce the 5-HT precursor, tryptophan (Trp). To test our hypothesis, mice were treated daily for one week by oral gavage of *Bacillus (B.) subtilis* (R0179), heat-inactivated R0179, or a tryptophan synthase-null strain of *B. subtilis* (1A2). Tissue levels of Trp, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were determined and changes in motility were measured. Mice treated with *B. subtilis* R0179 exhibited greater colonic tissue levels of Trp and increased 5-HT signaling, as indicated by an elevated 5-HIAA/5-HT ratio, compared to vehicle-treated mice. Furthermore, *B. subtilis* treatment accelerated colonic motility in both healthy mice as well as in a mouse model of constipation. These effects were not observed with heat-inactivated R0179 or the live 1A2 strain that does not express tryptophan synthase. Lastly, we found that the prokinetic effects of *B. subtilis* R0179 were blocked by co-administration of a 5-$HT_4$ receptor (5-$HT_4$R) antagonist and were absent in 5-$HT_4$R knockout mice, demonstrating the involvement of the 5-$HT_4$R in mediating the bacteria's prokinetic actions. Taken together, these data demonstrate that serotonergic signaling and intestinal motility can be augmented by treatment with bacteria that synthesize Trp. Our findings provide mechanistic insight into a transient and predictable bacterial strategy to promote GI motility.

**Poster 33**

**Protective role of ursodeoxycholic acid (UDCA) on gallbladder smooth muscle in gallstone disease.**

Brigitte Lavoie*, Isabella C. Ley, Matthew J. Kwasnik*, Onesmo B. Balemba†, Grant W. Hennig and Gary.M. Mawe*

* University of Vermont, Burlington, VT; †University of Idaho, Moscow, ID.

Hallmarks of gallstone disease include increased cholesterol and hydrophobic bile salts in bile, inflammation (cholecystisis), and gallbladder smooth muscle (GBSM) dysfunction. Cholesterol and hydrophobic bile salts can disrupt GBSM activity by activating distinct pathways (PMIDs 10564108 and 20624794). Increased cholesterol concentrations attenuate GBSM function by inhibiting Ca2+ channel activity and the binding of excitatory agonists to their receptors. Hydrophobic bile salts disrupt GBSM activity by binding to G-protein bile acid receptor 1 (GPBAR1, also known as TGR5), which activates cAMP-PKA pathway leading to an inhibition of GBSM via KATP channel-mediated hyperpolarization. Protecting GBSM function from these effects could help prevent cholestasis, which is linked to gallstone formation (cholelithiasis). A number of animal and human studies have suggested that the hydrophilic bile salt, ursodeoxycholic acid (UDCA), can preserve gallbladder function and prevent cholelithiasis, but a direct effect of UDCA on GBSM function have not been determined. In the current study we used Ca2+ imaging (mouse and guinea pig gallbladders) and intracellular recording techniques (guinea pig gallbladders) to test if UDCA can elicit a protective effect on GBSM from mice fed a lithogenic diet (LD) or in ex vivo preparations of GBSM treated with cholesterol or bile salts. In LD mice, GBSM function was relatively normal GBSM when mice received UDCA in their drinking water (12 mg/kg/day) as compared to LD-fed mice with untreated water. UDCA (100 µM by itself) had little or no direct effect on GBSM function in ex vivo preparations. Pretreatment of gallbladder preparation with UDCA protected GBSM from the disruptive effects of hydrophobic bile salts CDCA (50 µM) and LCA (10 µM), or cholesterol (50 µg/ml). Interestingly, the protective role of UDCA in other systems has been attributed to the activation of PI3K/Akt signaling pathway. Similarly, we found that UDCA increased Akt phosphorylation in GBSM preparations, as detected by immunohistochemistry and immunoblotting, and this effect was blocked by the PI3K inhibitor, LY294002 (50 µM). Furthermore, in the presence of LY294002, UDCA failed to protect GBSM from the disruptive actions of CDCA (50 µM). Collectively, these data support a role for UDCA in protecting GBSM function and suggest that the mechanisms of action involve the PI3K/Akt signaling pathway. This study is supported by NIH grant DK131044

**Poster 34**

# Gold nanoparticle modification of carbon fiber microelectrodes for *in vivo* acetylcholine detection using fast scan cyclic voltammetry

Owen Neilan

University of Vermont

Millions of people suffer from Alzheimer's disease (AD) and symptoms of dementia. AD and dementia are characterized by loss of cognitive function, which decreases quality of life for patients and their caretakers. As the baby boomer generation approaches the age at which these symptoms arise, not only is the disease detrimental to the quality of life, but society as a whole is also faced with healthcare and economic challenges. The role of the neurotransmitter acetylcholine (ACh) in AD is currently being explored, as it is suspected to play a role in learning and memory (Hasselmo, 2006). Specifically, a decrease in cholinergic transmission has been correlated with cognitive decline seen in AD and dementia (Blokland, 1996). Despite its importance, the available technology to measure ACh *in situ* is currently not adequate in detecting the neurotransmitter in such a small spatial and short time scale. We aim to increase the sensitivity of this type of sensor by modifying the surface of carbon fiber microelectrodes (CFMs) with gold nanoparticles (AuNPs). The increased sensitivity can be verified using fast scan cyclic voltammetry (FSCV), which will read the signal output of a CFM in a flow cell. Our hypothesis is that lower ACh levels will be found in animal models with greater cognitive decline. This project aims to construct a small, biocompatible sensor that can measure ACh *in vivo* in dementia models to better understand the neurotransmitter's role in the cognitive decline associated with AD.

**Poster 35**

## Frequency of spontaneous neurotransmission at individual boutons corresponds to the size of readily releasable pool of vesicles

Amelia J Ralowicz, **Mizuki Tojo**, Sasipha Hokeness, Michael B Hoppa*.

Synapses maintain two forms of neurotransmitter release to support communication in the brain. First, evoked neurotransmitter release is triggered by the invasion of an action potential across en passant boutons that form along axons. The probability of evoked release (Pr) varies substantially across boutons, even within a single axon. Such heterogeneity is the result of differences in the probability of a single synaptic vesicle fusing (Pv) and in the number of vesicles available for immediate release, known as the readily-releasable pool (RRP). Spontaneous release (also known as a mini) is an important form of neurotransmission that occurs in the absence of action potentials. Because it cannot be triggered with electrical stimulation, much less is known about potential heterogeneity in the frequency of spontaneous release between boutons. We utilized a photostable and bright fluorescent indicator of glutamate release (iGluSnFR3) to quantify both spontaneous and evoked release at individual glutamatergic boutons. We found that the rate of spontaneous release is quite heterogenous at the level of individual boutons. Using a new optical method to measure RRP at individual boutons, we found that this heterogeneity in spontaneous release was strongly correlated with the size of the RRP, but not related to Pv. Calcium plays an important role in both Pv and the RRP, so we went on to quantify contributions of voltage-gated calcium channels and ryanodine receptors to evoked release and spontaneous release. We conclude that the RRP is a critical and dynamic aspect of synaptic strength that contributes to both evoked and spontaneous vesicle release.

**Poster 36**

## Characterization of SH protein family interactions using proteomic analysis

Phoebe M. Cousens, Brendan W. Chandler, Anna M. Schmoker, Jaye L. Weinhart, Charlotte A. Kearns, Amila Semic, Alicia M. Ebert, & Bryan A. Ballif.

Department of Biology, University of Vermont

Chicken tumor virus #10 regulator of kinase (CRK) and CRK-like (CRKL) function in several phosphotyrosine-dependent signaling pathways that mediate cell adhesion, proliferation, and motility during neurodevelopment [1-6]. CRK and CRKL are adaptor proteins that bind to other proteins with their Src Homology 2 (SH2) and Src Homology 3 (SH3) domains. CRK has two isoforms, CRKI, which has one C-terminal SH3 and one N-terminal SH2 domain, and CRKII, which, like CRKL, has two C-terminal SH3 domains and one N-terminal SH2 domain [7-9]. The SH3 domains often bind to upstream or downstream signaling proteins, while interactions between Crk family SH2 domains and other proteins are responsible for the localization of Crk family members [7, 8]. Understanding what proteins an SH2 domain binds to is essential for understanding the protein's functions and interaction mechanisms. CRK family SH2 domains selectively bind to a phosphorylated motif pYXXP (pY = phosphorylated tyrosine; X = any amino acid; P = proline) [8]. Among all YXXP containing proteins, the Src homology 2 domain-containing (SH) protein family is one protein family enriched in YXXP sites, suggesting that they bind to CRK- and CRKL-SH2 domains in a phosphorylation dependent manner. The SH protein family consists of four proteins, SHB, SHD, SHE, and SHF, each with an SH2 domains and 6, 5, 4, and 3 YXXP sites, respectively. Our studies have shown that in the presence of the kinase Abl, nearly all YXXP sites within the SH protein family can be phosphorylated. This phosphorylation is both necessary and sufficient to induce direct binding to the SH2 domain of CRKL. Since SH family proteins also have SH2 domains, this suggests that SH family members may also act as adaptor proteins. Surprisingly, our experiments demonstrate a lack of functionality in SH family SH2 domains, leading us to believe that the SH family may be a family of pseudo-SH2 domain-containing proteins.

**Poster 37**

## Delay of habitual development following gonadectomy in female rats

Zaidan Mohammed*, Hannah Schoenberg, Daisy Powers, Russell Dougherty, Adina Kraus, Sarah Van Horn, Sarah VonDoepp, Ella Hilton-Vanosdall, Francesca Carasi-Schwartz, and Donna Toufexis

Abstract
This study explores the expression of habit in ovariectomized (OVX) female rats. Two experimental groups of OVX female rats were trained to 160 response outcomes (R-Os), a threshold of training where intact female rats usually have transitioned to habit, and to 240 R-Os, where intact females show strong habitual behavior. A separate experimental group of intact females trained to 240 R-Os was included as a control. Results replicated our earlier finding that intact females show robust habitual behavior at 240 R-Os. Removal of ovarian hormones caused OVX females to remain goal-directed at both 160 and 240 R-Os, indicating that ovarian hormones are involved in enhancing habitual behavior in intact female rats. These findings hold significant clinical implications for the development of therapeutic interventions aimed at targeting hormonal pathways to modulate habit-like processes such as addiction in females.

**Poster 38**

## Determining the mechanism by which testosterone enhances place and response learning in adult male rats

Mark D. Spritzer, Hieu H.M. Nguyen, Jessica D. Guo, Thomas D. Khodadad, Tinglin Shi, Em M. Luber, Abigail G. Griffin, Nicholas J. Tuta, Kevin J. Zhang, Michael B. Dash

Department of Biology, Department of Psychology, and Program in Neuroscience, Middlebury College, Middlebury, Vermont, U.S.A.

Testosterone has complex dose-dependent effects on spatial working memory in male rats, with a low physiological dose enhancing response learning and a high physiological dose enhancing place learning. The mechanism by which testosterone has these effects remains unknown but may involve direct actions on the brain through androgendependent and/or estrogen-dependent molecular pathways. In two experiments, we tested whether the effects of testosterone on place and response learning are androgen dependent. Adult male rats (3-8/group) were bilaterally castrated and given daily injections of various drugs starting 7 days prior to maze habituation and continuing through habituation and testing. A plus-maze was used to test rats on either a place task (learning the position of a goal arm relative to distal cues) or a response task (learning to turn a particular direction). Our first experiment involved testing the effects of 5α-dihydrotestosterone (DHT) at three doses (0.5, 1.0, and 2.0 mg/kg) along with a negative control group (sesame oil) and positive control groups (0.5 or 2.0 mg/kg testosterone propionate). On the place task, a high dose of DHT (2.0 mg/kg) significantly improved performance relative to the oil-injected control group. On the response task, both low (0.5 mg/kg) and intermediate (1.0 mg/kg) doses of DHT improved performance. In our second experiment, rats were given testosterone doses that were previously shown to enhance place or response learning along with an aromatase inhibitor (1 mg/kg letrozole) to reduce testosterone's conversion to estradiol. A high dose of testosterone (2.0 mg/kg) was again found to enhance place learning, whereas letrozole had no effect on place learning. In contrast, neither letrozole nor a low dose of testosterone were found to influence response learning, but this experiment needs further replication (n = 3-6/group). Both experiments indicated that testosterone enhances place learning through an androgen-dependent pathway. The results for response learning are less clear but suggest that androgens are only partially responsible for testosterone's effects on response learning. In a third experiment, we injected testosterone intra-cranially into either the dorsal hippocampus or dorsolateral striatum, followed by testing on the place task (n=12-14/group) or response task (n = 6-8/group), respectively. Subjects were assigned to one of three intracranial doses of testosterone (0.1, 1.0, or10 $\mu$g/hemisphere) or a control injection of DMSO, and all groups received three infusions at 2, 24, and 48 h before testing. We found that a high dose of testosterone infused into the hippocampus impaired place learning slightly. In contrast, low and high doses of testosterone infused into the striatum significantly improved response learning, indicating that androgens can have direct actions upon the striatum. Our results with intracranial testosterone injections do not correspond directly with our results with systemic testosterone injections, but it is unclear how the systemic doses are metabolized in the brain, and the duration of injections was longer for our systemic experiments.