SPEAR 2026
Schedule

Sunday, March 22, 2026	
-	*Arrive at University of Calgary Barrier Lake Field Station*
7:30 - 10:00 PM	Pizza Dinner Meet & Greet in the Dining Hall

Monday, March 23, 2026	
7:30 - 8:45 AM	Breakfast in the Dining Hall
8:45 - 9:00 AM	**Introduction** Matthew Hill, PhD (UofC)
Session 1 - Stress, Aversion, and Pain Session Chair: Matthew Hill, PhD (UofC)	
LONG FORM TALKS	
9:00 - 9:20 AM	Raymond Shim, PhD (Kubes Lab, UofC) *Sympathetic Nerves Maintain Kupffer Cell Identity, Phenotype, and Function During Homeostasis and Stress*
9:20 - 9:40 AM	Mijail Rojas-Carvajal, PhD (Hill Lab, UofC) *Paradoxical Effects of THC on the Consequences of Acute Stress*
9:40 - 10:00 AM	Aoi Ichiyama, PhD (Cembrowski Lab, UBC) *Network Mechanisms Regulating Functional States of Hypothalamic Stress Effector Neurons*
SHORT FORM TALKS	
10:00 - 10:07 AM	Patrick Grovue (Gordon Lab, UofC) *Stress-Induced Sensitization of CRH PVN Neurons*
10:07 - 10:15 AM	Alex Garcia (Stuber Lab, UW) *Lateral Habenula Neurons Code Different Timescales of Stress*

10:15 - 10:22 AM	Jessie Yu (Zamponi Lab, UofC) *LC–mPFC Noradrenergic Signaling and Peri-LC Microcircuits Differentially Regulate Anxiety and Pain*
10:22 - 10:30 AM	Christopher Dedek (Prescott Lab, UofC) *Using RAMalgo the Robot to Remove Human Variability in Rodent Pain Testing*
10:30 - 11:00 AM	Coffee Break in the Dining Hall
Session 2 - Anxiety and Emotion Session Chair: Michael Bruchas, PhD (UW)	
LONG FORM TALKS	
11:00 - 11:20 AM	Marta Trzeciak (Stuber Lab, UW) *Dynorphinergic Modulation of Paraventricular Thalamic Circuits Across Aversive States*
11:20 - 11:40 AM	Brandy Briones, PhD (Stuber Lab, UW) *Posterior Paraventricular Thalamus Modulates Socially-biased Aggression*
11:40 - 12:00 PM	S. Gibson Cook, PhD (Bains/Mayo/Hill Labs, UofC) *Psilocybin Induces Sex- and Context-specific Recruitment of the Stress Axis*
SHORT FORM TALKS (Tools & Techniques)	
12:00 - 12:07 PM	Jamie Sanson (Cone Lab, UofC) *An Open-Source Closed-Loop Behavioural Control System for the Raspberry Pi*
12:07 - 12:15 PM	Lily Torp (Berndt Lab, UW) *FentSense1.7: A Genetically Encoded Fluorescent Opioid Sensor for Characterizing Fentanyl Pharmacology in vivo*
12:15 - 12:22 PM	Aida Moghadasi (Berndt Lab, UW) *Engineering a Protein-based Fluorescent Sensor for Real-time Monitoring of Estradiol in the Brain.*
12:22 - 12:30 PM	Abhi Aggarwal (Lohman Lab, UofC) *Engineering High-Performance Biosensors for Real-Time Neural Circuit Imaging*
12:30 - 2:00 PM	Lunch in the Dining Hall
Session 3 - Pain and Cannabinoids Session Chair: Benjamin Land, PhD (UW)	
LONG FORM TALKS	
2:00 - 2:20 PM	Erika Harding, PhD (Zamponi/Trang Labs, UofC) *Locus Coeruleus Microcircuitry Processes Periaqueductal Grey Inputs into Distinct Outputs for Regulation of Pain and Anxiety*

2:20 - 2:40 PM	Madalyn Critz (Land/Bruchas Labs, UW) *Imaging the Neural Encoding Dynamics of Chronic Pain Transition in the Ventrolateral Periaqueductal Gray*
2:40 - 3:00 PM	Frank Salazar (Delevich Lab, WSU) *Investigating the Role of Cannabinoid 1 Receptor (CB1R) in Mediating Hypolocomotor, Antinociceptive, and Hypothermic Effects of Vaporized Cannabis Extract in Mice*
SHORT FORM TALKS	
3:00 - 3:07 PM	Matteya Proctor (McLaughlin Lab, WSU) *Impacts of Minor Cannabinoids and Terpenes on Perimenopause & Menopause Symptoms*
3:07 - 3:15 PM	Erika Lutz (Cuttler Lab, WSU) *Psychological and Physiological Effects of Cannabigerol (CBG): Results from a Clinical Trial*
3:15 - 3:22 PM	Katarzyna Anna Dudek, PhD (Hill Lab, UofC) *Astrocytic 2-AG Metabolism in Chronic Stress Related Neuroinflammation*
3:22 - 3:30 PM	Stephanie Rha (Teskey Lab, UofC) *The Distinct Sex- and Route-Specific Effects of Δ9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) on Postictal Hypoxia*
3:30 - 5:30 PM	**Coffee and Posters**
-	Dinner *(self-arranged)* Coordinate amongst yourselves to drive into Canmore or Kananaskis Nordic Lodge

Tuesday, March 24, 2026

7:30 - 9:00 AM	Breakfast in the Dining Hall
Session 4 - Addiction Chair: Leah Mayo, PhD (UofC)	
LONG FORM TALKS	
9:00 - 9:20 AM	Sierra Schleufer, PhD (Coffey Lab, UW) *Lateral Habenula Activity During Negative Affective States of Fentanyl Use in Rats*
9:20 - 9:40 AM	Ari Peden-Asarch (Neumaier Lab, UW) *Lateral Habenula Dynamics Encode the Shift in Internal Attitude for Fentanyl Seeking Behavior as Adverse Consequences Rise*
9:40 - 10:00 AM	Todd Appleby, PhD (Golden Lab, UW) *Strain and Sex Differences in Oral Fentanyl Self-administration in Mice*
SHORT FORM TALKS (Psychedelics & Opioids)	
10:00 - 10:07 AM	Micaela Ruiz (Land/Chavkin Labs, UW) *Serotonin and Dopamine in the Nucleus Accumbens is Modulated by Dynorphin/ Kappa Opioid Receptor (KOR) Activation of p38α Mitogen-activated Protein Kinase*
10:07 - 10:14 AM	Hadi Semizeh (Borgland Lab, UofC) *Optogenetic Activation of LH Orexin/Dynorphin Inputs to the VTA Enhances BLA Dopamine Dynamics and Reveals Morphine-induced Sensitization*
10:15 - 10:22 AM	Kyra Diaz (postbac, Ferguson Lab, UW) *Characterizing the Effect of Psychedelics on Social Motivation After Polysubstance Methamphetamine and Fentanyl Withdrawal*
10:22 - 10:30 AM	April Contreras, PhD (Hill Lab, UofC) *Role of Endocannabinoids in the Behavioral Effects of Psychedelics*
10:30 - 11:00 AM	Coffee Break in the Dining Hall
Session 5 - Reward Session Chair: Stephanie Borgland, PhD (UofC)	
LONG FORM TALKS	
11:00 - 11:20 AM	Ryann Tansey, PhD (Mayo Lab, UofC) *The Role of Endocannabinoids in the Acute Effects of Alcohol on Reward Function in the Brain: A Within-subjects fMRI Study*
11:20 - 11:40 AM	Drew Neyens, PhD (Borgland Lab, UofC) *Insulin Induces Synaptic Depression in PPTg Inputs to VTA Dopamine Neurons via a CB1R- and Astrocyte-mediated Mechanism*

11:40 - 12:00 PM	Catalina Zamorano (Bruchas Lab, UW) *Isolating the Role of Endogenous μ-Opioid Activity in the Ventral Tegmental Area During Natural Reward*
SHORT FORM TALKS	
12:00 - 12:07 PM	Adriana Quintero Narvaez (Cone Lab, UofC) *Probing Codes for Subjective Values and Costs in Brain Reward circuits*
12:07 - 12:15 PM	Ritwik Das (Gruber Lab, UofC) *Neural Encoding and Causal Basis of Competitive Decision-Making in Striatal Subcircuits*
12:15 - 12:22 PM	Lucy Tian (Stuber Lab, UW) *Dopamine Modulation of Nucleus Accumbens Dynamics Across Cue-Reward Learning*
12:22 - 12:30 PM	Amanda Pasqualini (Bruchas Lab, UW) *Characterizing the Role of Central Amygdala Nociceptin Opioid Neurons in Reward Salience Encoding*
12:30 - 2:00 PM	Lunch in the Dining Hall
Session 6 - Intersection of Stress, Reward, and Aversion Session Chair: Garret Stuber, PhD (UW)	
LONG FORM TALKS	
2:00 - 2:20 PM	Zach Pennington, PhD (junior faculty, UBC) *An Amygdala-Hypothalamic Circuit Gates Negative Valence and Stress Vulnerability*
2:20 - 2:40 PM	Matt Dawson (Sargin Lab, UofC) *Role of Hypocretin/Orexin Neurons in Social Behavior and Social Stress*
2:40 - 3:00 PM	Siyi Ma, PhD (Soden Lab, UW) *Response of Leptin Receptor Expressing Dopamine Neurons in the Ventral Tegmental Area to Appetitive and Aversive Stimuli*
SHORT FORM TALKS	
3:00 - 3:07 PM	Morgan Sotzen (Skibicka Lab, UofC) *From the Pancreas to the Dorsal Raphe – A Novel Neural Substrate for Amylin's Effects on Ingestive and Motivated Behavior*
3:07 - 3:15 PM	Camila Saenz (Borgland Lab, UofC) *Oral Cannabis Consumption-induced Hyperphagia Requires Ghrelin Receptor Signalling in Female and Male Mice*
3:15 - 3:22 PM	Abigail Lunge (Mayo Lab, UofC) *Exploring the Relationship between Childhood Maltreatment, Social processing, and Endocannabinoids*
3:22 - 3:30 PM	Madison Martin (Bruchas Lab, UW) *Characterizing the Function of Locus Coeruleus and Pericoerulear Zone Activity During Avoidance Behavior*

3:30 - 4:00 PM	**Closing thoughts and debrief**
-	Depart Field Station or Dinner *(self-arranged)*

Wednesday, March 25, 2026	
by 10:00 AM	Depart Field Station

SPEAR 2026
Poster Abstracts

Poster session
Monday, March 23rd at 3:30 - 5:30 PM
* = *presenting author*

Hazardous Cannabis Use and the Misalignment Between Objective Sleep and Sleep Perception

*Jane Lopez**, Joshua E. Gonzalez, LaTroy D. Robinson, Ayeisha Haswarey, Cohen Williams, Steven A. Shea, Nicole P. Bowles

Background: Cannabis is widely used as a sleep aid, despite growing evidence that habitual cannabis use may contribute to sleep disruption. Sleep perceptions often diverge from objective measures of sleep, and such misperceptions may reinforce continued cannabis use. However, little is known about how cannabis use relates to objective sleep and subjective-objective sleep discordance.

Methods: We studied 70 young adults (26.9±3.8 years;41 female) across a range of potentially hazardous cannabis use, assessed with the Cannabis Use Disorder Identification Test–Revised (CUDIT-R). Participants reported daily subjective sleep quality and daytime sleepiness and had sleep objectively assessed via wrist actigraphy for up to 20 consecutive days. Associations between CUDIT-R scores, objective sleep parameters, and any subjective–objective sleep discordance were assessed using linear mixed-effects models adjusted for age and sex.

Results: CUDIT-R scores were not associated with objective total sleep time ($p > 0.10$). However, higher CUDIT-R scores (greater hazardous cannabis use) were associated with poorer sleep continuity (increased wake after sleep onset; β range = 15.4–18.7 min; $p < 0.05$). Individuals with the highest CUDIT-R scores reported poorer subjective sleep quality ($\beta = –0.38$, $p = 0.036$) and greater daytime sleepiness ($\beta = –0.68$, $p < 0.001$), despite similar objective sleep durations. Higher CUDIT-R scores were associated with greater discordance between objective sleep and both subjective sleep quality and daytime sleepiness (all $p <0.01$).
Conclusions: Greater hazardous cannabis use is associated with objective sleep fragmentation and altered sleep perception. These associations may reinforce habitual cannabis use.

Imaging Neural Dynamics in the Orbitofrontal Cortex During the Development of Obesity

*Samuel Burford**, Sam Livingston, Min Qiao, Stephanie Borgland

Weight loss drugs such as Ozempic have grown in popularity and effectiveness over the last decade. However, obesity rates continue to increase worldwide. Obesity is the leading cause of preventable death and affects an estimated 600 million people worldwide. Genetics and lifestyle choices play a role in this rise, yet easy access to calorie-dense foods is a key factor in these trends. This ease of access places stress on our internal decision-making and eating habits. The Orbitofrontal Cortex (OFC) plays a key role in decision-making based on our internal state. Our research aims to determine the specific timing and activation of neurons in the OFC

while eating. Using a mouse model, we have begun imaging neural dynamics from OFC pyramidal neurons using two-photon microscopy. Mice have learned through Pavlovian training to associate a reward consumption with a cue, followed by a devaluation task to reduce the reward through satiation. OHRBETS head fixation technology allows for tracking of individual cells across extended periods of time, throughout behaviour. Previous work from the Borgland Lab has shown that OFC neurons increase in excitability in obesity models, through disinhibition. We aim to clarify how this directly translates to cue learning behaviour during consumption. We also aim to determine which specific interneurons may play a critical role in the disinhibition and how this relates to the development of impaired decision making in obesity.

Sex Differences in Glial Involvement Delays Postoperative Pain Recovery in Female Rats

Churmy Fan, Nynke J van den Hoogen, Sierra Stokes Heck, Brendan McAllister, Peter Kamau, Annika Maj*, Flavia Tasmin Techera Antunes, Leah Heck, Mary Warren

Persistent postoperative pain affects a significant number of patients, yet its underlying causes remain poorly defined. Using the skin-muscle incision-retraction (SMIR) model, we observed that SMIR induced persistent mechanical hypersensitivity in both male and female rats. However, this hypersensitivity resolved by postsurgical day 35 in males, whereas it persisted beyond 42 days in females. In both sexes, spinal microglial reactivity was evident early during the postoperative period, and treatment with minocycline attenuated hypersensitivity at early, but not late, time points. In contrast, female rats displayed prolonged astrocyte reactivity, and inhibiton of astrocytes with fluorocitrate reduced the mechanical hypersensitivity during both early and late postoperative phases. These findings suggest that while microglia contribute to early postoperative hypersensitivity in both sexes, persistent pain in females specifically depends on sustained astrocyte reactivity. Notably, monocyte chemoattractant protein-1 (MCP-1) expression was differentially elevated in females during the persistent phase, and intrathecal administration of a MCP-1 neutralizing antibody reduced the postoperative mechanical hypersensitivity in females. Together, these findings implicate spinal astrocyte reactivity and MCP-1 signaling as a potential female specific mechanism in persistent postoperative pain.

Sex-dependent Modulation of the Lateral Hypothalamus-Dorsal Raphe Nucleus Pathway Following Acute Stress

*Nahid Rouhi**, Shi Chen Xu , Derya Sargin

Acute stress affects neural activity and emotional regulation, impacting circuits that maintain mood stability. Orexinergic inputs to the dorsal raphe nucleus (DRN) play a critical role in modulating serotonin-driven stress responses, which are essential for emotional balance. Using foot shock, a model of acute stress, we examined its effects on social, stress coping and anxiety-like behaviours in male and female C57Bl6 mice. Mice received 10 shocks (2 seconds each), followed by behavioural tests that assess socioemotional regulation. In vivo calcium imaging of orexinergic inputs to the DRN in stressed mice revealed a significant increase in neuronal activity of this pathway, and a threat-induced alteration in orexin activity. Ex vivo optogenetic stimulation of orexinergic terminals in the DRN, infused with light-activated opsins (ChR2), produced altered DRN firing patterns in stressed mice compared to control mice. Overall, our findings indicate that acute foot-shock disrupts orexin inputs to the DRN, and may result in dysregulation of orexins, which regulate sleep, appetite, and emotion, and is linked to disorders such as depression and anxiety, making this pathway promising target

for treatment. These findings also highlight that orexins contribute to sex differences in stress responses and subsequent mental health phenotypes, emphasizing the need to understand sex-dependent mechanisms underlying emotional dysregulation for the development of effective treatment strategies.

Redox Modulation of Corticostriatal Synapses: a Microfluidic Approach to Circuits of Motivation, Emotion, and Cognition

*Baillie Cej**

Elements that drive our actions - motivation, emotion, and cognition - converge upon the striatum where they are integrated to orchestrate complex behaviours. A primary afferent projection into the striatum arises from the cortex, forming the cortico-striatal circuit. At the heart of this circuit are multipartite synapses composed of cortical pyramidal neurons, striatal medium spiny neurons (MSNs), and glia. MSNs are GABAergic projection neurons in the striatum that receive glutamatergic input from the cortex, with their activity further shaped by dopaminergic modulation. One underexplored mechanism of modulation involves hydrogen peroxide (H_2O_2), a redox signal produced during metabolism and pro-inflammatory pathways. Highly expressed NMDA receptors (NMDARs) in the post-synaptic membrane of MSNs, contain redox-sensitive sites that fine-tune receptor activity when oxidized, thereby influencing the ability of synapses to respond to specific cues.

Based on evidence that neuroinflammation triggered by an abundance of reactive oxygen species (ROS) (e.g. H_2O_2) contributes to depressive symptoms including anhedonia, I am developing a microfluidic chip platform to uncover the mechanisms at the corticostriatal synapse that regulate this response. Using post-synaptic density-localized fluorophores to monitor NMDAR-mediated Ca2+ flux and H_2O_2 kinetics in combination with super-resolution microscopy, I will simultaneously track redox and synaptic signals in real time across different culture conditions and manipulations. This work will establish a novel platform for dissecting oxidative modulation of synaptic events in circuits central to motivation, emotion, addiction, and reward, providing mechanistic insight into synaptic vulnerability.

The Domain-specific Impact of Acute Social Stress on Cognitive Flexibility in Healthy Humans

*Keira Aubin**

Acute stress elicits coordinated physiological and behavioural responses through activation of the HPA and SAM axes, rapidly preparing the body to meet changing demands. Cognitive flexibility - the capacity to adapt thoughts and behaviours as circumstances shift - is central to effective executive functioning. While acute stress may be adaptive, chronic stress is known to bias executive functioning by increasing reliance on habitual patterns of thought and behaviour. The present study systematically examines how acute social stress influences three distinct components of cognitive flexibility: attention set-shifting (Wisconsin’s Card Sorting Task; WCST), reward-related reversal learning (Probabilistic Reversal Learning Task; PRL), and spontaneous thought dynamics (Think Aloud Task; TAT), while also examining potential sex differences.

100 healthy adults (50% female) complete baseline measures of mood, emotion regulation, reward responsiveness, resilience, substance use, and long-term endocannabinoid levels derived from hair samples. Participants then complete the WCST, TAT, and PRL tasks in a randomized order before undergoing either the Trier Social Stress Test or a control condition, during which psychophysiological activity is recorded.

Throughout the session, they provide six saliva samples for cortisol assessment and subsequently repeat the cognitive flexibility tasks.

Although data collection is ongoing, preliminary results are expected by the time of the conference. It is predicted that acute social stress, indexed by cortisol increases, will impair WCST performance and promote more flexible spontaneous thought patterns (TAT). Preliminary data from the current subsample (n = 36) indicate acute stress enhances PRL performance. Exploratory analyses will evaluate how sex may contribute to these effects.

Dopamine Dynamics in the Nucleus Accumbens Core Underlying Working Memory in the Odour Span Task

*Aiden E Glass**, Ronin G Sawitsky, Caleb LG White, Dylan J Terstege, Kirk Mulatz, Jonathan R Epp, Justin J Botterill, John G Howland

Working memory enables short-term retention and manipulation of information and is essential for daily functioning. Modulation of working memory capacity – the number of items that can be held in working memory – by striatal dopamine signaling is not thoroughly understood. The nucleus accumbens (NAc) core, a highly integrative subregion of the ventral striatum, mediates motivated working memory. In rodents, the odour span task (OST) is a well-established test of working memory and working memory capacity. However, temporal dopamine dynamics in the NAc during OST performance have not yet been characterized. In the OST, rats identify a session-novel odour to receive a food reward. A 1-minute delay follows each correct trial, during which another odour is added, incrementally increasing the number of odours the rat must hold in working memory. To investigate real-time bulk dopamine dynamics in the NAc underlying working memory in the OST, male and female rats underwent stereotaxic surgery to implant an optic fiber and infuse a viral vector containing the dopamine sensor GRAB-DA into the NAc core subregion. After excluding sessions with poor dopamine signal, we collected 16 fiber-photometry recordings synchronized with behavioural videos from five rats (n = 3 male, 2 female). On average, rats achieved a maximum span of 8.5 odours held in working memory before making an error. We are currently analyzing NAc core dopamine traces during OST trials using metrics of maximum peak height, dynamic range, and area under the curve. We are also doubling our sample size to increase confidence in our conclusions.

Dynamic Duo: Serotonin Transporter and Organic Cation Transporter 3 Contribute to Basolateral Amygdala Serotonin Clearance and Recent Fear Memory Recall

*Lauren E. Honan**, Sangbin Shin, W. Anthony Owens, Rebecca E. Horton, Yeon Ha Ju, Georgianna G. Gould, Lief E. Fenno, Glenn M. Toney, Lynette C. Daws

Converging evidence implicates dysregulation of serotonin in psychiatric disorders. Leading pharmacotherapies for psychiatric disorders target the serotonin transporter (SERT); yet, selective serotonin reuptake inhibitors (SSRIs) exhibit limited efficacy, suggesting additional mechanisms contribute to their etiology. Recent evidence suggests organic cation transporter 3 (OCT3) contributes to maintaining serotonin homeostasis; however, its contributions to serotonin clearance in basolateral amygdala (BLA), a central hub for emotional processing, are unknown. We utilized transgenic mice, in vivo neurochemical, and behavioral approaches to test the hypothesis that OCT3 contributes to serotonin clearance in BLA and influences

attendant fear behaviors. Depletion of OCT3 from serotonin neurons prolonged serotonin clearance in BLA across a range of concentrations, in comparison to depletion of SERT from serotonin neurons, which only prolonged serotonin clearance in BLA at relatively low concentrations. The SSRI fluvoxamine prolonged serotonin clearance in BLA of wildtype mice and OCT3 knockdown mice to a similar degree, and this effect was lost in mice with SERT depletion. Behaviorally, depletion of SERT or OCT3 from serotonin neurons did not impact fear learning; however, depletion of SERT trended to attenuate cued fear memory, and depletion of OCT3 modestly attenuated cued and contextual fear memory. These findings indicate that OCT3 could potentially serve as a novel therapeutic target for psychiatric disorders and encourage more research into its role in their etiology.

Causal Roles of Dopamine across Striatal Subregions in Consumption Behavior

*Adam Gordon-Fennell**, Elena Lu, Anthony Campuzano, Garret D. Stuber

Dopamine (DA) dynamics during consumption scale with value and consumption across the anterior–posterior axis of the striatum, yet the causal role of transient DA increases in shaping consumption behavior remains unclear. Here we tested how optogenetically evoked DA release at distinct striatal subregions alters ongoing licking. Head-fixed mice expressing ChR2 or mCherry control received implants of ten optical fibers targeting the ventral tegmental area (VTA), nucleus accumbens core (NAc), dorsomedial striatum (DMS), dorsolateral striatum (DLS), and tail of striatum (TS) bilaterally. Animals performed a brief-access taste task consuming 10% sucrose during 3 second windows. Stimulation was delivered in blocks of trials, and we quantified licking during time-locked stimulation. Preliminary data indicate that stimulation increased licking when DA was driven in the NAc, DMS, and DLS, whereas stimulation in the tail of the striatum had minimal behavioral effects. Ongoing work will assess the causal role of DA further by testing non-contingent stimulation, stimulation during free consumption, and quantifying how subregional position along the longitudinal axis relates to the behavioral effect. Together, these experiments begin to define how causal functions of DA signaling vary across striatal subregions during consumption, advancing beyond correlational structure to identify where DA transients are sufficient to shape consummatory behavior.

Impact of Fentanyl on Post-mTBI Behavior

*Madelyn T. Rice**, Alisa Coyne, Delaney S. Hurlimann, Bryan Schuessler, Abigail G. Schindler, and John F. Neumaier

Mild Traumatic Brain Injuries (mTBIs) are a prevalent condition that veterans experience during training and during their time in the field. Due to pain associated with this type of injury, opioid prescriptions are common, and this can open a pathway to opioid use disorder (OUD). Understanding the behavioral effects of repeated exposure to fentanyl after mTBIs could be a crucial step in understanding OUD. We hypothesize mice that undergo chronic fentanyl treatment after blast induced mTBI will develop greater hyperalgesia during withdrawal, increased anxiety-like behaviors following fentanyl administration, diminished motivational state, and increased fentanyl preference. This was modeled in mice that experienced multiple overpressure blast exposures that mimic fully body blast injuries that veterans may experience within the field. Shortly after inducing the mTBIs, minipump implants allowed a slow month-long administration of fentanyl treatment. One month after fentanyl exposure, an open field test and novelty-suppressed feeding assay were administered to see the long-term withdrawal effects on anxiety-like behaviors and motivational states. Throughout this

experimental paradigm, there were many timepoints for tail flick tests to examine how the levels of pain sensitivity changed with each condition. The last test conducted was a two-bottle choice assay, which examines fentanyl seeking by allowing the mouse free access to a water bottle and fentanyl water bottle during the dark cycle over 11 nights. The results are currently being analyzed. These results can contribute to finding novel and more effective interventions for OUD.

Identifying the Mechanisms of Noradrenergic Modulation of BLA-mediated Avoidance Behaviors

*Sayaka J Kenmochi**, Sean C Piantadosi, Madison M Martin, Veronica Porubsky, Avi K Matarasso, Heidi Neuman, Selena S Schattauer, Sarah Thai, Tammy K Nguyen, Larry S Zweifel, Michael R Bruchas

Acute stressors induce physiology anxiety, an adaptive survival response that influences how an organism reacts to environmental threats. Anxiety disorders are characterized by a maladaptive physiological anxiety response disproportionate to the perceived threat. A key neuromodulatory system that responds to stress exposure is the locus coeruleus noradrenergic system (LC-NE) via its projections to the basolateral amygdala (BLA). However, it is unclear how this circuit modulates anxiety-like behavior at the cell-type and receptor levels. We conducted two-photon calcium imaging of LC-NE neurons in response to a predator odor stressor and found synchronously increased LC-NE activity in a manner consistent with increased tonic activation. We then examined NE release in the BLA using a NE sensor (GRABNE2m) and mimicked the robust and sustained stress-induced NE release via tonic 5Hz optogenetic LC terminal activation (ChRimson). Using this stimulation paradigm and one-photon imaging, we found increased correlated BLA neuron activity during periods of stress-like NE release. Systemic pharmacology with the β-adrenergic receptor (AR) antagonist propranolol prevented increased BLA synchrony and the anxiogenic effect induced by LC-BLA stimulation. To further examine cell-type and receptor-type specificity, we used CRISPR-SaCas9 knockout of the gene encoding β2-AR (Adrb2) in the BLA of Vglut1-cre mice. CRISPR knockout of β2-AR from excitatory BLA neurons reduced stress-induced anxiety-like behavior. Using a combination of behavioral, optical, and molecular approaches we show that stress-like release of NE produces lasting alterations in BLA network activity and identify the receptor (β2-AR) that may be necessary for this type of network shift.

Investigating the Role of an aPVT-ZI Circuit in the Explore/Exploit Tradeoff

*Bailey A. Wells** , David Marcus, Maddie Critz, Rachel Oommen, Gunn Chun, Michael R. Bruchas

The Paraventricular Thalamus (PVT) is a midline thalamic structure involved in integrating interoceptive, affective, and motor signals to influence motivated behaviors. Recent studies of the PVT's role have produced conflicting results, partly due to its heterogeneity and lack of anatomical specificity. The neuromodulatory peptide neurotensin (NTS) is selectively expressed in the anterior PVT (aPVT) and our preliminary data demonstrates that these neurons send excitatory projections to multiple subcortical brain regions, including the Zona Incerta (ZI). The ZI, a largely inhibitory nucleus, regulates diverse functions such as sensory processing, arousal, and reward consumption. ZI projections to the periaqueductal gray (PAG) and midbrain dopamine neurons suggest it may play a role in balancing exploration and exploitation during reward-seeking behavior—a fundamental challenge in decision-making. In this study, we investigate how aPVT regulation of the ZI influences this tradeoff. To examine the aPVT-ZI circuit, we injected DIO-GCaMP6s into the aPVT of NTS-cre mice and recorded bulk calcium dynamics from the ZI during operant reward conditioning. In our

probabilistic reversal learning task, we found that the aPVT-ZI circuit was inhibited during reward consumption but excited during reward-seeking behavior. Future experiments, including retrograde tracing, electrophysiology, and optogenetics, aim to further investigate the mechanisms by which the aPVT modulates ZI outputs and action selection. Understanding how this circuit contributes to decision-making could provide insights into suboptimal motivation and cognitive flexibility, commonly seen in neuropsychiatric conditions.

Neurotensin Neurons in the Ventrolateral Periaqueductal Gray Drive Distinct Threat and Reward Responses

*Grace Davis**, Siyi Ma, McKenna Kernan, Dustin Sumarli, Mary Loveless, Kyle Schroeder, Su Cho, Zainab Nasir, Micaela Ruiz, & Marta E. Soden

Dopamine (DA) neurons in the ventral tegmental area (VTA) are central regulators of reinforcement learning, motivational drive, and adaptive decision-making. These neurons receive a wide array of excitatory and inhibitory inputs that convey information about both rewarding and aversive stimuli. Yet, the neural circuit integration of these opposing signals remains incompletely understood. One potential source of competing signals arises from the periaqueductal gray (PAG), a midbrain structure associated with nociception and threat detection but also increasingly implicated in appetitive processing. Within the PAG, a subset of VTA-projecting neurons expresses the stimulatory neuropeptide neurotensin, a potent modulator of VTA DA neuron excitability. Here, we investigated the molecular identity, anatomical organization, and functional properties of PAG-Nts inputs to the VTA. Using a combination of viral tracing and multiplex in situ hybridization, we defined the transcriptional markers and projection patterns of this population. We used fiber photometry to assess their activity dynamics during appetitive and aversive experiences and optogenetics to determine their contribution to motivated behavior. Our results revealed an unexpected dual role for PAG-Nts neurons in promoting both reward-seeking and threat-related responses, suggesting that they may serve as a flexible modulatory node that integrates diverse motivational signals. These findings indicate that PAG-Nts inputs to the VTA may contribute to a broader midbrain network that coordinates adaptive behavioral responses across motivational contexts.

Chronic Intermittent Ethanol Activates CHOP in Striatal Microglia and Exacerbates Ethanol Withdrawal

Rapheal G. Williams, Brett D. Dufour, *Kevin R. Coffey**, Atom J. Lesiak, William B. Nickelson, Aliyah J. Dawkins, Gwenn A. Garden, John F. Neumaier

Repeated cycles of alcohol intoxication and withdrawal induce profound changes in gene expression that can contribute to the physiological and behavioral consequences of ethanol. Since neuroinflammation is an important consequence of these changes, we used a novel strategy to investigate the impact of repeated cycles of chronic intermittent ethanol vapor and withdrawal on the RNAs actively undergoing translation in microglia in striatum, a key region involved in the relapse to ethanol consumption. We performed deep sequencing of the “translatome” from striatal microglia of male and female RiboTag mice, yielding a snapshot of RNA translation during alcohol intoxication and after 8 hours of withdrawal. Chronic intermittent ethanol produced robust changes in the translatome, with increases in genes and pathways associated with cytokine signaling, indicating increased neuroinflammation and microglial activation. After 8 hours of ethanol withdrawal, many inflammatory pathways remained upregulated and phagocytotic and proapoptotic pathways were

increased. Using unbiased network analysis, we identified gene modules that were differentially expressed in ethanol intoxicated vs. withdrawing animals. Genes associated with the unfolded protein response (UPR) were over-represented in one such module after withdrawal, including the transcription factor Ddit3 (CHOP), an important mediator of the UPR. We tested the impact of conditional knockout of CHOP from microglia specifically; following withdrawal from chronic intermittent ethanol, these mice had reduced thermoregulatory disturbances, anxiety-like behavior, and voluntary ethanol consumption compared to wild-type littermates. We conclude that CHOP and the UPR in microglia may be important targets for reducing the impact of withdrawal from chronic ethanol exposure.

Engineering an Enhanced, Bistable, Gq-coupled GPCR-based Opsin for Controlling Neuronal Activity in vivo

*Angela Wei**, Lily Torp, Yuxuan Wang, Yejin Kwon, Lila Jin, Justin Daho Lee, Bryan Copits, Michael Bruchas, Andre Berndt

Neuroscientists employ a myriad of protein-based tools to precisely study and control neuronal circuits in the brain. Optogenetic tools use light to activate genetically encoded ion channels, while chemogenetic tools require small molecules to activate engineered G protein-coupled receptors (GPCRs). Optopharmacology combines these approaches to enable optical control of GPCR-based neuromodulation without ligand addition. Copits et al. established lamprey parapinopsin (PPO) as a photoswitchable inhibitory (Gi-coupled) GPCR-based actuator activated by UV/blue light and inactivated with green/amber light. The Berndt Lab applied a structure-guided and high-throughput protein engineering approach to expand the available toolkit of excitatory (Gq-coupled) light-activated neuromodulatory tools. Mutating residues in the ICL2/ICL3 region of PPO, we generated a library of 160,000 PPO variants. Each variant was expressed in HEK293T cells and screened for enhanced Gq coupling using the red-shifted calcium indicator jRCaMP1b per the Opto-MASS protocol. We selected PPO-Gq1.0, a PPO variant which displays a nearly 2-fold increase in jRCaMP1b fluorescence following a 10 second 405nm light pulse (1.2mW/mm2) and is blocked with Gq-inhibitor YM-254890. PPO-Gq1.0 expresses well in the plasma membrane of HEK293 cells and primary cortical neurons. To further enhance Gq coupling we generated AlphaFold2-Multimer models of PPO bound to Gi- and Gq-proteins and identified residues within the binding region in close contact for mutagenesis. Additionally, we applied green-shifted cAMP indicator GFlamp1 and calcium indicator eGCaMP2+ to activate and image PPO-G-protein coupling with blue (440-488nm) light. Our engineering approach aims to expand the available excitatory tools to study neuronal circuits underlying stress, emotion, and addiction.

Introducing Probabilistic Threat of Shock during Liquid Fentanyl Oral Self-administration in Rats

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Opioid use disorder is a growing epidemic in the United States, yet the complexity of the disorder in humans renders it difficult to holistically research. Building off preliminary data from our previous experiments involving escalation of intake, extinction and withdrawal, and negative emotion following fentanyl self-administration, we are interested in the probabilistic threat of shock and its influence on fentanyl seeking in male outbred rats. Our two-lever operant boxes allow for volitional oral fentanyl self-administration–which has been successful in

modeling opioid seeking behavior in rats. Rats are conditioned to press an active lever for a liquid fentanyl reward via a paired tone and cue light along with an inactive lever. We will then implement a probabilistic threat of shock during the fentanyl administration trials, starting with a rising probability of shock at an unchanging mid-level shock intensity. This successfully recreates similar situations to stress-induced opioid use, highlighting an underutilized yet critical area of addiction research. Collecting the number of lever presses and the number of fentanyl infusions across time, video recordings for pose estimation, and ultrasonic vocalizations, we hypothesize a change in the pattern of escalation of fentanyl intake and aversion-resistant behaviors in the rats. Pose and ultrasonic vocalizations may provide critical insights into the emotional states of the rats following fentanyl administration and threat of shock. With our analysis of aversion-resistance and compulsive fentanyl seeking, we can gain a better understanding of the complex neural mechanisms of opioid addiction.

NAc Shell Pdyn Neurons Regulate Dopamine Signaling during Associative Learning

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Dopaminergic neurons in the ventral tegmental area (VTA) and their projections to the nucleus accumbens (NAc) play a central role in encoding both rewarding and aversive experiences. This modulation is influenced by neuropeptides, including dynorphin, which is primarily expressed by D1-type medium spiny neurons (MSNs), also known as prodynorphin (Pdyn)-expressing neurons, in the NAc. Dynorphin can act through kappa opioid receptors (KORs) on VTA neurons to regulate dopamine release. The NAc shell exhibits anatomical and functional heterogeneity, with its dorsal and ventral subdivisions contributing differently to motivated behaviors. Dysregulation of dynorphin and dopamine signaling in this region has been linked to neuropsychiatric disorders such as depression and addiction. Investigating how dynorphin release affects dopamine during associative learning could provide insight into these mechanisms.

We first monitored dynorphin release using the genetically encoded fluorescent sensor kLight1.3 in KOR-cre mice during a Pavlovian learning task and observed dynorphin release in both dorsal and ventral NAc shell in response to rewards. To examine the influence of Pdyn-expressing neurons on dopamine, we injected Pdyn-cre mice with a virus encoding Chrimson opsin and axonal dopamine sensor (GRAB-DA) and recorded dopamine dynamics via fiber photometry. First, we observed different dopamine dynamics in dorsal and ventral NAc shell. Unexpectedly optogenetic stimulation of Pdyn neurons in either NAc subregion enhanced cue-evoked dopamine release, and stimulation of Pdyn terminals in the VTA produced a similar effect, indicating modulation both locally and via NAc-to-VTA projections. We further investigated the specific contribution of dynorphin using pharmacological KOR manipulations.

Overall, these results demonstrate that dopamine and dynorphin are released in the NAc shell during associative learning and that Pdyn-expressing neurons modulate dopamine dynamics. Since these neurons also co-release GABA, future studies will dissect the relative contributions of dynorphin/KOR signaling to these effects.