SPEAR CONFERENCE 2025

Friday Harbor Labs

University of Washington

MARCH 10–12

PROGRAM BOOK

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Program Committee

Dr. Matt Hill (University of Calgary)
Dr. Stephanie Borgland (University of Calgary)
Dr. Garret Stuber (University of Washington)
Dr. Susan Ferguson (University of Washington)
Dr. Michael Bruchas (University of Washington)

Organizer: Dr. Lusine Eyde

We wish to thank Dr. Charles Chavkin for his support through the planning and organization process of this conference.

Thank you to organizers Jodi VanderYacht, Morgan Johnson, and the staff at Friday Harbor Labs.

Code of Professional Conduct

All participants are expected to treat others with appropriate respect and civility at all times. We strive to sustain an environment in which a free exchange of ideas and opinions can occur. Discrimination and harassment in any form will not be tolerated. If you witness or experience an interaction that makes you uncomfortable during the sessions or associated social events, please intervene immediately or report the incident to a member of the Program Committee (whichever you feel is appropriate).

Incidents of unprofessional conduct will be documented as completely as possible. Documented incidents will be reviewed by the Program Committee, and if the majority concurs, the alleged offender will be informed, and an incident report will be forwarded to their supervisor (e.g. Dean or Department Chair) for appropriate action.

This is a proactive policy statement. We have not been informed of any previous incidents, but by explicitly stating our expectations, we will hopefully reinforce everyone’s positive experience.

General Information

Conference on Stress, Pain, Emotion, Addiction, and Reward (SPEAR)
Friday Harbor Labs
March 10-12, 2025

Contacts & Emergency Information

- Fernald Front Office (M-F, 8:30 AM – 1:30 PM): 206-616-0702
- Morgan Johnson's Office (M-F, 8 AM – 4 PM): 206-616-0753
- Caretakers (Urgent Needs After Hours):
    - Mike & Michelle Herko: 360-298-0220 or 360-298-0800

Emergency Procedures

- For any emergency, call 911 first.
- Then, contact the Front Office during business hours or the Caretakers after hours.
- Fire**:** Do not attempt to fight a fire alone.
- Serious injuries: Go to Peace Island Medical Center & ER (1117 Spring St).
- Minor injuries**:** First aid supplies are available in all labs and the Stockroom.
- AED Locations**:** Fernald Lab (first floor, near stairs), R/V Kittiwake, and the phone booth near the apartments (relocated to Laundry Room in winter).
- In case of a disaster (earthquake, fire, etc.), meet in the main parking lot adjacent to Fernald Lab & Whiteley Center.

Housing Information

- **Check-In: After 3:00 PM.** Get your housing assignment/key and name badges from Lusine near the entrance of the dining hall.*** If you are arriving late, find your late arrival packet pinned to the bulletin board in the dining hall.

- **Check-Out: By 11:00 AM.**
    - Before you leave:
        - Wash dishes & clean out the refrigerator.
        - Dispose of trash & recycling in the outdoor bins.
        - Leave keys on the counter and lock the unit behind you

What's Provided in Your Unit?

- All units:
  - 2 blankets, pillows, sheets, towels, toilet paper
- Kitchen units:
  - Basic kitchen supplies & a coffee pot **(**bring your own coffee & filters**)**

Restrooms

- Huts & Dorm C residents: Use the bathrooms in Dorm A or B (Keypad: 217)
- Standard Dorms & Huts:
  - Men's & Women's restrooms in Dorm A & B and the Dining Hall
  - Gender-neutral bathroom in Dorm B
  - Single-user, gender-neutral bathroom with toilet & shower in Fernald Lab (first floor)

WiFi Access

FHL has three WiFi networks across campus. FHL WiFi Guide

1. EDUROAM/ANYROAM (Preferred)

- **Best option for UW students, faculty, & staff**
- Log in as usual if you've used Eduroam before.
- New users: Configure at **onboard.wifi.uw.edu**
- If affiliated with a non-UW Eduroam institution, contact your institution for setup instructions.
- If not affiliated with an Eduroam institution**,** connect via "Anyroam" (see links for MacOS & Windows setup). More details: FHL WiFi Guide

2. University of Washington Guest Network

- Grants 3 hours of access before requiring reconnection.
- Connect by selecting "University of Washington WiFi" in your settings, then choose "Connect as a Guest."

Campus Rules & Guidelines

- **Quiet Hours: 10 PM – 7 AM** (Please be considerate of researchers and students).
- Furniture & Equipment**:** Do not move furniture, kitchen supplies, or bedding between buildings.
- Pets & Wildlife: No pets allowed. Do not feed the wildlife.
- Camping & Vehicle Sleeping: Not permitted.
- Report Repairs: Contact the Office Coordinator for maintenance issues.

Dining Schedule & Menu

***For people who have previously mentioned their allergies/dietary restrictions: The cafeteria will need you to tell FHL servers your restrictions to get your specialty entree. All self-serve foods are labeled so you can see what is available to you.

All meals will be served at the designated Dining Hall during the following times:

Monday, March 10, 2025

Dinner (6:00 - 6:30 PM)

- Chicken Tamales
- **Vegetarian Option:** Green Chili Cheese Tamales
- Pinto Beans
- Corn Salsa, Sour Cream, Guacamole
- Salad
- Tortilla Chips
- Churro Donuts

Tuesday, March 11, 2025

Breakfast (7:45 - 8:15 AM)

- Home-Style Potatoes
- Scrambled Eggs
- Pork Sausage / Veggie Sausage

Lunch (12:00 - 12:30 PM)

- Caprese Sandwiches (Tomato, Basil, Mozzarella)
- Balsamic Vinegar & Olive Oil
- Soup

- Salad
- Cookies

Dinner (6:00 - 6:30 PM)

- Honey Mustard Chicken Thighs
- **Vegetarian Option:** Honey Mustard Tofu
- Mashed Potatoes
- Peas
- Dinner Rolls
- Salad
- Blackberry Pie

Wednesday, March 12, 2025

Breakfast (7:45 - 8:15 AM)

- French Toast with Syrup & Strawberries
- Pork Sausage / Veggie Sausage

Getting to Friday Harbor Labs (FHL)

- Address: 620 University Road, Friday Harbor, WA
- The ferry terminal is ~1.5 miles from FHL (~20-30 min walk or 6 min drive).
- **No Ubers or Lyfts** are available on the island.

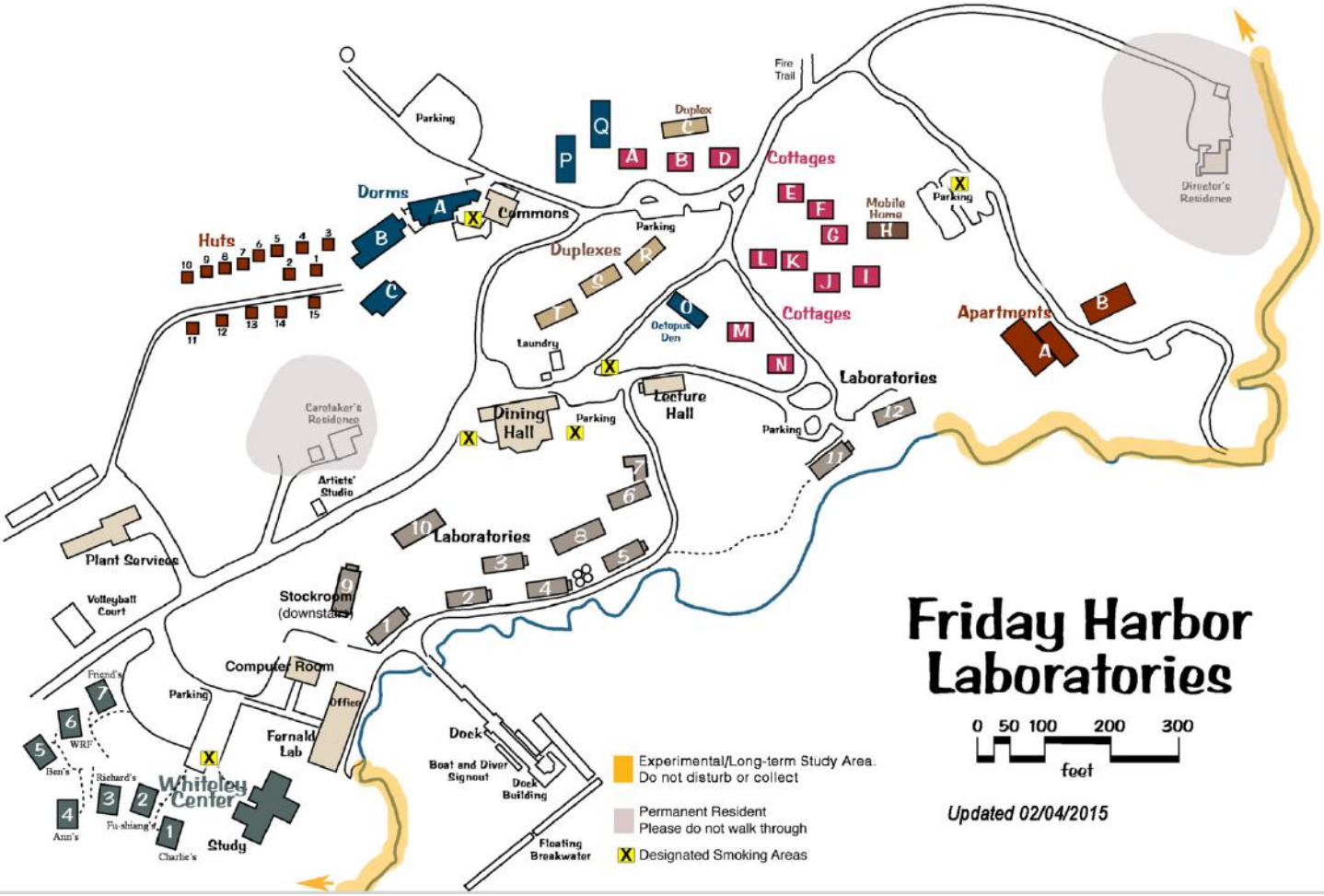
Transportation Options

- Drive or walk from the ferry.
- Taxis available in town.
- FHL Van Pickup:
    - Only for people arriving on the early afternoon Anacortes ferry**.**
    - Even if the ferry is late, the van will wait. If it's not at the terminal, stay put—it will return shortly.

Directions from the Friday Harbor Ferry Terminal

1. Turn right off the ferry, then immediately left onto Spring Street.
2. Go two blocks, turn right onto Second Street.
3. Follow Second Street for four blocks (it curves left).
4. At the second stop sign, turn right onto Tucker Ave.
5. Follow Tucker Ave for 0.3 miles to a fork in the road**.**
6. Take the right fork onto University Rd and proceed 0.3 miles to FHL**.**
7. For more details: **Visiting FHL as a Group Participant**


Walk off ferry and proceed as shown here with red arrows.
Van will be parked in traffic circle, on the right.
At end of pedestrian walkway, immediately turn right!


Friday Harbor Laboratories
Parking
Fire Trail
Duplex
Cottages
Director's Residence
Dorms
Commons
Huts
Duplexes
Mobile Home
Parking
Laundry
Octopus Den
Cottages
Apartments
Laboratories
Lecture Hall
Caretaker's Residence
Dining Hall
Artists' Studio
Laboratories
Plant Services
Volleyball Court
Stockroom (downstairs)
Computer Room
Office
Fornald Lab
Whiteley Center
Study
Friend's
WRF
Ben's
Richard's
Fu-shiang's
Ann's
Charlie's
Dock
Boat and Diver Signout
Dock Building
Floating Breakwater
Experimental/Long-term Study Area. Do not disturb or collect
Permanent Resident Please do not walk through
Designated Smoking Areas
0 50 100 200 300
feet
Updated 02/04/2015

SPEAR 2025 Conference Schedule

March 10, 2025 (Monday)

3:00 PM: Participant Arrival & Check-In
- Pick up conference materials, keys, and name tags.
- ALL Poster Presenters put their posters up - even and odd numbers

5:00 – 6:00 PM: Ice Breaker

6:00 - 6:30 PM: Dinner *(Dining Hall)*

6:30 - 8:00 PM: Poster Session Part I – odd numbers

March 11, 2025 (Tuesday)

7:45 AM - 8:15 AM
Breakfast *(Dining Hall)*

8:20 AM – 8:30 AM
Introduction: Matthew Hill

Session 1 – Dopamine, Reward, and Addiction
Session Chair: Garret Stuber

8:30 AM - 8:50 AM: **Adam Gordon-Fennell** (Postdoc; Stuber Lab, UW)
Dopamine Signals Across the Anterior-Posterior Axis of the Striatum Represent Unique Components of Consummatory Behavior

8:50 AM - 9:10 AM: **Aida Mohammadkhani** (Postdoc; Borgland Lab, UofC)
Effects of Opioids on LH Orexin and Dynorphin Modulation of VTA Dopamine Neurons

9:10 AM - 9:30 AM: **Mollie Bernstein** (Graduate Student; Zweifel Lab, UW) *Investigating the Role of TRPC6 Channels in Reward and Uncertainty Encoding in Midbrain Dopamine Neurons*

9:30 AM - 9:50 AM: Leah Salinsky (Postdoc; Ferguson Lab, UW)
Examining the Impact of Methamphetamine and Fentanyl Polysubstance Administration on Locomotor Sensitization and Social Interactions: Can a Psychedelic Compound Mitigate Drug-Induced Social Deficits?

9:50 AM - 10:10 AM: Break

10:10 AM – 10:40 AM: Data Blitz Presentations:

Session Chair: Michael Bruchas

10:10 AM - 10:17 AM: **Avi Matarasso** (Graduate Student; Bruchas Lab, UW)
LC-mediated DA Dynamics in Appetitive and Aversive Stimuli

10:17 AM - 10:24 AM: **Matt Dawson** (Graduate Student; Sargin Lab, UofC)
Role of Hypocretin/Orexin Neurons in Social Behavior and Isolation

10:25 AM - 10:32 AM: **Selena Fu** (Graduate Student; Dyck Lab, UofC)
A Role for Vesicular Zinc in the Regulation of the Social Reward Learning Critical Period

10:32 AM - 10:40 AM: **Monica Tschang** (Graduate Student; Schindler Lab, UW)
An Alternative Fecal Microbiota Transplant Method for Interrogating the Role of the Gut Microbiome on Trauma Outcomes in Blast Polytrauma

Session 2 – Stress and Pain
Session Chair: Michael Bruchas

10:45 AM - 11:05 AM: **Carlee Toddes** (Postdoc; Golden Lab, UW)
Social Interaction Reveals Dynamic Pain Recovery Window Following Neuropathic Injury in Mice

11:05 AM - 11:25 AM: **Ryan Phillips** (Postdoc; Baertsch Lab, UW)
Disentangling Pain Modulation and Respiratory Depression at the Level of the Rostral Ventromedial Medulla

11:25 AM - 11:45 AM: **Gavin Petrie** (Postdoc; Mayo Lab, UofC)
Endocannabinoid Upregulation Influences the Behavioral and Neuroendocrine Response to Stress

12:00 – 12:30 PM
Lunch *(Dining Hall)*

Session 3 – Cannabis and Endocannabinoids
Session Chair: Stephanie Borgland

1:00 PM - 1:20 PM: **Jessica Scheufen** (Graduate Student; Hill Lab, UofC)
How Cannabis Use Patterns Differentially Affect Adolescent Neurodevelopment

1:20 PM - 1:40 PM: **Sara Westbrook** (Postdoc; McLaughin Lab, WSU)
Vaporized Cannabis During Adolescence Impacts Parvalbumin Interneurons in the Medial Prefrontal Cortex in Adulthood

1:40 PM - 2:00 PM: **David Marcus** (Postdoc; Bruchas Lab, UW)
Endocannabinoids Facilitate Transitory Reward Engagement through Retrograde Gain- Control

2:00 PM - 2:20 PM: Break

2:20 PM – 2:50 PM: Data Blitz Presentations:

Session Chair: Stephanie Borgland

2:20 PM - 2:27 PM: **Catherine Hume** (Postdoc; Hill Lab, UofC)

Investigating the Appetitive and Motivational Effects of Vaporised Tetrahydrocannabinol (THC)

2:27 PM - 2:34 PM: **Kaylin Ellioff** (Graduate Student; Land and Bruchas Lab, UW)

Characterizing the Effects of Select Phytocannabinoids and Terpenes for Pain Relief

2:34 PM - 2:41 PM: **Anthony English** (Graduate Student; Bruchas and Stella Lab, UW)

THC-Dependent Increases in Neuronal and 2-AG Activity in Mouse Prefrontal Cortex at the Initiation of Locomotion

2:42 PM - 2:50 PM: Lucia Javorcikova (Graduate Student; Hill and Lohman Lab, UofC)

Investigating the Response of the Endocannabinoid System Following Repetitive Mild Traumatic Brain Injury in Adolescent Male and Female Rats

Session 4 – Opioids

Session Chair: Susan Ferguson

2:50 PM - 3:10 PM: Kentaro Ishii (Postdoc; Stuber Lab, UW)

Brain Wide Neuronal Ensembles Engaged by Opioid

3:10 PM - 3:30 PM: Lily Torp (Graduate Student; Berndt Lab, UW)

Engineering a Genetically Encoded Fluorescent Sensor for in vivo Fentanyl Detection

3:30 PM - 3:45 PM: Break

3:45 PM - 4:05 PM: Yuxuan Wang (Graduate Student; Berndt Lab, UW)

High-throughput Engineering of Genetically Encoded Fluorescent Sensor for Detecting Opioids in vivo

4:05 PM - 4:25 PM: Todd Appleby (Postdoc; Golden Lab, UW)

Individual Variability in Fentanyl Abuse and Relapse Using an Oral Self-Administration Model

4:25 PM – 4:55 PM: Data Blitz Presentations:

Session Chair: Susan Ferguson

4:25 PM - 4:32 PM: **Micaela Ruiz** (Graduate Student; Chavkin and Land Lab, UW)
Dynorphin Regulation of Dorsal Raphe Nucleus Encodes Stress Induced Dysphoria

4:32 PM - 4:39 PM: **Raajaram Gowrishankar** (Postdoc; Bruchas Lab; UW)
Endogenous Dynorphin Dynamics in the Dorsal Striatum Shape Neural Activity for Goal- directed Behavior.

4:40 PM - 4:48 PM: **Ari Peden-Asarch** (Graduate Student; Neumaier Lab, UW)
LHb Neural Dynamics During Drug Cessation from a Novel Delayed Punishment Paradigm

4:48 PM - 4:55 PM: **Marilena DeMayo** (Postdoc; McGirr Lab, U of C)
Clinical Outcomes in Fibromyalgia Following a Four Week Non-invasive Brain Stimulation Treatment with Adjunctive D-Cycloserine

5:00 PM - 6:00 PM: Conference Discussion

6:00 PM - 6:30 PM: Dinner (Dining Hall)

7:00 PM - 9:00 PM: Poster Session II – even numbers

March 12, 2025 (Wednesday)

7:45 AM - 8:15 AM
Breakfast (Dining Hall)

Session 5 – Stress and Aversion
Session Chair: Matthew Hill

8:30 AM - 8:50 AM: Jingyi Chen (Postdoc; Bruchas Lab, UW)
Endogenous Opioids Facilitate Stress-Induced Binge Eating via an Insular Cortex-Claustrum Pathway

8:50 AM - 9:10 AM: Ibukun Akinrinade (Postdoc, Bains Lab, UofC) Friend or Foe: Role of CRH-PVN neurons in Social Threat Detection

9:10 AM - 9:30 AM: Ryann Tansey (Postdoc; Mayo Lab, UofC)
Fear Generalization and Functional Connectivity of the Dorsolateral Prefrontal Cortex in Posttraumatic Stress Disorder

9:30 AM - 9:50 AM: Ekayana Sethi (Graduate Student; Zweifel Lab, UW)
Serotonin Release in the Central Nucleus of the Amygdala in Appetitive and Aversive Learning

9:50 AM - 10:10 AM: Weston Fleming (Postdoc; Palmiter and Stuber Lab, UW) Central amygdala acetylcholine signaling in conditioned taste aversion

10:10 AM - 10:30 AM: Mijail Rojas Carvahal (Graduate Student; Bains Lab, UofC) Exercise Erases the Behavioral and Synaptic Consequences of Stress

11:00 AM Departure (ferry at 12:10 PM)

Poster Session I (March 10, 6:30 – 8:00 PM)			
Name	**PI**	**Poster Title**	**Station**
Abigail Elerding	Zweifel/Stuber	*Differential responses to reward and aversion by genetically distinct GABA subpopulations of the VTA*	1
Anthony English	Bruchas/Stella	*THC-Dependent Increases In Neuronal and 2-AG Activity in Mouse Prefrontal Cortex at the Initiation of Locomotion*	3
Ari Peden-Asarch	Neumaier	*LHb Neural Dynamics During Drug Cessation from a Novel Delayed Punishment Paradigm*	5
Avi Matarasso	Bruchas	*LC-mediated DA dynamics in appetitive and aversive stimuli*	7
Bailey Wells	Bruchas	*Investigating the Role of an aPVT-ZI Circuit in the Explore/Exploit Tradeof*	9
Brandy Briones	Stuber	*Posterior paraventricular thalamus modulates socially-biased aggression*	11
Bryce Lecamp	Dhaka	*Investigating the analgesic properties of Cannabidiol in larval Zebrafish*	13
Carrie Stine	Bruchas	*Lateral hypothalamic projections to the ventral tegmental area modulate the activity of stress-sensitive nociceptin opioid peptide neurons.*	15
Catherine Hume	Hill	*Investigating the appetitive and motivational effects of vaporised tetrahydrocannabinol (THC)*	17
Charles Zhou	NAPE IC	*Characterization of microprisms for deep-brain 2-photon imaging*	19
Elora Reilley	Baertsch	*Emotional Modulation of Breathing: Mapping projections to the preBötzinger complex*	21
Ethan Ancell	Witten	*Post-selection inference for networks*	23
Jenna Sanders	Schindler	*Seasonal and circadian effects on stress research in male and female mice*	25
Kaylin Ellioff	Land/Bruchas	*Characterizing the effects of select phytocannabinoids and terpenes for pain relief*	27
Lucia Javorcikova	Hill/ Lohman	*Investigating the Response of the Endocannabinoid System Following Repetitive Mild Traumatic Brain Injury in Adolescent Male and Female Rats*	29
Madalyn Critz	Land/Bruchas	*Pain-encoding neurons in the periaqueductal gray during chronic neuropathic pain and cannabinoid treatment*	31

Poster Session II (March 11, 7:00 – 9:00 PM)			
Name	**Lab**	**Title**	**Station**
Madalyn Rice	Neumaier	*Investigating the Structure of Perineuronal Nets During Fentanyl Exposure and Withdrawal*	2
Madison Martin	Bruchas	*Characterizing locus coeruleus and pericoerulear zone activity in response to aversive and appetitive stimuli*	4
Maja Johnson	Geng	*EpiBrain: the brain's epigenetic landscape in a snapshot*	6
Mar Borrego	Ferguson	*Female rats show a greater behavioral response to heroin across self administration and locomotor sensitization compared to males*	8
Marilena DeMayo	McGirr	*Clinical outcomes in fibromyalgia following a four week non-invasive brain stimulation treatment with adjunctive D-Cycloserine*	10
Matthew Dawson	Sargin	*Role of Hypocretin/Orexin Neurons in Social Behavior and Isolation*	12
Micaela Ruiz	Chavkin/Land	*JWT-101 as a long-lasting KOR antagonist*	14
Monica Tschang	Schindler	*An alternative fecal microbiota transplant method for interrogating the role of the gut microbiome on trauma outcomes in blast polytrauma*	16
Myesa Travis	Li	*Elucidating the Role of Locus Coeruleus in Sleep Disturbances from Chronic Opioid Use*	18
Raaj Gowrishankar	Bruchas	*Endogenous dynorphin dynamics in the dorsal striatum shape neural activity for goal-directed behavior.*	20
Sara Saavedra	Ferguson	*Exploration of the behavioral profile of sequential opioid-stimulant polysubstance use disorders in a translational rodent model.*	22
Selena Fu	Dyck	*A role for vesicular zinc in the regulation of the social reward learning critical period*	24
Stefan Sandberg	Phillips	*Head-to-head comparison of fast-scan cyclic voltammetry and dLight 1.3b*	26
Victoria Hones	Mizumori	*mPFC dynamics during rat model of opiate withdrawal and subsequent psilocybin treatment*	28
Amanda Pasqualini	Bruchas	*Investigating the role of nociceptin-expressing central amygdala neurons in reward seeking*	30
Jonathan Sedano	Baerstch	*Individual Variability in Fentanyl Abuse and Relapse Using an Oral Self-Administration Model*	32

Abstracts for Oral Presentations

Dopamine signals across the anterior-posterior axis of the striatum represent unique components of consummatory behavior

Gordon-Fennell A., Benowitz B.M., Barbakh J.M., Montequin I., Campuzano A., Stevenson H., Hjort M.M., Critz M., Ancell E., Witten D. M., Stuber G.D.

Dopamine (DA) release at subregions throughout the striatum regulates unique components of behavior but DA signaling across the striatum during consumption remains poorly understood. Extensive research into reinforcement learning and motivation has determined DA dynamics during consumption of fixed volumes, but there has been relatively less investigation into how the DA system signals during periods where animals must shape their ongoing consumption response following the detection and valuation of solutions. To model consummatory behavior, we employed a head-fixed multiple solution brief-access taste task to elicit a range of consumption responses to a spectrum of rewarding solutions (0-30% sucrose) and aversive solutions (0-1.5M NaCl). To measure striatum-wide DA dynamics during consumption, we employed multi-site fiber photometry paired with GRAB-DA2m to record DA release up to 6 striatal sites across the anterior-posterior axis. To assess the components of the task that DA represents, we employed a generalized linear model to measure the unique contribution of each task component to the recorded signal. These experiments have revealed that DA release at subregions of the striatum show distinct activity that encodes unique components of consummatory behavior including initiation, solution value, and solution history. Despite widespread scaling of the DA response during consumption, there is an anterior-posterior distribution of encoding of solution value independent of licking behavior, with greater encoding of value in the rostral portions of the striatum. Altogether, our work outlines a spatiotemporal map of DA dynamics during consumption of rewarding and aversive solutions.

Effects of opioids on LH orexin and dynorphin modulation of VTA dopamine neurons

Aida Mohammadkhani, Ijeoma Ifionu, Min Qiao, Stephanie L. Borgland

The misuse of opioids has risen rapidly and remains a major health issue worldwide. Addiction is associated with neural circuit dysfunction, characterized by changes in synaptic transmission in the ventral tegmental area (VTA). Orexins (ox) and dynorphin (dyn) are co-expressed lateral hypothalamic (LH) neuropeptides that project to VTA. It is unclear how these co-released peptides affect DA neuron activity in physiological and pathological states. This study examined the effects of optically driven LHox/dyn release on VTA DA neuronal activity and how opioid dependence alters the selective contributions of LHox/dyn to the firing of VTA DA neurons that project to either the basolateral amygdala (DA-BLA) or the lateral shell of the nucleus accumbens (DA-AcbSh). We first observed a diverse response of LHox/dyn photostimulation on DA neuronal firing rate. In the presence of synaptic transmission blockers, 30-Hz optical stimulation increased firing in 60% of DA-lAcbSh neurons and decreased firing in 72% of DA-BLA neurons. An ox1 receptor inhibitor or a KOR inhibitor reversed the potentiation or inhibition of firing, respectively. In opioid (morphine and fentanyl) dependent mice, 30-Hz stimulation increased firing in 62% of DA-BLA neurons. Taken together, LHox/dyn corelease may tune VTA output by simultaneously inhibiting and activating different VTA projection neurons, with this tuning shifting under opioid dependence. This raises two hypotheses: opioid exposure upregulates dynorphin-degrading enzymes, leading to dynorphin inactivation, or it desensitizes KORs, reducing dynorphin's effect on DA-BLA neuronal firing. Current experiments address these hypotheses.

Investigating the role of TRPC6 channels in reward and uncertainty encoding in midbrain dopamine neurons

Mollie X. Bernstein, Sage Cho, Andrew Fan, Daniel McAuley, Scott Ng-Evans & Larry S. Zweifel

Dopamine (DA) neurons within the ventral tegmental area (VTA) are enriched in a variety of neuropeptides. These neuropeptides and their receptors, which are G-protein coupled receptors (GPCRs), differentially modulate the DA system and are sufficient to promote reward reinforcement. GPCR activation initiates a signaling cascade that results in an increase in intracellular calcium ions through multiple types of channels, such as transient receptor potential canonical (TRPC) channels. Based on the sufficiency of dopaminergic neurons in the VTA to promote reinforcement and the enrichment of TRPC6 channels in VTA-DA neurons, we hypothesize that calcium signals associated with GPCR receptor activation are mediated in part by TRPC6 channels and that these signals contribute to the encoding of reward-related information in VTA-DA neurons.

Examining the Impact of Methamphetamine and Fentanyl Polysubstance Administration on Locomotor Sensitization and Social Interactions: Can a Psychedelic Compound Mitigate Drug-Induced Social Deficits?

Leah M. Salinsky*, Kyra C. Diaz, Joshua L. Fox, Susan M. Ferguson

As opioid-use disorder (OUD) numbers continue to climb, polysubstance usage among users has become ever-apparent. Notably, while a substantial proportion of OUD patients also report psychostimulant usage , research has primarily focused on opioids or psychostimulants in isolation, leading to a gap in knowledge on how polysubstance usage may differentially impact users. Thus, we sought to evaluate the impact of single versus polysubstance exposure on locomotor sensitization and social interaction in vivo. We hypothesized that Sprague Dawley rats sequentially exposed to both methamphetamine and fentanyl would display a distinct locomotor response over time from subjects exposed to methamphetamine or fentanyl alone. We found that daily administration of methamphetamine resulted in locomotor sensitization over time in males but not females, while daily administration of fentanyl resulted in locomotor sensitization in both males and females. In rats exposed to both methamphetamine and fentanyl, males had significantly increased methamphetamine- and fentanyl-induced locomotion, while females had significantly decreased methamphetamine-induced locomotion and no significant changes in fentanyl-induced locomotion. Our results indicate that polysubstance exposure differentially impacts drug-induced locomotor sensitization compared to single-substance exposure in a sex-dependent manner. We are currently evaluating the impact of single versus polysubstance exposure on social interaction and hypothesize that polysubstance exposure will exacerbate the social interaction deficits that occur following single substance use. Lastly, recent evidence has suggested the potential for psychedelic compounds to decrease facets of both opioid and stimulant use disorders. Thus, we are testing the hypothesis that the psychedelic compound R-(-)2,5-dimethoxy-4-iodoamphetamine (DOI) will reverse social interaction deficits in both single and polysubstance exposed animals.

LC-mediated DA dynamics in appetitive and aversive stimuli

Avi Matarasso, B.S.; Elena Seaholm, B.S.; Itzel Rodriguez Reyes; Ashritha Cheeyandira; Sean C. Piantadosi Ph.D.; Li Li, Ph.D.; Michael R. Bruchas, Ph.D.

The locus coeruleus (LC) is a major source of norepinephrine (NE) that projects to distinct, functional targets, influencing arousal, anxiety, and learning. Recent pharmacological data suggest dopamine (DA) is also released from the LC, yet definitive measures of release across regions, paradigms, and behaviors typically associated with LC have not been characterized, due to difficulty in separating DA from NE using traditional sensing methods. Here, we used fluorescent biosensors to isolate monoamine release following selective photo-stimulation of LC terminals in hippocampus (CA1) and amygdala (BLA). We also stimulated ChrimsonR-expressing LC terminals at several frequencies (for 3s between 1-20hz) to mimic physiological activity, revealing NE and DA release increase nonlinearly with stimulation frequency. Ex vivo 2-photon imaging revealed optogenetic stimulation evoked DA release from LC. We also investigated the endogenous dynamics of NE and DA release in CA1 in response to appetitive and aversive stimuli with and without Gi-DREADD inhibition of LC and VTA to isolate LC and VTA contributions to dopamine release. In experiments without inhibition, we found more DA than NE is released in BLA and more NE than DA in CA1 in all behaviors. Inhibiting LC led to a decrease in DA released during free reward and shock in BLA. Further, VTA inhibition did not affect optically-evoked release of LC-DA. This work suggests that LC releases dopamine across stimulation frequencies, and the LC is contributing to DA release in response to natural stimuli.

Role of Hypocretin/Orexin Neurons in Social Behavior and Isolation

Matthew Dawson, Dylan J Terstege, Naila Jamani, Van Anh Lee, Kartikeya Murari, Jonathan R Epp, Derya Sargin

Chronic social isolation during adolescence disrupts normal social behavior and is a risk factor for anxiety and depression. Proper social functioning during adolescence is also essential for development of adult social behavior. Yet, our knowledge of which brain regions and circuits are affected by social isolation is incomplete. Based on our previous work (Dawson et al., 2023), the activity of hypocretin neurons - a cluster of neurons endemic to the lateral hypothalamus that govern arousal and motivation - is essential for normative social behavior. Our project builds on these findings to test our hypothesis that chronic social isolation produces deficits in social interaction by disrupting the normal functioning of hypocretin neurons. To do this, we first performed in vivo calcium recordings from hypocretin neurons in control (group-housed) and isolated (single-housed) mice and examined the differences in hypocretin activity during social interaction. We quantified social interaction behavior using an automated behavioral classifier. Here, we show that hypocretin neuron activity increases in female and male control and isolated mice upon initial interaction with a same-sex stranger conspecific. However, the amplitude of interaction-induced hypocretin activity is significantly reduced in female and male isolated mice, compared with controls. Quantification of social behavior showed that isolated mice displayed deficits in social interaction when compared with control mice. Finally, we used the novel hypocretin GRAB biosensor OX0.9 to examine how hypocretin signaling is altered in isolated mice and in response to social fear in postsynaptic regions.

A role for vesicular zinc in the regulation of the social reward learning critical period

Selena Fu*, Richard Dyck

Critical periods describe a developmental period during which the nervous system displays heightened sensitivity and increased plasticity, with closure of these critical periods limiting the ability of the brain to adapt. Recently, a novel critical period for social reward learning was discovered, and psychedelics have been shown to reopen this period. Our previous research suggests that vesicular zinc, which acts as a neurotransmitter, plays a role in several critical periods of plasticity, though its involvement in social reward learning remains unexplored. Thus, the aim of the current study is to investigate the role of zinc signaling in social critical periods and its potential modulation by psychedelics, by examining and comparing behavioural and anatomical phenotypes in normal mice and transgenic mice lacking vesicular zinc.

An alternative fecal microbiota transplant method for interrogating the role of the gut microbiome on trauma outcomes in blast polytrauma

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The microbiota-gut-brain axis (MGBA) is a key bidirectional communication network that is vulnerable to trauma and injury, mediating adverse outcomes including post-traumatic stress disorder (PTSD) and comorbid risky substance use. We previously established an association between gut microbiota and PTSD-like behaviors in mice exposed to repetitive blast polytrauma. Blast polytrauma is a significant health concern for Veterans and bystanders in ongoing international conflict. Critically, the contribution of the MGBA to the clinical effects of blast is understudied, and current animal models to establish MGBA causality are confounded by stress-inducing procedures (i.e., oral gavage). As an alternative to oral gavage, we recently developed the “poopsicle” method of fecal microbiota transplant that relies on self-administration instead of restraint. Results demonstrate this method effectively transfers gut microbiota without increasing stress (n=12 per group). Using our poopsicle method, we next tested MGBA causality in blast-related behavioral outcomes by transferring feces from blast mice to naïve mice - results demonstrate significant increases in anxiety-like behaviors in recipient mice that are reminiscent of blast-induced behavioral effects (n=6-11 per group). Together, our results demonstrate the viability of the "poopsicle" as a non-stressful alternative method to establish causality between trauma-induced changes in gut microbiota and PTSD-like outcomes. Ongoing and future projects will use this method to interrogate the role of the MGBA in comorbid outcomes like risky alcohol use. Together, this work has the potential to fill critical knowledge gaps and significantly expand our understanding of the role of the MGBA in blast polytrauma.

Social interaction reveals dynamic pain recovery window following neuropathic injury in mice

Carlee Toddes, Kevin Bai, Isabel Halperin, Riley Keeler, Michael Mosquera, Mitra Heshmati, Sam Golden

Following prolonged exposure to painful stimuli, functional and anatomical alterations occur in the neural circuits mediating both pain and social cognition. In humans, the nucleus accumbens (NAc), a key hub in the mesolimbic reward circuit, undergoes alterations following persistent pain that are accompanied by changes in affect and motivation. In mice, peripheral injury alters synaptic activity in the NAc by suppressing both glutamatergic and dopaminergic input, altering reward-related behaviors. While it is well reported clinically that humans develop maladaptive social behavior, such as social withdrawal and aggression, following traumatic injuries, little is known about what neural modifications may be causing these aversive social changes.

Current preclinical models evaluating pain-modulated social behaviors focus on procedural variations of resident-intruder pairing, where experimental mice are subjected to forced, involuntary social interactions. These procedures fail to incorporate volitional social decision-making and motivation metrics, nor do they account for individual variability, ultimately making them a poor tool for understanding changes to volitional social behavior that is relevant for human patients with chronic pain. In the following study, we aim to narrow this gap in preclinical understanding by pairing a novel volitional social self-administration procedure in mice with single cell resolution in-vivo recordings of NAc neural ensembles following sciatic nerve injury.

Disentangling pain modulation and respiratory depression at the level of the rostral ventromedial medulla

Ryan Phillips, Joe Arthurs, Elora Reilly, Alyssa Huff, and Nathan Baertsch

Opioid medications are pivotal in pain management but are often accompanied by severe side effects—most notably opioid-induced respiratory depression (OIRD), the primary cause of death in opioid overdoses. The respiratory side effects and pain-relieving properties of opioids are typically attributed to the respective activation of μ-opioid receptors (μOR) in respiratory and pain modulatory circuits. However, the rostral ventromedial medulla (RVM)—the primary output node of the descending pain modulatory system and a key mediator of opioid analgesia—also plays a significant role in OIRD, raising questions about whether analgesia and respiratory depression can be fully decoupled. Using intersectional genetic techniques in mice, we explored the roles of RVM subpopulations in descending pain modulation and respiratory control, focusing on their excitatory/inhibitory phenotypes and expression of Oprm1 (the gene encoding μOR) or proenkephalin (a polypeptide hormone that produces endogenous opioid peptides). We show that RVM subpopulations exert bidirectional, state-dependent control over breathing—sometimes causing prolonged apneas through upper airway collapse—and strongly influence pain thresholds. Notably, we identified sex differences in pain modulation and the time course of respiratory effects, providing potential mechanistic insights into sex-specific vulnerabilities in OIRD, differences in descending pain modulation, and the roles of endogenous opioid signaling. These findings highlight the complex intersection of pain and respiration at the level of the RVM, pointing to new therapeutic targets for reversing OIRD or achieving pain relief without respiratory depression.

Endocannabinoid Upregulation Influences the Behaviorl and Neuroendrocrine Response to Stress

Gavin Petrie, Georgia Balsevich, Hiulan Lee, Robert Aukema, Tamás Füzesi, Samantha Baglot, Jaideep Bains, Matthew Hill

Introduction: Endocannabinoid (eCB) signaling regulates stress responses, including hypothalamic-pituitary-adrenal (HPA) axis activity. The paraventricular nucleus of the hypothalamus (PVN), home to corticotropin-releasing hormone (CRH) neurons, is central to this process. eCB signaling mediates glucocorticoid-dependent negative feedback on CRH neurons, suggesting that pharmacologically enhancing this system may reduce stress-related outcomes. This study explored how upregulating eCB signaling mitigates stress responses using cellular, endocrine, and behavioral measures.

Methods: Endogenous Anandamide (AEA) and 2-AG levels were increased by inhibiting their respective enzymes, FAAH and MAGL, before foot shock stress. Outcomes included grooming behavior, PVN CRH neuron activation, and corticosterone release, assessed via fiber photometry, immunohistochemistry, and ELISA. Cannulae enabled targeted MAGL inhibitor (JZL184) delivery into the PVN.

Results: Foot shock stress increased grooming, CRH neuron activity, and corticosterone levels. Systemic FAAH inhibition had no effect, but MAGL inhibition reduced stress-induced grooming in a time-dependent manner. Fiber photometry revealed that MAGL inhibition enhanced CRH neuron responses during stress and accelerated their shutdown upon returning to safety. Intra-PVN, but not intra-BLA, MAGL inhibition reduced stress-induced grooming and HPA axis activation.

Conclusions: Pharmacological enhancement of 2-AG in the PVN modifies behavioral and HPA axis stress responses. MAGL inhibition produces temporally specific effects, amplifying CRH neuron activity during stress while facilitating recovery afterward. These findings support the potential of cannabinoid-based therapies for stress-related disorders.

How cannabis use patterns differentially affect adolescent neurodevelopment

Jessica Scheufen*, Savannah Lightfoot, Samantha Baglot, Catherine Hume, and Matthew Hill

Introduction: This project aims to investigate the effects of varied patterns of vaporized cannabis inhalation on adolescent rodent neurodevelopment and behaviour.

Methods: Adolescent (P34) male and female rats were split into four usage cohorts: 1) control non-users, 2) weekly users, 3) daily users, and 4) high frequency (HF) users (daily exposure up to up to 3 times/day). Magnetic resonance imaging (MRI) scans were taken pre- and post-vapour exposure, to quantify individual volumetric differences in corticolimbic regions of interest (ROI). Rats were also subject to three behavioral tests: 1) light-dark box (LDB), 2) fear conditioning, and 3) novel-object-context-mismatch (NOCM).

Results: MRI: In some of the ROIs analysed, HF-exposed rats had significantly more volumetric growth than controls, and the daily and weekly exposed rats had significantly less growth than controls. Behaviour: During LDB, the weekly- and daily-exposed rats showed significantly less anxiety-like behaviour than controls and HF. During FC extinction retrieval, the daily- and weekly-exposed rats had a stronger association with the fear memory than controls. During NOCM, there were no significant effects of treatment.

Conclusions: Here we present structural and behavioural evidence for a biphasic effect of cannabinoids, where at low frequencies cannabis had one effect, and at higher frequencies cannabis had different effects.

Vaporized cannabis during adolescence impacts parvalbumin interneurons in the medial prefrontal cortex in adulthood

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Cannabis use has significantly increased in recent years, particularly in adolescents, as the illicit drug becomes legal in many states. This is alarming as the long-term neurobehavioral consequences of adolescent cannabis use remain poorly understood. Recently, we reported that vaporized cannabis in adolescence led to long-lasting impairments in medial prefrontal cortex (mPFC)-dependent cognitive flexibility. Parvalbumin interneurons (PV) mediate cognitive flexibility as their inhibitory function tightly regulates mPFC output neurons. Thus, we hypothesized that vaporized cannabis during adolescence leads to long-lasting aberrant PV function in the mPFC, thereby leading to the cognitive flexibility deficit. To test this hypothesis, adolescent (postnatal day [P]35-55) Sprague-Dawley rats of both sexes received daily 1-h non-contingent vaporized cannabis extract or vehicle sessions for three weeks. After a two-week washout period, cognitive flexibility testing began on ~P70. After behavioral testing, the brains of littermates were either collected for immunohistochemistry (IHC) or whole cell patch clamp slice electrophysiology to record from viral-mediated fluorescently tagged PV cells in the mPFC. Our findings indicate that PV cells from cannabis-exposed rats were more excitable (increased spiking and lower rheobase) than PV cells from vehicle vapor-exposed rats. Moreover, in the IHC littermates, cannabis-exposed rats were impaired in cognitive flexibility and had reduced fluorescent intensity of PV cells in the mPFC compared to vehicle-exposed rats. These findings support the hypothesis that adolescent vaporized cannabis exposure leads to mPFC dysfunction in adulthood through altering PV interneurons and suggest that normalizing PV cell function may be a promising target to alleviate adolescent cannabis-induced mPFC dysfunction.

Endocannabinoids facilitate transitory reward engagement through retrograde gain-control

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The endogenous cannabinoid (eCB) signaling system represents the most widely distributed neuromodulatory system in the mammalian brain, yet we have a limited understanding of how this system regulates motivated behaviors. All major components of the eCB system are expressed in the Nucleus Accumbens, a ventral striatal brain region proposed to serve a role as a 'limbic-motor interface'. Using the fluorescent sensor of eCB release, GRABeCB 2.0, we report that eCB release in the NAc is evoked by salient rewarding and aversive stimuli, and that release dynamics evolve as animals learn task parameters. Given that eCBs function retrogradely, we identified the anterior paraventricular thalamus (aPVT) as the brain region which expresses the highest % of NAc projecting neurons which also highly coexpress the CB1 receptor. Using fiber photometry in mice expressing GCaMP in aPVT, we observed that aPVT-NAc projections are inhibited during engagement in reward-seeking behaviors and activated upon behavioral disengagement. Nest, using a CRISPR/Cas9 strategy to delete the CB1 receptor from aPVT neurons, we found that CB1 deletion was sufficient to reduce engagement in reward-seeking behaviors and concomitant aPVT-NAc terminal inhibition. Lastly, using an anterograde transsynaptic labeling technique combined with 1-photon miniscope imaging, we observed that specific clusters of NAcaPVT D2/PENK+ neurons track eCB release dynamics or aPVT terminal activity, providing a putative link between single cell neural activity, eCB release, and terminal modulation. Collectively, hese studies reveal a novel eCB mechanism for regulation of engagement in reward-seeking behaviors through modulation of a genetically and anatomically defined thalamo-striatal circuit.

Investigating the appetitive and motivational effects of vaporised tetrahydrocannabinol (THC)

Catherine Hume (presenting author), Samantha Baglot, Lucia Javorcikova, Victoria Melts, John Bieber & Matthew Hill

It's well established that cannabis drives food intake, commonly referred to as 'the munchies'. These appetitive effects have putative therapeutic benefit for assisting in treating malnutrition and wasting, however the underlying mechanisms remain unknown.

Evidence suggests that cannabis can trigger appetite when there is no physiological drive to eat (i.e., when satiated or nauseous), thereby the aim of this project was to investigate if cannabis drives feeding by increasing food motivation and reversing food reward devaluation.

We used a tetrahydrocannabinol (THC) vapour inhalation rat model where animals were exposed to THC or vehicle vapour for 15min/day and subsequent appetitive behaviour measured; food motivation measured using an operant paradigm were animals lever press for sucrose, satiety induced through palatable food access, and food aversion induced by pairing food access with lithium chloride-induced nausea.

We showed that THC vapour robustly drives food intake whether animals are satiated or not. Further, THC increased food motivation, even when food was devalued in satiated or aversion conditions, demonstrating that the effects of THC on appetite are motivation based, where THC can reduce food reward devaluation, inducing feeding in circumstances where there is no physiological drive to eat. This sheds light on the behavioural mechanisms by which cannabis alters feeding patterns. Future studies will delve into the neuronal mechanisms underlying these effects.

Characterizing the effects of select phytocannabinoids and terpenes for pain relief

Kaylin Ellioff, Keming Qiu, Anthony English, Sean C. Piantadosi, Nephi Stella, Michael R. Bruchas, Benjamin B. Land

Chronic pain affects nearly 100 million individuals in the US, and current analgesics have significant drawbacks including opiate use disorder and overdose deaths. Cannabidiol (CBD) and terpenes, two constituents of Cannabis, act within the endocannabinoid (eCB) signaling system and hold promise as alternative analgesics. Recent studies suggest that CBD and terpenes may act via an “entourage effect” where their combined effect is greater than each individually. It is also known that eCB signaling within the basolateral amygdala (BLA) mediates pain. It remains unclear how terpenes interaction with CBD affects pain states and whether these compounds act within the BLA to regulate nociception. To answer these questions, we employed a gelatin, self-administration model in combination with classical pain behaviors and cutting-edge machine-learning approaches to classify novel neuropathic chronic pain phenotypes. Results indicate that mice consistently consume gelatin that contains four terpenes (α-pinene, β-myrcene, linalool, β-caryophyllene) and/or CBD ad libitum and that these gelatin treatments can alleviate allodynia weeks following chronic pain induction. Cannabinoid receptor 1 (CB1R) and serotonin 1A receptor (5-HT1A) antagonists do not reverse this effect. To investigate how CBD acts within the BLA, we performed in-vivo 2-photon imaging during chronic pain after CBD and terpene gelatin administration. We observe blunted nocifensive responses and reduced excitatory cell activity in mice given this treatment, supporting the role of the BLA in pain regulation. With the growing use of Cannabis and lack of non-opioid pain treatments, these results provide important information regarding both CBD and terpenes in the context of pain.

THC-Dependent Increases in Neuronal and 2-AG Activity in Mouse Prefrontal Cortex at the Initiation of Locomotion

Anthony English, David Marcus, Khushi Yadav, Yassin Elkhouly, Fleur Uittenbogaard, Rayna Simons, Victoria Corbit, Yulong Li, Benjamin B. Land, Nephi Stella, and Michael R. Bruchas

THC triggers a dose-dependent reduction in spontaneous locomotion in mice, characterized by immobility interrupted by brief, random movement. We used fiber photometry, pose estimation, and genetic interventions to investigate how THC alters locomotor kinematics by measuring neuronal activity and cannabinoid signaling in the medial prefrontal cortex (mPFC) of treated mice. WT mice were injected with AAV5-hSyn-GRABeCB2.0, while VGAT-Cre and VGLUT1-Cre mice were injected with AAV5-DIO-GCaMP6f and implanted with an optic fiber into the mPFC. Using SLEAP, we tracked behavior in a dual-view linear track chamber, with positional data fed into a machine learning model to classify behaviors, revealing photometry-linked activity and nuanced behavioral effects. VGAT-Cre and VGLUT1-Cre mice also received AAV5-DIO-CRISPR-Cnr1 to knock out CB1 receptors in specific neurons. Locomotion initiation increased glutamatergic and GABAergic mPFC activity, with higher GCaMP6f signaling in VGAT-Cre (Z-Score: 8.64 ± 0.48) than VGLUT1-Cre (Z-Score: 1.97 ± 0.24) mice treated with THC. GRABeCB2.0 activity rose gradually and spiked during locomotion, more so in THC-treated than VEH-treated mice. Pharmacological inhibitors JZL-184 and DO34 modulated these spikes. THC impaired kinematic features, such as gait, indicating motor impairment, partially reversed by CB1R knockout in VGAT neurons. These findings highlight THC's differential effects on mPFC neuronal activity, with GABAergic depolarization driving locomotion initiation. Pharmacological results implicate 2-AG as a key endocannabinoid. Behavioral tracking revealed nuanced kinematic impairments beyond reduced locomotion, emphasizing THC’s modification of mPFC activity's role in regulated motor coordination.

Investigating the Response of the Endocannabinoid System Following Repetitive Mild Traumatic Brain Injury in Adolescent Male and Female Rats

Lucia Javorcikova, Samantha L. Baglot, Catherine Hume, Tom Carr, Aly Muhammad Salim, Jessica Scheufen, Alexander W. Lohman, Matthew N. Hill

Repetitive mild traumatic brain injuries (RmTBI) are common in adolescents and lead to neuroinflammation, motor deficits, and behavioral changes. The endocannabinoid (eCB) system plays a key role in cognitive and behavioral responses. The eCB system is primarily anti-inflammatory offering a potential therapeutic target for RmTBI. This study aims to quantify eCB expression and assess cognitive and motor outcomes of RmTBI using a rodent model. Adolescent (P34) male and female Sprague-Dawley rats were administered a total of 5 mTBI at 72-hour intervals using the lateral impact device. Brain regions including the hippocampus, amygdala, motor and prefrontal cortex were analyzed for eCB levels (2-arachidonoylglycerol [2-AG] & anandamide [AEA]) via mass spectrometry immediately and one week post injury. Anxiety like behaviour and object-context discrimination was assessed, along with motor strength. After behavioral testing, brains were processed to evaluate microglia density and morphology. Results showed that RmTBI significantly reduced motor strength in males but not in females. RmTBI did not influence anxiety like behaviour in males. Notably 2-AG levels were significantly decreased in the prefrontal cortex of male rats one week after injury while AEA levels were elevated in the hippocampus immediately post injury. Female molecular and behavioral analysis is ongoing. Overall, this study is anticipated to expand the current understanding of the response of the eCB system following RmTBI in both males and females.

Brain wide neuronal ensembles engaged by opioid

KENTARO ISHII, Meha Shah, Yizhe Zhang, Garret Stuber

Opioid use disorder is a worldwide societal problem and public health burden. The number of prescription opioids and related deaths has been increasing for a decade. The consumption of opioid drugs starts as reinforcing, gradually becomes more dependent and then results in a negative emotional state when the drug is withdrawn. Understanding how the brain functions as a system before, during and after the development of opioid addiction is essential for the discovery of more efficient treatments. Here, we utilized an unbiased approach to map the whole brain response to opioids and opioid withdrawal using tissue clearing and light sheet microscopy methods. Furthermore, by using genetic tools that allow activity dependent labeling, we compared the input-output structure of the neurons which are recruited during opioid administration or withdrawal. Overall, our results highlight that the brain utilizes different sets of neural circuits depending on the stage of addiction.

Engineering a genetically encoded fluorescent sensor for in vivo fentanyl detection

Lily Torp, Yuxuan Wang, Sarah Wait, Mikayla Gargantiel, Lila Jin, Cat Zamorano, Mary Loveless, Marta Soden, Michael Bruchas, and Andre Berndt

Opioid-based analgesics are the most widely used treatments for chronic pain. Within the past decade, fentanyl has rapidly infiltrated the drug supply, leading to a rise in opioid use disorder (OUD) cases and overdose deaths. Fentanyl is a potent synthetic opioid and binds to the μ-opioid receptor (μOR), but the pharmacological and spatiotemporal profile of fentanyl distribution in the brain is poorly understood. To address this need, the Berndt Lab developed μMASS, a genetically encoded μOR-based fluorescent opioid sensor coupling real-time opioid detection to an increased fluorescence response. However, μMASS senses multiple opioids, making it difficult to detect specific ligands. To interrogate μMASS-ligand binding, we generated AlphaFold2 μMASS models and docked fentanyl in the μMASS binding pocket. We observed fentanyl did not maintain a conserved salt bridge with residue D147. Altering this residue to glutamate (D147E) rendered μMASS selective for fentanyl while greatly reducing affinity for endogenous opioid peptides, and we dubbed this sensor FentMASS1.0. FentMASS1.0 detects fentanyl in HEK293 cells and primary cortical neurons. Importantly, the fluorescence signal is rapid and reverses upon application of μOR antagonist naloxone. To enhance sensor signal and neuronal expression we introduced alternative μOR receptors (zebrafish, killifish) and applied homologous mutations (D139E, D124E) to each sensor. Both variants demonstrated increased response to fentanyl while maintaining fentanyl selectivity over met-enkephalin. Ultimately, this sensor will enable cell-type specific fentanyl pharmacokinetics measurements to determine if there are appreciable differences in the timing, accumulation, and potency of fentanyl across μOR-positive brain regions, such as the VTA and PAG.

High-throughput Engineering of Genetically Encoded Fluorescent Sensor for Detecting Opioids in vivo

Yuxuan. Wang, Lily Torp, Sarah Wait, Mikayla Gargantiel, Lila Jin, Catalina Zamorano, Mary Loveless, Marta Soden, Michael Bruchas, Andre Berndt

Genetically encoded fluorescent indicators (GEFIs) are powerful tools for real-time monitoring of neural activity and enable high sensitivity, specificity, and spatiotemporal resolution. Engineering GEFIs has become a primary goal for many seeking to understand neuromodulation. However, it is challenging to study opioid signaling due to the vast mutational landscape inherent to large proteins. To address this problem, the Berndt Lab developed the optogenetic microwell array throughput screening system (Opto-MASS), a high-throughput screening platform capable of screening thousands of GEFIs in a single day. We previously leveraged Opto-MASS to identify an improved opioid sensor μMASS, which displays an increased fluorescence response to endogenous (met-enkephalin) and exogenous (fentanyl) opioid ligands. To improve the baseline fluorescence, signal-to-noise ratio, and opioid selectivity of μMASS, we tested μ-opioid receptors from different species (Zebrafish and Killifish), leading to μMASS1.5(Zebrafish) and μMASS1.7(Killifish). These sensor variants display enhanced responses to met-enkephalin (~1.5 fold and ~3 fold). Additionally, to engineer a μMASS variant with optimal neuronal expression, we engrafted protein expression tags to μMASS1.5 and μMASS1.7. We found the HA-FLAG and PRC tags resulted in greater fluorescence response (~1.5 fold) to met-enkephalin in neurons compared to μMASS. Taken together, we leveraged the high-throughput capabilities of Opto-MASS to engineer novel tools to detect opioids in real-time which can be applied in future in vivo experiments in freely behaving animals.

Individual Variability in Fentanyl Abuse and Relapse Using an Oral Self-Administration Model

Todd Appleby, Kevin Coffey, Sam Golden

Synthetic opioids like fentanyl are endemic in the United States and are currently the major contributing factor causing high rates of relapse and overdose. Opioid addiction is characterized behaviorally by compulsive drug-seeking and drug-taking. However, the rate at which these maladaptive behaviors manifest after initial exposure and during withdrawal varies across individuals. The neural basis for individual variability in resilience to opioid addiction and relapse is poorly understood, which contributes to a lack of clinical interventions.

To study individual variability in fentanyl self-administration and relapse, our labs (Golden and Coffey) have developed an oral fentanyl self-administration model. This approach integrates SimBA, an open-source machine-guided behavioral classification pipeline developed by our team. We also use unsupervised learning approaches in behavioral classification to determine new behavioral phenotypes across the longitudinal progression of our addiction model.

Here, we first validate this model by showing robust acquisition of oral fentanyl self-administration sessions in male and female CD1 and C57 mice. We then show behavioral classification data from our self-administration model for 10 male and female CD1 and 8 male and female C57 mice. Behavioral classifications during acquisition, extinction, and relapse will provide new indicators of maladaptive behavior onset across the longitudinal progression of addiction. Moreover, comparisons of behavior across animals will help identify neural markers for individual vulnerability to onset and relapse of addiction behavior.

Dynorphin regulation of the dorsal raphe nucleus encodes stress-induced dysphoria

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Stress activates the release of neuropeptide transmitters, including endogenous dynorphin (Dyn) opioids that activate $G\alpha_{i/o}$-coupled Kappa Opioid Receptors (KOR) to encode the dysphoric properties of stress. KOR activation leads to the recruitment of the three major mitogen-activated protein kinases (MAPK), and a unique role for p38α MAPK in regulating serotonin (5-HT) tone has been established by p38α inhibitor and gene deletion studies. Further, p38α MAPK mediates dysphoria and promotes relapse of drug seeking during periods of drug abstinence. Preclinical evidence suggests that dysphoria may be a consequence of a transient, stress-induced hyposerotonergic tone in the nucleus accumbens (NAc) dependent on Dyn/KOR/p38α MAPK signaling in 5-HT neurons of the dorsal raphe nucleus (DRN). Characterization of the pharmacological and behavioral role of stress-induced activation using *in vivo* fiber photometry and the genetically-encoded sensor, GRAB-5_{HT} in combination with CRISPR gene deletion allows us to monitor real-time 5-HT DRN neurons projecting to the NAc during repeated forced swim (rFSS). On day 1, mice are injected with either vehicle or the long-lasting KOR antagonist, norBNI (10 mg/kg). 48 h later, mice are tethered to the photometry rig and run for a baseline for 10 minutes followed by a 15-min swim. 24 h following, mice are again tethered to the photometry rig and run for 6-min swim, followed by a 6-min rest, repeated 4 times (4x6). During periods of swim in control mice, there are robust changes in 5-HT fluorescence that can be blocked with either p38a excision in the DRN or KOR antagonism. Understanding how stress-induced dysphoria is regulated by dynorphin in the dorsal raphe 5-HT neurons will provide critical insight into the mechanisms by which stress disrupts the 5-HT system to increase susceptibility to subsequent reward.

Endogenous dynorphin dynamics in the dorsal striatum shape neural activity for goal-directed behavior.

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Endogenous neuropeptides, signaling via G protein-coupled receptors, are uniquely poised to regulate neuronal activity and behavior. Yet, how neuropeptide dynamics control behavior is unclear. The endogenous neuropeptide dynorphin is highly enriched in the dorsal striatum, known to be critical for regulating goal-directed behavior. However, the locus, the precise timescale, or functional role of dynorphin signaling via the kappa opioid receptor on goal-directed behavior is unknown. Here, we report that dynorphin neuron activity evolves during behavioral learning, leading to synchronized, time-locked dynorphin release at strikingly fast timescales in the dorsomedial striatum. Further, we demonstrate that dynorphin release results in retrograde presynaptic GPCR-mediated inhibition of glutamatergic axon terminals from the basolateral amygdala, thereby promoting and refining behavior. Collectively, our findings isolate a causal role for endogenous opioid release at rapid timescales, and a novel mechanism whereby subsequent GPCR activity at excitatory terminals shape fundamental goal-directed behaviors. Future work will elucidate how endogenous neuropeptide signaling is maladapted during goal-directed behavior for misused substances, and delineate the impact of neuropeptide dynamics in the basal ganglia on circuit activity during goal-directed behavior.

LHb Neural Dynamics During Drug Cessation from a Novel Delayed Punishment Paradigm

Ari Asarch, Neethi Belur, Will Nickelson, Michele Kelly, Kevin Coffey, John Neumaier.

Opioid Use Disorder remains a critical public health concern, particularly in understanding the neural mechanisms underlying the decision to stop drug use despite increasing negative consequences. While it is well-established that aversive stimuli can modulate drug-taking behavior, the neural dynamics driving this shift remain poorly understood due to technical limitations. Here, we investigated how the lateral habenula (LHb), a key decision-making center known for processing aversive stimuli, responds during this critical transition using miniscopes to capture neuronal activity. Our study presents the first large-scale in vivo calcium imaging investigation of LHb activity during drug-seeking behavior, providing unprecedented insight into the neural basis of punishment-mediated drug cessation. To probe the neuronal mechanisms underlying this behavioral shift, we developed a novel delayed punishment paradigm. Rats were trained to self-administer oral fentanyl and then subjected to increasing shock intensities following a drug-loading phase. Interestingly, we observed that rats self-administering fentanyl exhibit increased drug intake when presented with low-intensity shocks (0.2 mA) compared to no shock, suggesting a paradoxical escalation in drug-seeking behavior under mild aversion. However, at higher shock intensities (0.8 mA), fentanyl self-administration decreased below baseline levels, indicating a tipping point where the negative consequences outweigh the drug's rewarding effects. Preliminary analyses of LHb activity suggest a distinct neuronal signature correlating with the cessation of drug-seeking behavior under high aversion. Notably, global activity signals appeared misleading, underscoring the importance of analyzing individual neuronal patterns. We also observed behavioral validation through ultrasonic vocalizations (USVs), with preliminary analyses showing 22 kHz calls increasing alongside shock intensity, confirming their association with negative events. These findings provide insights into the neural and behavioral dynamics of addiction and aversion, offering potential targets for interventions. Understanding the neural basis of this shift may inform targeted, time-sensitive treatments for addiction and advance philosophical perspectives on decision-making in Opioid Use Disorder.

Clinical outcomes in fibromyalgia following a four week non-invasive brain stimulation treatment with adjunctive D-Cycloserine

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Fibromyalgia is a chronic pain condition that affects approximately 2-3% of the population It is characterized by ongoing, widespread pain, along with accompanying symptoms such anxiety, mood, disrupted sleep, and cognitive difficulties. While there are some effective treatments, many individuals with fibromyalgia continue to experience significant impairment from their symptoms.

For these individuals, one novel treatment proposed is non-invasive brain stimulation. In particular, Transcranial Magnetic Stimulation (TMS) targeting the dorsolateral prefrontal cortex has a strong evidence base in fibromyalgia, with high rates of tolerability. While many individuals with fibromyalgia benefit from TMS, however, not all do.

TMS involves generating magnetic fields outside of the body to change the firing of neurons in the brain. This is hypothesized to occur via synaptic plasticity. Previous data in major depression indicates that we can enhance the clinical benefit of TMS by pairing it with D-Cycloserine, a medication that enhances synaptic plasticity. There is a significant overlap between stimulation target and TMS protocols used for depression and for fibromyalgia, so hypothesize that the combination of D-Cycloserine and TMS will lead to greater improvements in fibromyalgia than TMS alone.

In this trial, all participants with fibromyalgia received 4-weeks of intermittent theta burst stimulation (iTBS), a type of TMS. In addition, participants were randomized to receive either adjunctive D-Cycloserine or placebo, and this assignation was double-blinded. Results will be presented from the interim analysis planned at 45 participants on clinical and pain measures, examining if adjunctive D-Cycloserine enhances outcomes following 4-weeks of iTBS.

Endogenous Opioids Facilitate Stress-Induced Binge Eating via an Insular Cortex-Claustrum Pathway

Jingyi Chen, Leandra Mangieri, Sophia Mar, Sean Piantadosi, David Marcus, Phoenix Davis, Benjamin Land, Michael Bruchas

Stress has been shown to promote the development and persistence of binge eating behaviors. However, the neural circuit mechanisms for stress-induced binge-eating behaviors largely unreported. The endogenous dynorphin (dyn)/kappa opioid receptor (KOR) opioid neuropeptide system has been well established to be a crucial mediator of and anhedonic components of stress. Here we aimed to dissect the basis of dynorphinergic control of stress-induced binge-like eating behavior. We first established a mouse behavior model for stress-induced binge-like eating behaviors and found that mice exposed to stress increased their food intake of familiar palatable food (high fat, high sugar, HPD) compared to non-stressed mice. Following a brain-wide analysis we isolated robust cFos-positive cells in the Claustrum (CLA), a subcortical structure with highly abundant KOR expression following stress-induced binge-eating behavior. We report here, that KOR signaling in CLA is necessary for this elevated stress-induced binge eating behavior. In vivo cell recordings in glutamatergic neurons of the CLA using fiber photometry revealed increased neural activity during initiation of HPD feeding bouts. Local disruption of KOR signaling in CLA, blocked stress-induced binge eating, indicating that endogenous dynorphin release mediates this behavior. We further established the dynamics of endogenous dynorphinergic control of this behavior using using a genetically encoded dynorphin biosensor Klight. Combined with 1-photon single-cell calcium imaging, we report significant heterogeneity with the CLA population during stress-induced binge eating. Furthermore, we isolate the anterior Insular cortex (aIC) as the potential source of endogenous dynorphin afferents intoto the CLA. By characterizing neural circuit and peptidergic mechanisms within the CLA, we uncover a pathway which implicates endogenous opioid regulation stress-induced binge eating.

Friend or Foe: Role of CRH-PVN neurons in social threat detection

IBUKUN AKINRINADE, Meenakshi Pardasani, Toni-Lee Sterley, Tamás Füzesi, Matt Hill, Jaideep Bains

Our understanding of how animals detect and respond to threat rely on a framework of predator-prey interactions. Threat, however, can also arise from conspecifics. Accurately discerning the potential threat posed by others is vital for building social relationships, yet the mechanisms underlying this process remain unclear. The corticotropin-releasing hormone neurons of the paraventricular nucleus in the hypothalamus (CRHPVN) control physiological and endocrine responses to threat. They are necessary for the social investigation of a stressed partner that results in the transmission of stress from one individual to another. Whether these neurons are also involved in assessing the potential threat posed by conspecifics is unknown. Using behavioral analysis and fiber photometry, we examined CRHPVN activity in resident mice during interactions with intruders. CRHPVN activity increased during approach behavior, with a significantly larger response to unfamiliar intruders compared to familiar ones. This was followed by elevated anogenital sniffing toward unfamiliar intruders. When intruders exhibited a negative affective state (via foot-shock), residents spent more time investigating stressed familiar intruders compared to naïve ones, accompanied by corresponding changes in CRHPVN activity. However, CRHPVN responses were similar for stressed and naïve unfamiliar intruders. These findings suggest CRHPVN neurons play a key role in social threat detection and safety assessment, modulating responses based on familiarity and affective state. Differences in CRHPVN activity associated with familiarity and affective state reflect its role in guiding appropriate social investigative behaviors. Overall, our study highlights CRHPVN neurons as critical mediators in identifying potential social threats and directing adaptive social responses.

Fear generalization and functional connectivity of the dorsolateral prefrontal cortex in posttraumatic stress disorder

Ryann Tansey, Gavin N. Petrie, Irene Perini, Matthew N. Hill, Markus Heilig, and Leah M. Mayo

BACKGROUND: Traumatic experiences can have substantial effects on mental and physical health outcomes. Trauma disorders, such as posttraumatic stress disorder (PTSD), can develop from dysfunctional fear generalization, which can result in an association between neutral stimuli and the fearful stimulus. In a recent study of adult participants both with and without a history of childhood maltreatment, individuals with greater functional connectivity between the dorsolateral prefrontal cortex (DLPFC) and the amygdala had lower levels of fear generalization. Here, we explored whether DLPFC functional connectivity is associated with fear generalization in PTSD.

METHODS: Participants with PTSD (n = 100) completed a fear conditioning task and a separate resting-state fMRI scan. Facial electromyography (EMG) was used to measure the startle response of participants in the fear conditioning task. Following acquisition of fear conditioning, fear generalization was assessed as the difference between startle responses to the CS+ and the CS-, with scores near 0 representing more fear generalization. Whole-brain functional connectivity was calculated using the DLPFC as the seed. Clusters were defined at a voxel-wise significance of $p = 0.002$ and a cluster-forming threshold of $\alpha = 0.05$.

RESULTS: There was a significant positive relationship between fear generalization and right DLPFC connectivity to the bilateral inferior parietal lobule, middle cingulate cortex, left temporal pole, frontal eye fields, and right posterior cingulate cortex, fusiform gyrus, and putamen, such that greater connectivity to the DLPFC was indicative of less fear generalization.

CONCLUSIONS: The right DLPFC may play an important role in fear generalization in PTSD.

Serotonin Release in the Central Nucelus of the Amygdala in Appetitive and Aversive Learning

Ekayana Sethi, Mi-Seon Kong, Marta Soden, Larry Zweifel

Anxiety and substance use disorders frequently co-occur and have overlapping neural mechanisms contributing to maladaptive fear and reward learning. The central nucleus of the amygdala (CeA), which integrates emotional signals, plays a crucial role in these processes. Dysregulation of 5HT signaling is implicated in anxiety and addiction, yet its role in dynamic reward and fear conditioning remains unclear.

Here we used a novel 5HT biosensor to measure real-time serotonergic release in the CeA during operant reward and fear conditioning. Our data show that there is an increased 5HT release during food consumption and sustained increase of 5HT signals while waiting for food delivery. This sustained 5HT release during the waiting period diminished as rewards became less consistent in an omission paradigm or were devalued by pairing it with a mild footshock in our novel FoodShock paradigm. In a fear conditioning paradigm, 5HT release increased to a footshock and was elevated in response to a cue paired with the footshock.

These results reveal that CeA 5HT signaling dynamically tracks reward anticipation, consumption as well as aversive stimuli like footshocks and its associated cues, suggesting a role on 5HT in appetitive as well as aversive learning. Interestingly, our data demonstrate that sustained increase in 5HT release during reward anticipation can be decreased by making reward delivery less consistent as well as devaluing the reward, indicating a role of CeA 5HT in tracking consistent and highly rewarding stimuli, providing further insights into the shared neural mechanisms underlying anxiety and substance use disorders.

Central amygdala acetylcholine signaling in conditioned taste aversion

Weston Fleming, Marta Trzeciak, Aurora Liu, Jordan Pauli, Sekun Park, Cassidy Burke, Richard Palmiter, Garret Stuber

Conditioned taste aversion (CTA) is a powerful form of learning that involves associating a novel food (CS) with visceral malaise (US). CTA can occur even when CS consumption and malaise occur hours apart. We propose that the neurobiological mechanisms that make such an association possible over such a time difference involve acetylcholine (ACh) signaling through muscarinic GPCRs in central amygdala (CeA). Using a novel head-fixed CTA behavior, we observe that ACh is released during exposure to a novel solution; that ACh signaling is altered following CTA conditioning; and that CeA neurons show increased recruitment and activity during exposure to the CS following conditioning. Critically, we also show that ACh levels in CeA increase dramatically following injection of a visceral malaise inducing agent, LiCl. Pharmacology experiments using slice electrophysiology and 2-photon calcium imaging indicate ACh can promote widespread activation of CeA neurons, primarily via m3 and m5 muscarinic receptors. We hypothesize that increases in ACh tone during visceral malaise are key for generating plasticity underlying CTA. To this end, we demonstrate that the parabrachial nucleus sends a functional cholinergic projection to CeA, and that CRISPR-mediated knockdown of ACh synthesis in PBN greatly reduces ACh released following LiCl injection. Ongoing experiments will test whether this same knockdown approach also impairs CTA in our head-fixed assay, and how impairment of ACh signaling in CeA affects CeA neuronal activity at each stage of CTA.

Exercise erases the behavioral and synaptic consequences of stress

Mijail Rojas-Carvajal, Tamás Füzesi, Dinara Baimoukhametova, Núria Daviu, Govind Peringod, Patrick Grovue, Sarah Cook, Grant Gordon, and Jaideep S. Bains

Stress imprints biochemical, molecular, and synaptic changes in the brain to promote adaptation. However, these changes can become maladaptive and foster neuropsychiatric diseases. Surprisingly, there is limited understanding on how these imprints can be reversed. In humans, exercise is used to cope with stress despite inducing physiological stress itself. Here we examined the effects of exercise on stress-induced short-term potentiation (STP) of glutamate synapses on corticotropin release hormone cells in the paraventricular nucleus of the hypothalamus (CRH-PVN). Exercise (treadmill) for one hour after foot shock (FS) increased CRH-PVN activity and circulating corticosterone (CORT). Next, we obtained electrophysiological recordings from CRHPVN neurons in hypothalamic slices and evaluated the effects of exercise after FS on STP. Following FS, high frequency stimulation of glutamate synapses elicited STP. Exercise after FS blunted STP. Exercise after FS increased brain-derived neurotrophic factor (BDNF) in the PVN. And incubation of brain slices from FS mice with a TrkB agonist and CORT blunted STP. At a behavioral level, mice subjected to FS showed lower exploration of the light compartment in a Dark/Light box. Exercise after FS reversed this phenotype. Our findings demonstrate that exercise increases BDNF in PVN and decreases STP induced by stress This is accompanied by a decrease in stress-induced anxiety.

Abstracts for Poster Presentations

(*Some poster presenters are also giving oral presentations; their abstracts can be found in the previous section.*)

Differential responses to reward and aversion by genetically distinct GABA subpopulations of the VTA

Abigail J. Elerding, Adam Gordon-Fennell, Josef Dostal, Eleanor Mauk, Marta E. Soden, Garret D. Stuber, Larry S. Zweifel

GABA neurons in the ventral tegmental area (VTA) are a potent regulator of the dopamine
system, and many medicinal and non-medicinal compounds affect GABAergic transmission
which can lead to substance misuse and the development of substance use disorders. Current research has revealed complex roles for these neurons, identifying them as critical mediators of reward, aversion, and associative learning. Previous single-nucleus RNA sequencing has revealed genetically distinct GABAergic subpopulations within the VTA, but the function of these genetic subtypes still needs to be resolved. Here, we explored the broader impact of VTA GABA neurons on reward-related behaviors and the cellular and circuit heterogeneity within these inhibitory populations. Using intersectional genetic strategies in mice, we selectively targeted GABA neurons expressing Pnoc, Crhbp, or Cbln4. Analysis of calcium dynamics during head-fixed presentations of appetitive and aversive stimuli revealed distinct patterns of activity in these subtypes. Preliminary results suggest that Pnoc-GABA neurons are more active in response to aversive stimuli, while Crhbp and Cbln4 subtypes are primarily engaged during reward signaling. Further investigation into the function and diversity of VTA GABA neurons will yield deeper insights into how these populations coordinately regulate the mesolimbic dopamine system and contribute to reward processing and motivation.

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

Wells B. A., Marcus D. J. Critz M., Oommen R., Chun G., Bruchas M. R.

The Paraventricular Thalamus (PVT) is a key brain structure involved in integrating interoceptive, emotional, and motor signals, and influencing motivated behaviors. Recent studies of the PVT's role have produced conflicting results, partly due to its heterogeneity and lack of anatomical specificity. Notably, the neuropeptide neurotensin (NTS) is selectively expressed in the anterior PVT (aPVT), which projects to several subcortical 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.

Posterior paraventricular thalamus modulates socially-biased aggression

Brandy Briones, Nico Masputra, Dechen Sakya, Marta Trzeciak, Alondra E. Torres, Zoe Garret, Raihana Oien, Adam Gordon-Fennell, Prabhat Aluri, Marissa Borrego, Garret D. Stuber

Social biases influence whether we act affiliative, neutral, or aggressive towards strangers. In unfamiliar circumstances learned biases can affect which strangers are perceived as safe or threatening. These oversimplified perceptions can disrupt the formation of healthy social relationships and, in some cases, result in unsubstantiated intergroup violence. In our study, to enhance an “in-group” identity, we reared mice with other phenotypically similar mice (homogeneous environment) and used the resident-intruder assay to assess their behavior towards strangers with familiar (in-group) versus unfamiliar (out-group) physical traits. This revealed robust out-group-specific aggression, with male mice attacking out-groups significantly more than in-group strangers. However, aggression was equally distributed amongst out- and in-group strangers when mice were reared in heterogeneous environments, suggesting that biased aggression is mediated by learned familiarity from their homecage environment. We then identified a dense population of neurons co-expressing estrogen and androgen-related genes in the paraventricular thalamus (PVT), a sensory and emotional state relay center that coordinates defensive responses to stress and predators. Recognizing the importance of sex steroid hormones as neuromodulators of social behaviors, we designed a set of experiments to explore the potential involvement of PVTEsr1 neurons during biased aggression, utilizing fiber photometry calcium recordings, optogenetics, chemogenetics, and viral-induced genetic knockout. High-frequency optogenetic stimulation of PVTEsr1 neurons during the resident-intruder test enhanced out-group-specific aggression, while DREADD inhibition reduced it. Cre-induced PVT Esr1 knockout reduced spontaneous neural activity and excitability ex vivo, and similar to DREADD inhibition, also reduced out-group attacks, altogether suggesting PVTs involvement in biased aggression.

Investigating the analgesic properties of Cannabidiol in larval Zebrafish

Bryce Lecamp, Kali Esancy, Gloria Shen, Quinn Bianucci, Sidhant Rauniyar

Pain is the number one reason why patients seek medical treatment and over 20% of the US population is affected by chronic pain. Current therapeutics have limited efficacy and produce harmful side effects. Cannabidiol (CBD) shows potential as an analgesic, but its mechanism of action is not well understood. We aim to validate larval zebrafish as a model system to investigate CBD-mediated analgesia as well as to discover new analgesics in the cannabinoid family.
We show that, through a battery of behavioral experiments, CBD can alternately increase or decrease sensitivity to noxious stimuli. In accordance with prior literature, we demonstrate that CBD activates Trpa1+ sensory afferent neurons, and at similar doses will induce thermal hypersensitivity. Both effects are eliminated by genetic knockdown of the pro-nociceptive ion channel Trpa1.
Meanwhile, lower doses of CBD than induce thermal hypersensitivity profoundly reduce aversion to the Trpa1 agonist allyl isothicyanate (AITC) as well as to optogenetic activation of Trpa1+ sensory neurons.
Our work highlights the need for multiple, independent assays for pain sensitivity—given CBD can appear to either reduce or enhance pain depending on the dosage and the way in which pain sensitivity is measured. By understanding the mechanistic action of CBD on its targets, including Trpa1, we may be able to rationally design new compounds with improved analgesic properties, while limiting unwanted side effects.

Lateral hypothalamic projections to the ventral tegmental area modulate the activity of stress-sensitive nociceptin opioid peptide neurons.

Carrie Stine, Amanda L. Pasqualini, Ananya S. Achanta, Joseph C. Johnson, Xuehan Zhou, Tommaso Patriarchi, Michael R. Bruchas

Nociceptin/orphanin FQ (N/OFQ), an endogenous opioid neuropeptide, and its G-protein coupled receptor NOPR are broadly implicated in behavioral states such as motivation, aversion, and sleep. Stress-induced dysfunction in these states is central to the development of numerous psychiatric disorders, driving interest in NOPR as a therapeutic target. However, the circuits and mechanisms by which nociceptin signaling impacts these states remain largely unknown. To this end, we previously identified a candidate population of N/OFQ neurons in the paranigral ventral tegmental area (pnVTA-Pnoc) that restrict motivation and drive aversion (Parker et al, Cell 2019). Here we expand on these findings by characterizing pnVTA-Pnoc sensitivity to stress exposure and identifying pnVTA-Pnoc afferent input from the lateral hypothalamus (LH). We hypothesize that stress engages the pnVTA N/OFQ system via the LH to disrupt motivation and enhance aversion. Using fiber photometry and Pnoc-Cre mice, we recorded pnVTA-Pnoc GCaMP activity and found robust, transient activation in response to multiple acute stressors. Similar recordings with NOPLight, a genetically-encoded nociceptin sensor, revealed corresponding transient changes in VTA nociceptin levels after stress. Using approaches in retrograde tracing and electrophysiology, we identified inhibitory and excitatory connectivity from the LH onto pnVTA-Pnoc neurons. Ongoing studies are evaluating the stress-responsivity of LH terminals in the VTA. Here we provide evidence that acute stress engages pnVTA N/OFQ release and identify LH afferent modulation of pnVTA-Pnoc activity. These findings support our hypothesis that this circuit acts as a critical bridge between stress and motivation, encouraging further exploration of the therapeutic potential of NOPR antagonists.

Characterization of microprisms for deep-brain 2-photon imaging

Zhe Charles Zhou, Madalyn Critz, Samantha Lee, Sean Piantadosi, Lucy Tian, Benjamin Land, Michael R Bruchas, Garret D Stuber

Optical elements, such as microprisms and gradient index lenses, are critical for fluorescence imaging of deep brain regions that extend beyond the cortical surface. Microprisms have been used to achieve unprecedented numbers of recorded cells within a single FOV. Despite initial adoption of microprisms, more research is necessary to understand the optical properties of microprisms. Here we 1) assess the optical qualities of microprisms, and 2) demonstrate longitudinal tracking of hundreds of cells in mice implanted with microprisms.
For optical characterization of microprisms, an assembly of optomechanical components was constructed to hold both the microprism (1.5x1.5x8mm) and sample slide in varying orientations relative to the microscope. To measure the resolution of the optical train, we utilized a motorized stage to generate 0.2 um step z-stacks of diffraction limited beads. For in vivo imaging, mice expressing GCaMP6s implanted with a microprism lens in nucleus accumbens underwent 2-photon imaging sessions.
The resolution of the optical system was 0.907 um and 71.188 um in the lateral and axial dimension respectively (n=5 beads). We also observed that tilting the microprism away from the optical axis (5-10 degrees) resulted in a 2.27-fold reduction of image brightness. For in vivo 2-photon imaging, the maximum single FOV cell yield was 1087. The stable FOV and image quality enabled long-term tracking of individual neurons (mean=662±170 cells, n=4 animals) over the course of a month. Overall, we 1) found a broad axial excitation profile when imaging through microprisms and a dependence of optical performance on tilt relative to the optical axis, and 2) demonstrate stable longitudinal tracking of large-scale neural populations. We conclude that microprisms are powerful tools for deep-brain neural recordings; however caution is recommended when interpreting signals in the axial dimension and when attempting single-cell optogenetic stimulation.

Emotional Modulation of Breathing: Mapping projections to the preBötzinger complex

Elora Reilly, Joe Arthurs, Grigory Loginov, Nathan Baertsch

Breathing is altered by behavior and emotion, yet little is known about the cell-types that transmit these non-homeostatic breathing commands to the respiratory rhythm generator, the preBötzinger complex (preBötC). Interestingly, higher-order brain regions cannot be distinguished based on whether they project to excitatory or inhibitory preBötC neurons. We hypothesized that preBötC inputs would map to distinct brain regions based on the inhibitory or excitatory phenotype of the projecting neurons. To test this, mice that express tdTomato only when both Cre and FlpO are present (Ai65) were bred with mice that express Cre in either inhibitory (VgatCre) or excitatory (Vglut2Cre) neurons. Adult offspring received a unilateral preBötC injection of a retrograde AAV that expresses FlpO (AAVrg-FlpO), thereby specifically and efficiently activating tdTomato expression in either inhibitory or excitatory neurons that project directly to the preBötC. Whole-brain imaging revealed distinct populations of neurons in many mid- and forebrain regions with little overlap between inhibitory and excitatory projections. Example inhibitory projections include CeA, BNST, Zona Incerta, and RVM, whereas projections from LC, PBN, Hypothalamus, and CTX are excitatory. Defining the transcriptional phenotypes of these preBötC inputs establishes an essential foundation for future functional studies to determine the role of these circuits in the control of breathing.

JWT-101 as a long-lasting KOR antagonist

Micaela Ruiz, Charles Chavkin, Benjamin Land

Kappa opioid receptors (KOR) ligands have been explored for anti-anxiolytic, anti-depressive, pain, and substance use disorder therapeutics. These therapeutic effects are partly attributed to biased signaling through the cJun N-terminal Kinase (JNK) pathway, which involves complex molecular interactions and downstream effects. JWT-101, a novel KOR ligand, has demonstrated long-lasting therapeutic effects; however, its underlying mechanism remains poorly understood. I assessed KOR agonist-induced analgesia by measuring the latency of tail withdrawal from 52.5°C water after treatment with U50,488, a KOR agonist. Pretreatment with JWT-101 (15 mg/kg) 24 hours before U50,488 injection effectively blocked KOR-induced analgesia in wild-type male mice. This effect was reversed by the short-acting, KOR-selective antagonist Aticaprant (5 mg/kg), suggesting that JWT-101's action is specifically mediated through KOR. Further investigation using in-vivo fiber photometry and ex-vivo slice imaging with the novel peroxide sensor oROS-Gr reveal that JWT-101 (15 mg/kg for fiber photometry and 10 uM for slice imaging) significantly increases ROS production in KOR-expressing cells of the prefrontal cortex that can be blocked with KOR antagonism. These findings indicate that JWT-101 activates the KOR/JNK signaling pathway, leading to increased ROS levels. The enhanced ROS production and subsequent receptor inactivation suggest a mechanism where JWT-101's therapeutic effects may be attributed to its ability to modulate KOR signaling through oxidative stress pathways. Understanding this mechanism could provide valuable insights into the development of more targeted KOR-based therapies and advance our knowledge of how JWT-101 exerts its long-lasting effects.

Post-selection inference for networks

Ethan Ancell, Daniel Kessler, Daniela Witten

Networks—collections of edges representing pairwise links among a collection of nodes—arise in numerous applications in the social and biological sciences. Given a single realization of a network, we consider selecting a parameter in a data-driven manner, and then conducting inference on this data-driven parameter. For instance, suppose that we perform community detection, i.e. we learn a partition of nodes into communities such that nodes within a given community have similar connectivity patterns. Conducting inference on the connectivity within and between estimated communities poses a challenge, since the communities are themselves estimated from the data. Of course, using the same network realization to select a parameter and to conduct inference on it is problematic; furthermore, since only a single realization of the network is available, sample splitting is not possible. In this work, we show that it is possible to split a single realization of a network consisting of p nodes into two (or more) networks involving the same p nodes; the first network can then be used to select a data-driven parameter, and the second to conduct inference on that parameter. In the case of weighted networks with Poisson or Gaussian edges, we obtain two independent realizations of the network; by contrast, in the case of binary edges, the realizations are dependent, and so extra care is required. We establish the theoretical properties of our estimators, in the sense of confidence intervals that attain the nominal (selective) coverage, and demonstrate their utility in numerical simulations.

Seasonal and circadian effects on stress research in male and female mice

Jenna Sanders, Makenzie Patarino, Bryan Schuessler, Abigail Schindler

Circulating hormone levels are influenced by seasonal and circadian rhythms which can in turn impact response to stress. While circadian rhythm biology has received increased focus within the stress research community, how seasonal changes might influence experimental results has received little focus. This is of particular interest to stress neuroscientists given the complex interplay between stress, hormones, and behavior. Therefore, increased efforts to establish if/how stress is impacted by time of year and/or time of day is required to increase rigor and reproducibility in stress and trauma research.

Here we detail results from over 2,500 male and female adult C57Bl6 mice that were exposed to either single or repetitive (3x) blast polytrauma (or sham control). Blast polytrauma is a full-body injury that results in complex pathophysiological and behavioral outcomes including mild traumatic brain injury, posttraumatic stress disorder, chronic pain, and risky substance use. Acute outcome variables examined include loss of righting reflex time, weight loss, and mortality. Sub-acute outcome variables examined include locomotor and anxiety/risk-taking like behavior in the open field test. Covariates included sex, time of year (month), time of day (hour), initial body weight, and blast parameters (PSI). Initial results highlight a complex interaction between sex, time of year, and time of day and ongoing work now focuses on using hierarchical multivariable regression models to understand how these factors are working to contribute to adverse blast outcomes. Together these results highlight the importance of considering seasonal and circadian rhythms as covariates in stress-related research.

Pain-encoding neurons in the periaqueductal gray during chronic neuropathic pain and cannabinoid treatment

Madalyn Critz, Benjamin Land, Michael Bruchas

Chronic pain affects up to 100 million people in the United States, and severely impacts quality of life. Current analgesic treatments such as opioid drugs have significant drawbacks, making the management of chronic pain uniquely difficult. There is a significant need for new chronic pain treatments, as well better understanding of the biological mechanisms governing the development of chronic pain. Cannabinoids (CBs) have been implicated as effective treatments for chronic neuropathic pain, but the mechanisms by which CBs confer their analgesic properties are not well known. The ventrolateral periaqueductal gray (vlPAG) is an important hub for the descending modulation of pain signals, and may be dysregulated by chronic pain states. Additionally, the vlPAG expresses an abundance of cannabinoid receptors, and may therefore be an important target for CBs to relieve chronic pain. Here, we used 2-photon resonance microscopy to observe calcium dynamics of vlPAG neurons during acute and chronic pain in vivo. We also investigated the impact of acute cannabinoid treatment on the activity of these neurons and the manifestation of pain responses. These studies will inform how cannabinoids might act on neurons in the vlPAG to confer analgesia, and eventually help to develop novel intervention strategies for chronic pain.

Investigating the Structure of Perineuronal Nets During Fentanyl Exposure and Withdrawal

Madelyn T. Rice (presenting author), Nathaniel S. Rieger, Ari Peden-Asarch, Alisa Coyne, Delaney Hurlimann, John Neumaier

Perineuronal nets are specialized net-like structures of the extracellular matrix that are involved with adult brain plasticity. They surround specific neurons in the brain and the reticular structure acts as a physical barrier to new incoming synaptic inputs. However, the perineuronal nets can be remodeled due to experience and environmental factors. While there has been some research on the involvement of perineuronal nets in opioid responses, much remains to be done on what could be an important plasticity marker. The existing literature on morphine self-administration found that during acute withdrawal, there was an increase in perineuronal nets in the infralimbic cortex. Given this, we hypothesize that there will be a decrease in the density of perineuronal nets in the hippocampus during fentanyl exposure and an increase in the density of perineuronal nets in the hippocampus and infralimbic cortex during withdrawal. To test the hypothesis, we plan to look at perineuronal net density in mice during acute exposure to fentanyl. This will be modeled with 1 cycle of escalating doses, as well as during early withdrawal (16 hours), late withdrawal (30 days), and naloxone precipitated withdrawal. At each of these points, we will use histology to directly examine the perineuronal nets. We plan to utilize a novel approach to analyze the density of perineuronal nets in our brain regions of interest. If fentanyl exposure and/or withdrawal does impact perineuronal nets, future research into perineuronal net interventions may have positive implications on patients who are addicted to fentanyl.

Characterizing locus coeruleus and pericoerulear zone activity in response to aversive and appetitive stimuli

Madison M Martin, Michael R Bruchas

Acute stressors induce physiological anxiety and behavioral avoidance, which can be necessary for survival in the face of environmental threats. However, excessive avoidance of both stressful and innocuous situations is a common symptom of anxiety disorders. A key neuromodulator known to respond to stress exposure is norepinephrine (NE), which is released from the locus coeruleus (LC) to mediate behavioral responses to aversive as well as appetitive stimuli. Recent evidence suggests that GABAergic pericoeruleus (peri-LC) neurons directly inhibit the LC and respond heterogeneously to different types of stimuli, but it is unknown how peri-LC GABA activity modulates LC NE neurons in vivo, nor how changes in LC and peri-LC activity drive avoidance behaviors. Using 2-photon imaging to record calcium activity of individual LC and peri-LC neurons in response to various stimuli, we found that most LC neurons were synchronously activated by aversive and appetitive stimuli across sensory modalities, but they responded more strongly to aversive stimuli. LC neurons also displayed variability in the offset and magnitude of their activity, the latter of which scaled with stimulus intensity. Compared to the LC, the peri-LC responded less intensely but more heterogeneously to each stimulus, with a relatively balanced proportion of activated, inhibited, and non-responsive neurons. We have also begun to explore how changes in peri-LC GABA activity alter LC activity to drive subsequent changes in behavior. By understanding the local circuit-based mechanisms of how LC activity is regulated, we can better understand how LC activity becomes dysregulated during maladaptive behavioral states.

Female rats show a greater behavioral response to heroin across self administration and locomotor sensitization compared to males

Mar Borrego*, Nailyam Nasirova, Timothy O'Neal, Zackari Murphy, Umme Habiba, Susan Ferguson

Preclinical data from animal models of opioid use disorder (OUD) suggest sex-based differences in reward circuitry and drug-related behaviors; however, it remains unclear which facets of OUD are particularly dependent on sex. We directly compared male and female rats across an array of heroin-induced behaviors in order to provide insight into the nuances of sex differences in OUD. We first used a 6-hour intermittent access heroin self administration paradigm to quantify six distinct drug-taking and -seeking behaviors and from these classified rats as having high- or low-severity phenotypes. In a separate group of rats, we adapted this classification system to a 1-hour continuous access self administration paradigm. In both models, high-severity males and females only differed in a subset of the behaviors measured, yet a greater proportion of females fell into the high-severity phenotype classification compared to males. We next examined locomotor sensitization following daily heroin injections in two groups of rats. The first were given 2 mg/kg/day i.p. heroin for 9 days and the second were given 0.55 mg/kg/day i.v. heroin for 20 days. Following i.p. heroin treatment, males had lower overall locomotion, but sensitization was not dependent on sex. In response to i.v. heroin, locomotor escalation occurred more rapidly in females compared to males. Our results indicate a pattern of females having a greater behavioral response to heroin compared to males and support the need to consider sex as a biological variable in our development of treatments for OUD.

EpiBrain: the brain's epigenetic landscape in a snapshot

Maja Johnson, Yijie Geng

Epigenetics connect nature with nurture and can help explain how experiences and the environment influence gene expression to promote individual differences in behavior and mental health. However, how a particular perturbation induce epigenetic changes across various regions of the brain remains largely unknown. To visualize changes in brain’s epigenetic landscape at a glance, we developed EpiBrain, a method utilizing whole-brain imaging and registration to capture epigenetic changes in the entire zebrafish brain in a snapshot. Using this method, we have uncovered brain-wide epigenetic changes induced by chemical treatment, brain activity, and genetic mutation. RNA sequencing detected brain activity-induced immediate early genes (IEGs) with epigenetic modulatory function, indicating a potential molecular mechanism mediating experience-induced epigenetic changes. EpiBrain enables rapid and unbiased assessment of whole-brain epigenetic changes following physiological and disease-relevant perturbations as well as mechanistic discoveries at the molecular level.

Elucidating the Role of Locus Coeruleus in Sleep Disturbances from Chronic Opioid Use

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An important contributor to opioid use disorder is opioid-induced sleep dysfunction, but its underlying brain circuit mechanisms remain incompletely understood. The locus coeruleus (LC), the brain's principal noradrenergic arousal circuit, is a well-known target of opioids, but its role in opioid-induced sleep dysfunction is unclear. To address this question, we examined light-cycle sleep by recording LC-norepinephrine (NE) activity, electroencephalogram (EEG), and behavior in an escalating opioid dosing model in mice (intraperitoneal morphine administration in 10 mg/kg/day increments up to 50 mg/kg in treatment group versus corresponding saline administration in the control group). We found that the fraction of LC-NE activity that was elevated did not change significantly during the escalating dosing, but did on the first withdrawal day before resolving. Interestingly, we did not observe a significant change in the sleep-awake pattern, though the first withdrawal day trended toward a decreased awake time. We further investigated the correlation between LC-NE activity and EEG. While we found low correlation overall, we observed relatively higher correlation in the delta and gamma band frequencies, and relatively higher correlation on first withdrawal day compared to day 4 of the escalating dosing. These preliminary results suggest that the LC-NE system likely plays a nuanced role in opioid-induced sleep changes. We are currently working to examine peri-LC inhibitory neuron activity and thalamic NE activity in this escalating opioid dosing model to examine an upstream LC regulator and the downstream effect, respectively.

Exploration of the behavioral profile of sequential opioid-stimulant polysubstance use disorders in a translational rodent model.

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Concurrent opioid and stimulant use is responsible for increasing numbers of overdoses and relapses, but their combined effects on drug-seeking and -taking behavior is poorly understood. The goal of this study was to compare polysubstance and single substance addiction behavioral patterns using a rodent self-administration model.
Adult rats with indwelling jugular catheters were trained to lever press for fentanyl (FENT, 7 days/week), methamphetamine (METH, 5 days/week) or both (POLY). After acquisition and escalation of drug intake over 3 weeks, animals completed either an extinction and cue-induced reinstatement of drug-seeking test, or a behavioral economics thresholding test. POLY rats completed tests for one drug.
Overall, drug intake and lever pressing were similar in POLY and single-drug groups. However, POLY rats exhibited higher drug-seeking behavior during extinction and reinstatement for methamphetamine-associated cues compared to METH rats. No differences were seen in fentanyl-seeking behaviors between POLY and FENT rats. In the behavioral economics test, POLY rats preferred a higher initial dose of fentanyl and exerted more effort to obtain fentanyl than FENT rats. Fentanyl demand elasticity also decreased with a history of polysubstance use. There were no differences in demand between METH and POLY animals.
Our findings suggest that there are bidirectional and substance-specific trends in addiction—related behaviors in animals with a history of polysubstance use. These results may indicate the recruitment of distinct neural circuits compared to those implicated in single substance addiction. Ongoing experiments will use light sheet microscopy to map neurocircuit activation patterns that underlie these behavioral differences.

Head-to-head comparison of fast-scan cyclic voltammetry and dLight 1.3b

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Recent developments of genetically expressed sensors have afforded great advances in the detection selectivity of neurochemical monitoring. Here we benchmark dLight 1.3b (dLight) against an established technique has not been demonstrated in vivo.
To this end, we affixed an optical fiber to a carbon fiber electrode (for fast-scan cyclic voltammetry, FSCV), allowing for in-situ comparison. Electrical stimulation of the medial forebrain bundle in urethane (1.5 g/kg, i.p.) anesthetized Sprague-Dawley rats were used to elicit dopamine release in the nucleus accumbens.
dLight has significantly faster response kinetics and greater signal to noise ratio. dLight signals saturates at high dopamine release events caused by 60 Hz stimulations longer than 30 pulses. Raclopride (RAC) administration (20 mg/kg, i.p.) resulted in a significant increase in electrically evoked dopamine amplitudes with FSCV, but not with dLight. AUC was significantly increased post RAC for both techniques. dLight measured lidocaine sensitive spontaneous transients, previously only been observed with FSCV post amphetamine administration.
dLight sensor has significantly faster response time kinetics, and sensitivity compared to FSCV, but saturates at lower concentrations. The higher sensitivity of dLight could be due the expression level of the dLight sensor and/or the location of expression with respect to the synaptic cleft. However, the carbon fiber electrode is smaller compared the optical fiber (7 μm vs 400 μm in diameter) and allow for measuring from a smaller sample volume. The larger sample volume of the dLight sensor could be underlying the larger signal to noise ratio and higher sensitivity.

Exploration of the behavioral profile of sequential opioid-stimulant polysubstance use disorders in a translational rodent model.

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The rising prevalence of opioid addiction has propelled a significant increase in mortality and morbidity in the U.S (Kolodny et al., 2015). Currently available medication-assisted treatments for opioid use disorder are inefficient given their addictiveness and risk of relapse, which result in only short-lasting abstinence and require a long-term commitment to treatment (Volkow et al., 2018). Psilocybin, a serotonergic agonist, has shown efficacy in treating depression, alcoholism, and nicotine addiction with a rapid-acting and non-addictive profile (Bogenschultz et al., 2015, Johnson et al., 2014, Carhart-Harris et al., 2017). The medial prefrontal cortex (mPFC) contributes to reward processing and drug-induced sensitization, and is subject to long-lasting modifications as a result of opiate exposure (Jiang, et al., 2019; Qu et al., 2020). Additionally, the mPFC exhibits significant morphological changes, modulation of activity, and improvements in flexible behavior in response to psilocybin (Shao, et al., 20021; Morgan, et al., 2023). In this study, we used a rat model of opiate withdrawal, combined with the analysis of basic behaviors (DeepLabCut; SimBA) and one-photon calcium imaging in the mPFC, to discern whether psilocybin treatment restores the behavioral and neural alterations following opiate withdrawal to baseline conditions. Our initial observations show hypofunction of the mPFC and altered behavioral phenotypes following opiate withdrawal. Single-dose psilocybin treatment, compared to saline treatment, appears to increase activation in the mPFC. Additionally, we see alterations in novel object recognition between groups and treatment types, which correlated with differences in mPFC activation. Full results will be presented.

Investigating the role of nociceptin-expressing central amygdala neurons in reward Seeking

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The motivated actions animals undertake to achieve rewards are heavily influenced by neuromodulation. Neuropeptides and their respective G-protein coupled receptor (GPCR) signaling cascades play crucial roles in modulating neural activity and a wide array of resulting behavioral outcomes. One such neuropeptide, nociceptin, is an endogenous opioid that binds to an inhibitory GPCR, opioid receptor-like 1 (OPRL1), to influence motivation, reward processing, and aversion. A unique subpopulation of GABAergic central amygdala neurons expressing nociceptin (CeA_{Pnoc}) was recently identified to promote palatable food consumption and reward behaviors (Hardaway et al., 2019). However, the mechanisms by which CeA_{Pnoc} neurons modulate reward processing and motivation remain unknown. We hypothesize CeA_{Pnoc} neurons promote motivation by reinforcing reward-seeking behaviors via nociceptin-mediated disinhibition of downstream reward circuitry. We found optogenetic activation CeA_{Pnoc} neurons is reinforcing, and mice will exert progressively demanding effort to self-administer intracranial CeA_{Pnoc} optogenetic stimulation. Current work aims to characterize CeA_{Pnoc} neural activity during motivated reward seeking by utilizing fiber photometry calcium sensor recordings in mice during Pavlovian and operant reward conditioning. Similarly, to investigate in vivo nociceptin signaling in the central amygdala during these tasks, we recorded NOPLight, a novel genetically-encoded nociceptin biosensor, from Oprl1-expressing central amygdala neurons (CeA_{Oprl1}). These studies will provide novel insight on reward circuitry mediated by endogenous nociceptin opioid signaling originating in the central amygdala.

Role of Opiodergic Neurons in the Solitary Nucleus in Respiration and Pain Circuits

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Opioid drugs are highly effective analgesics that can also produce life-threatening respiratory depression, illustrating the intimate relationship between breathing and pain. Yet, little is known about the circuits that link these vital functions.

The nucleus tractus solitarius (NTS) is a major relay for sensory signals from the lungs, plays a role in the transmission and perception of pain, and contains transcriptionally heterogeneous cell-types that are functionally diverse. We hypothesize that NTS neurons that express the endogenous opioid ligands enkephalin (Penk) or Beta-endorphin (POMC) form circuits that integrate breathing and pain by linking the preBötzinger Complex (preBötC), parabrachial nucleus (PBN), and rostral ventral medulla (RVM), key brainstem sites involved in respiration and nociception. To test this, we used a dual recombinase dual transgenic strategy to achieve cell-type and projection specific circuit mapping. PenkCre/Cre or POMCCre/Cre mice were crossed with Ai65 mice, which express tdTomato only when both Cre and FlpO are present. In offspring, separate groups of mice received an injection of retroAAV-FlpO into either the preBötC, PBN, or RVM, labeling only neurons that express Penk or POMC (Cre) and have synapses within the injected region (FlpO). We found that the preBötC, PBN, and RVM all receive substantial synaptic inputs from opioidergic neurons in the NTS. Ongoing and future work will utilize optical manipulation and recording techniques combined with respiratory and nociceptive assays to determine how and when these opioidergic NTS circuits affect breathing and pain.