2026 NAPE Spring Symposium

Thursday, May 7, 2026

Schedule

Thursday, May 7, 2026

1:30 - 2:00 PM	*Poster presenters are free to arrive early to put up their poster*
2:00 - 2:15 PM	Arrive and Check-In
2:15 - 2:20 PM	Introduction by Dr. Charles Chavkin, Professor of Pharmacology and Director of the NAPE Center
2:20 - 3:00 PM	Dr. Karel Svoboda, Vice President and Executive Director of the Allen Institute for Neural Dynamics
3:00 - 5:00 PM	*Appetizers catered by Bay Laurel (just outside SOCC 316)*
3:00 - 4:00 PM	Poster Session 01 (odd numbered posters)
4:00 - 5:00 PM	Poster Session 02 (even numbered posters)

Location

South Campus Center (SOCC)
1601 NE Columbia Rd, Seattle, WA 98195
Room 316

Transportation

Parking

$5/hr surface parking right outside SOCC

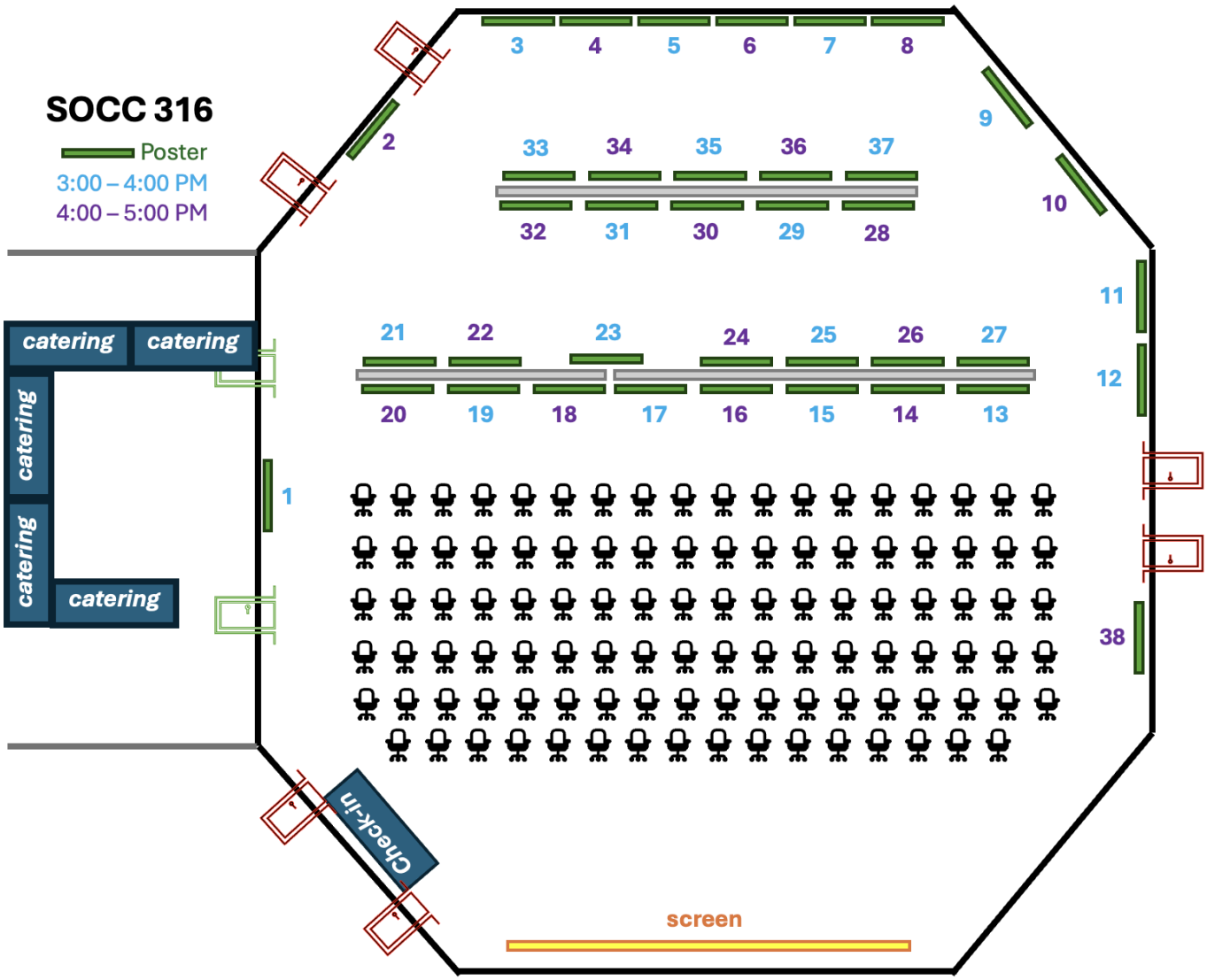
Transportation from South Lake Union

You can take the South Lake Union -> UWMC free shuttle.
Stop UWMC RR-Wing is just past SOCC.
Additional details found here.

Access to SOCC

UW students/employees should have access to SOCC with their husky card (reminder sometimes it requires you to tap twice with 10 sec between taps).
If you do not have access, the northwestern doors will be unlocked from 1:00 - 3:00 PM (see diagram below).

Room Layout & Poster Locations



Poster Abstracts

Presentation Time Slots
odd poster numbers, 3:00 - 4:00 PM
even poster numbers, 4:00 - 5:00 PM

Poster List

Click on poster number to see abstract details

Poster	Presenter	Title
01	Ethan Ancell	Statistical inference after estimating communities in a network
02	Alex He	ASD-relevant neurodevelopmental toxicities of the monoterpenes Thymol and p-Cymene
03	Alli Brezinski	State-dependent Norepinephrine signaling in the preBötzinger Complex
04	Jonathan Sedano	Dissecting preBötzinger Complex circuits for pain-breathing coordination
05	Elora Reilly	Investigating the function of forebrain projections to the preBötzinger complex in modulating breathing.
06	Micaela Ruiz	Serotonin and Dopamine in the nucleus accumbens is modulated by dynorphin/ Kappa opioid receptor (KOR) activation of p38α mitogen-activated protein kinase (MAPK)
07	Eunice Lee	Forward genetic identification of regulators of Mu-Opioid Receptor signaling and behavioral responses to opioids
08	Carlie Neiswanger	Emergence from anesthesia recruits spatially distributed neural ensembles in mice
09	Matt Becker	Topographic organization of locus coeruleus activity from learning to vigor
10	Jerome Lecoq	OpenScope : The first observatory in systems neuroscience
11	Nick Ressler & Amelia Li	Decoding animal behavioral states using wireless implantable accelerometers
12*	Minke Nota	Activity-dependent capture using a transgenic mouse system reveals brain-wide signatures of anesthesia-induced unconsciousness
13	Carlee Toddes	Identifying and decoding pain mediated shifts in volitional social behavior following neuropathic injury.
14	Sayaka Kenmochi	Identifying the mechanisms of noradrenergic modulation of BLA-mediated avoidance behaviors
15	Mike Seibert	Neuropeptidergic control of striatal acetylcholine release during reward learning
16	Pradumna Sachdeva	Role of opioid receptors on fentanyl-seeking and neuronal activity in the Ventral Tegmental Area
17	Madison Martin	Using in vivo 2-photon calcium imaging to characterize the function of locus coeruleus activity during avoidance behavior

18	Marcelina Wezik	Accumbens mShell Pdyn neurons' control over dopamine dynamics
19	Mary Loveless	In vivo testing of fluorescent μ-opioid sensor variants in pain and drug administration assays
20	Madalyn Critz	Imaging the neural encoding dynamics of chronic pain transition in the ventrolateral periaqueductal gray
21	Kaylin Ellioff	Characterizing the effects of select phytocannabinoids and terpenes for pain relief within the BLA
22	Kayla Kittrell	JWT-101: a novel long-lasting KOR antagonist
23	Loveleen Tripathi & Shawn Panh	Uncovering phenotypic diversity and cell-type-specific prefrontal cortex calcium dynamics in rat fentanyl self-administration
24	Ekayana Sethi	Serotonin encoding of reward value and HTR2A-dependent modulation of behavior in the Central Amygdala
25	Yuxuan Wang	High-throughput engineering of genetically encoded μ-Opioid sensors with enhanced sensitivity and neuronal expression for detecting opioids in vivo
26	Anirudh Ramadurai	Multi-modal physiological and behavioral modeling to assess individualized opioid withdrawal severity in mice
27	Iris Xu	Impaired noradrenergic signaling in ketamine-induced dissociation in mice
28	Brandy Briones	Posterior paraventricular thalamus regulates defensive aggression amidst social uncertainty
29	Cassidy Burke	Multimodal interrogation of cell type-specific transcriptional states and brain-wide activity profiles in maladaptive opioid use
30	Abi Elerding	Diversity of GABAergic cell types in the mesolimbic system
31	Lucy Tian	Dopamine modulation of Nucleus Accumbens dynamics across cue-reward learning
32	Weston Fleming	A role for central amygdala acetylcholine in conditioned taste aversion
33	Megan Melavic	Modulation of mGluR5 alters heroin self-administration behavior
34	Madelyn Rice	Impact of fentanyl on post-mTBI behavior
35	Christina Gao	Identifying and validating neuronal PKA inhibitors as targets of inflammatory pain treatment
36	Alvin Fu	Engineering antigen-dependent stability in nanobodies: A bioinformatics tool for tuning intracellular nanobody stability.
37	Marco Pravetoni	Protein based therapeutics for opioid use disorders and overdose
38	Nicole Miranda	Noradrenergic Modulation of Breathing: Implications for Opioid-Induced Respiratory Depression

Poster 01

Statistical inference after estimating communities in a network

Ethan Ancell, Daniela Witten, and Daniel Kessler

Given a dataset consisting of a single network, we consider inference on a parameter selected from the data. We focus on the setting where the selected parameter is a linear combination of the mean connectivities within and between estimated communities. Inference in this setting poses a challenge, as the communities are themselves estimated from the data. Furthermore, since only a single realization of the network is available, sample splitting is not possible. We show how to split a single realization of a network with n nodes into two (or more) networks involving the same n nodes; the first network can 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 Bernoulli edges, the two realizations are dependent, and so extra care is required.

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Poster 02

ASD-relevant neurodevelopmental toxicities of the monoterpenes Thymol and p-Cymene

Baixi He, Ping Zhang, Catherine Hong, Annabelle Chen, Emma Kong, and Yijie Geng.

The increasing prevalence of autism spectrum disorder (ASD) has drawn significant attention to its environmental origin. Environmental factors are estimated to contribute to 40% of ASD risk. However, the identities of these factors and their mechanisms of action remain poorly understood due to limitations of current research approaches. To bridge this gap, we screened the ToxCast library using a high-throughput social behavioral assay platform, Fishbook, to discover emerging ASD-relevant toxicants. From the 140 toxicants screened, we identified Thymol and p-Cymene, both belonging to the chemical class monoterpenes, as potent inhibitors of social behavioral development through embryonic exposure. Monoterpenes are natural compounds commonly found in food flavorings, essential oils, and personal care products. Through a proteome-wide reverse molecular docking screen, we discovered lysine demethylase 5 (KDM5) family proteins, in particular KDM5C and KDM5D, as predicted binding targets of Thymol and p-Cymene. The KDM5 family proteins are histone demethylases that selectively remove methyl groups from histone H3 lysine 4 (H3K4me 2/3), which are epigenetic marks typically associated with active gene transcription. Embryonic exposures to Thymol and p-Cymene increased the global level of H3K4me3, indicating that these toxicants likely inhibited KDM5 activity. RNA-sequencing detected profound up-regulation of genes associated with genetic risk factors of autism following thymol and p-cymene exposure. In summary, this work discovered thymol and p-cymene as risk factors for social behavioral development and revealed KDM5 as a potential mediator of their neurodevelopmental toxicity.

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Poster 03

State-Dependent Norepinephrine signaling in the preBötzinger Complex

Allison N Brezinski, Jonathan R Sedano, Nathan A Baertsch

Opioid-induced respiratory depression (OIRD) is the leading cause of death in opioid use, and its severity is tightly linked to arousal state. Norepinephrine (NE) is a critical neuromodulatory neurotransmitter involved in sympathetic nervous system regulation and is a key mediator of the “fight-or-flight” response, influencing multiple physiological systems, including heart rate, blood pressure, and importantly arousal state and respiration. Despite its widespread effects and known link to arousal and respiratory control, the real-time dynamics of norepinephrine release within respiratory circuits remain poorly understood. Here, we employ the genetically encoded G protein–coupled receptor activation–based norepinephrine sensor (GRABNE) to monitor norepinephrine activity in the preBötzinger complex during the Hargreaves thermal nociception test and respiratory challenges. Experiments were conducted in both awake and isoflurane-anesthetized mice to compare state-dependent neuromodulatory responses. We observed a moderate increase in norepinephrine release in the preBötzinger complex in response to hind-paw heat stimulation under anesthesia, accompanied by correlative changes in respiratory patterns. The NE signal displayed a prolonged decay lasting several minutes and a long refractory period, potentially reflecting rapid depletion of releasable NE. These findings suggest that nociceptive stimuli can engage neuromodulatory pathways that influence breathing, highlighting a potential mechanism by which pain and stress states modulate respiratory rhythm. Ongoing experiments aim to further delineate state dependencies and the neural circuits involved, particularly in the context of fentanyl and other opioids, with the goal of clarifying mechanisms underlying OIRD.

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Poster 04

Dissecting preBötzinger Complex circuits for pain-breathing coordination

Jonathan Sedano, Alli Brezenski, Elora Reilly, Nathan Baertsch

Opioids remain essential for strong pain relief, yet the neural systems that regulate pain are closely tied to those that control breathing. As a result, opioid-induced respiratory depression and apnea are the leading causes of death in overdoses — a crisis intensified by the rise of synthetic opioids, which contributed to roughly 80,000 deaths in the United States in 2023. To mitigate this life-threatening side effect, we need a deeper understanding of the specific circuits that couple pain and breathing, and how these circuits respond to opioids.

The preBötzinger Complex (preBötC) is the central brainstem locus that generates the respiratory rhythm. It contains transcriptionally diverse neurons, including populations expressing the μ-opioid receptor (Oprm1) which influence breathing when manipulated. With a quarter of these preBötzinger neurons expressing the Penk gene — which encodes for a ligand for the μ-opioid receptor — we hypothesize that these Penk preBötzinger¬ neurons play a pivotal role in the interactions between breathing and pain.

Through fiber photometry and optogenetic experiments, the data suggests that Penk neurons are integrated into the rhythmogenic preBötzinger network and are also active participants in nociception. Comparing the overlap of Penk expression with the expression of excitatory (Vglut2) and inhibitory (Vgat) vesicular transporters, we then use an INTRSECT opsin to explore the distinct breathing and pain effects of stimulating these two different Penk preBötC populations.

Altogether, our data supports a role for Penk neurons reacting to and controlling breathing and pain functions of the preBötzinger Complex.

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Poster 05

Investigating the function of forebrain projections to the preBötzinger complex in modulating breathing

Reilly E, Arthurs J, Brezinski A, Sedano J, Phillips R, Baertsch N

In previous work mapping direct excitatory and inhibitory inputs to the respiratory rhythm–generating preBötzinger complex (preBötC), we identified several forebrain projections, including a prominent inhibitory input from the central amygdala (CeA). In epilepsy patients, electrical stimulation of the amygdala can elicit apnea without distress, implicating an inhibitory pathway to vital respiratory centers. We therefore hypothesized that, in mice, cell-type–specific activation of inhibitory CeA neurons would suppress breathing.

To test this, we injected Cre-dependent channelrhodopsin into the CeA of Vgat-Cre mice and placed optical fibers either above the CeA or over the preBötC. Contrary to our prediction, activation of CeA cell bodies during awake plethysmography produced a slight increase in breathing rate. Video analysis revealed that this effect co-occurred with a stimulation-evoked behavioral shift. Consistent with this, terminal stimulation in the preBötC did not affect breathing rate and open-field assays showed reduced locomotion during cell-body—but not terminal—stimulation.

To separate direct respiratory effects from behaviorally driven ones, we repeated stimulation under anesthesia. Neither manipulation altered breathing rate. However, both increased the likelihood of sighs during or shortly after stimulation which are associated with affect, arousal, and physiological state. This shared effect of cell-body and terminal stimulation suggests that the inhibitory CeA→preBötC pathway contributes specifically to sigh modulation rather than apnea or overt respiratory suppression. Finally, Hargreaves testing revealed increased pain thresholds during stimulation in both awake and anesthetized animals.

Together, these findings refine functional models of CeA–preBötC communication and motivate further exploration of forebrain inputs to respiratory circuits.

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Poster 06

Serotonin and Dopamine in the nucleus accumbens is modulated by dynorphin/ Kappa opioid receptor (KOR) activation of p38α mitogen-activated protein kinase (MAPK)

Micaela V. Ruiz, Sophia Rodriguez, Kayla Kittrell, Aaron Valdez, Benjamin B. Land, Charles Chavkin

Stress triggers the release of neuropeptides, including endogenous dynorphin (Dyn) opioids, which activate Gαi/o-coupled kappa opioid receptors (KOR) to produce the dysphoric effects of stress. KOR activation of arrestin recruits mitogen-activated protein kinases (MAPKs), and p38α MAPK has emerged as a key regulator of serotonin (5-HT) and dopamine (DA) signaling based on inhibitor and gene-deletion studies. In serotonin neurons, KOR-driven p38α activation mediates stress-induced potentiation of cocaine preference and increases serotonin transporter (SERT) function in the dorsal raphe nucleus (DRN). This enhanced SERT activity is hypothesized to produce a hyposerotonergic state in the nucleus accumbens (NAc), though this has not been directly demonstrated. In DA neurons of the ventral tegmental area (VTA), KOR-p38α signaling is also required for stress-induced aversion, as pharmacological inhibition or selective deletion of p38α blocks aversive responses. Thus, the Dyn/KOR/p38α system is a powerful modulator of both 5-HT and DA circuits involved in stress. Using a repeated forced-swim paradigm to examine the role of stress combined with in vivo fiber photometry using the genetically-encoded GRAB5HT and GRABDA sensors, in combination with CRISPR-mediated deletion of p38α, we found that both 5-HT and DA in the NAc exhibit dynamic fluctuations during swim and rest periods. These signals are abolished when p38α is selectively deleted in DRN 5-HT neurons or VTA DA neurons projecting to the NAc. Further defining how KOR/Dyn signaling shapes 5-HT and DA interactions will provide insight into how stress disrupts the 5HT/DA system to increase susceptibility to subsequent reward.

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Poster 07

Forward genetic identification of regulators of Mu-Opioid Receptor signaling and behavioral responses to opioids

Eunice Lee, Elizabeth Kwan, Karla J. Opperman, Brock Grill

The opioid epidemic is one of the most severe public health crises in US history. While opioids are the most effective analgesics, their clinical use is significantly constrained by dependence and tolerance highlighting the urgent and ongoing need to develop new treatment strategies that mitigate the adverse consequences of opioids. The mu-opioid receptor (MOR) is the primary molecular target of opioids that mediates both analgesia and harmful side effects. To investigate MOR signaling, our lab built novel transgenic C. elegans models (tgMOR) expressing either mouse or human MOR (MtgMOR or HtgMOR). These opioid-sensitive, whole-animal models are coupled to computationally automated assays to measure behavioral sensitivity to opioids and facilitated unbiased, large-scale forward genetic screens targeting MOR. We completed our first forward genetic screen using MtgMOR and successfully mapped multiple MtgMOR hypersensitive mutants by combining whole-genome sequencing and phenotypic selection. To broaden the human relevance of our forward genetic approach, we recently generated a humanized tgMOR C. elegans model (HtgMOR). This second-generation HtgMOR model has been used in a separate forward genetic screen for modifiers of opioid sensitivity and identified multiple HtgMOR hypersensitive mutants. Overall, our innovative tgMOR C. elegans model will facilitate unbiased, large-scale forward genetic approaches to better understand the gene networks that regulate MOR signaling and opioid-induced behavior.

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Emergence from anesthesia recruits spatially distributed neural ensembles in mice

Neiswanger C, MacMillen LK, Murry AD, Szelenyi ER, Navarrete J, Nota MHC, Lazaro H, Shin CC, Zhang J, Apley EA, Zhang Y, Diaz C, Schneider KN, Goodwin NL, Jin M, Nilsson SRO, Ishii K, Stuber GS, Bruchas MR, Golden SA, Heshmati M

General anesthesia provides an evolutionarily conserved, pharmacologic model for studying conscious state transitions. Emergence, defined as the recovery of consciousness following anesthesia, remains a passive process dependent on drug clearance. A better understanding of the brain-wide neural circuitry engaged during conscious state transitions will contribute to advancing consciousness research and improve clinical anesthetic recovery. Here we analyze brain-wide neural activity associated with emergence from isoflurane anesthesia in mice using Fos immunolabeling combined with intact brain clearing and light-sheet microscopy. This approach enables unbiased quantification of neural activity at cellular resolution across the intact whole brain and supports subsequent network analysis. By comparing emergence to two behaviorally distinct awake states, home cage and open field context-matched controls, we identify spatially distributed neural ensembles selectively activated by emergence. Overall neural activity in emergence is reduced relative to controls, with significant suppression of cortical regions and activation of subcortical regions. We perform a community analysis of the functional difference network to identify the most highly significant brain regions to focus future study, indicating high centrality of the ventral orbital cortex in emergence, a brain region in the orbitofrontal area that integrates sensory and autonomic information to modulate behavior via limbic circuit connectivity. Together, these data shows engagement of distributed cortical to subcortical circuitry underlies recovery of consciousness from isoflurane anesthesia. We provide a comprehensive cellular-resolution atlas of brain-wide activity in the emergence state. As a technical resource, this establishes a systems-level, cellular-resolution map of emergence and provides mechanistic insight into recovery of consciousness.

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Poster 09

Topographic organization of locus coeruleus activity from learning to vigor

Matthew I Becker, Zhixiao Su, Jeremiah Y. Cohen

The locus coeruleus (LC) is a small brainstem nucleus that distributes norepinephrine (NE) throughout the central neuraxis, implicating LC-NE in sensation, movement, learning, and arousal. Despite this broad influence, how LC spiking activity relates to the moment-to-moment execution of behavior remains poorly understood. Using electrophysiology and behavioral analyses in mice performing a dynamic foraging task, we characterize how LC single-unit activity relates to reaction time and movement kinematics across trials. LC neurons exhibit substantial heterogeneity in their spiking relationships to behavioral variables: neurons in dorsal LC projecting to cerebral cortex were excited by reward prediction errors and choice switches, signals driving flexible learning, while neurons in posterior and ventral LC encoded psychomotor measures of vigor, including reaction time and movement velocity. Background activity of ventral LC neurons was elevated when mice failed to respond to reward-predictive stimuli, suggesting a role in translating arousal into action initiation. These findings establish a functional map of the LC-NE system linking spiking activity to specific behavioral outputs, with implications for understanding how norepinephrine shapes psychomotor function in health and psychiatric disease.

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OpenScope : The first observatory in systems neuroscience

A. Amaya, A. Bawany, H. Belski, I. Bussi, S. Caldejon, M. Carlson, S. Daniel, S. Durand, C. Farrell, A. Fairhall, R. Gillis, S. Ghosh, C. Grasso, P. A Groblewski, W. Han, R. Howard, R. Hytnen, T. Johnson, J. Kenney, C. Kiselycznyk, K. Kording, C. Koch, **J. Lecoq**, C. Lennon-Jones, H. Loeffler, M. Mathis, R. Naidoo, K. Nguyen, S. R. Olsen, N. Orlova, B. Ouellette, J. Park, R. C. Peene, T. K. Ramirez, D. Rette, S. Seid, N. H. Smith, A. Steinmetz, J. H. Siegle, A. Stearns, L. Suarez, K. Svoboda, J. Swapp, M. Tom, O. Unokesan, X. Waughman, J. Weber, J. Wilkes, A. Williford, J. Zylberberg

Inspired by successful models in astronomy and weather forecasting, OpenScope was established as the first observatory in systems neuroscience: a centralized, open-access platform for large-scale, brain-wide recordings combined with standardized experimental design and public data sharing. OpenScope provides the global neuroscience community with access to state-of-the-art tools, including simultaneous multi-area electrophysiology and optical imaging, enabling the recording of thousands of neurons across the brain during behavior. Projects are selected through an annual, double-blinded peer-review process and executed with clear milestones and deliverables, ensuring rigor, reproducibility, and broad impact. All datasets are released publicly, fostering a growing ecosystem of open analysis and collaboration. To date, OpenScope has completed 11 projects spanning diverse experimental paradigms, with converging insights into how cortical circuits form and test predictions. These efforts have culminated in a large-scale, community-driven initiative on predictive processing, uniting researchers worldwide around shared experimental frameworks and data. By integrating impactful scientific questions, scalable infrastructure, and open data practices into a unified observatory framework, brain observatories have the potential to transform how systems neuroscience is conducted and to accelerate discoveries into brain computation in health and disease.

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Decoding animal behavioral states using wireless implantable accelerometers

Nick Ressler, **Amelia Li**, Mitra Heshmati, MD, PhD, Sam Golden, PhD

Integrating complex animal behavior with peripheral physiological recording is critical for revealing the neural basis of behavior. Traditional peripheral physiological recording methods constrain natural behavior due to cable tethers, and manually annotating behavior is time-intensive and often introduces subjectivity. We have recently published two pipelines that independently overcome these confounds: (1) mechano-acoustic (MA) devices that provide wireless, minimally invasive peripheral recording based on finely-tuned accelerometers, and (2) a computer vision based machine learning package (Simple Behavioral Analysis, SimBA) for supervised behavioral classification from recorded videos. Here, we developed a comprehensive machine learning model to classify behavioral states (e.g. rearing, running, grooming) using MA device accelerometer data, using SimBA to validate model predictions. We test this model by analyzing the effect of anesthesia and other consciousness-altering drugs on mice. Lastly, we extend this approach for closed-loop optogenetic applications. This work contributes to the growing field of bio-signal processing, offers a data-driven approach to automated behavior classification, and provides the groundwork for answering many diverse questions in neuroscience and related fields.

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Poster 12 presentation time 3:00 - 4:00 PM*

Activity-dependent capture using a transgenic mouse system reveals brain-wide signatures of anesthesia-induced unconsciousness

Minke H.C. Nota, Lukas K. MacMillen, Hallie Lazaro, Jaidyn O. Gaur, Ian Campuzano, Sam A. Golden1,2,3, Mitra Heshmati

Over 60,000 Americans experience general anesthesia (GA) daily. Inhaled anesthetics are commonly used to produce GA, but neural circuit mechanisms of anesthetic action remain poorly understood. Emergence from GA remains unpredictable with complications such as respiratory and cardiac events, delayed recovery of consciousness, agitation, delirium, and postoperative cognitive dysfunction. We previously showed that isoflurane alters brain-wide functional connectivity using viral approaches to target isoflurane-activated circuitry. We aimed to test the hypothesis that simultaneous recruitment of multiple neural networks will produce anesthetic effects using a transgenic mouse system. We first crossed R26-LSL-Gq-DREADD mice with Fos2A-iCreER2A mice to produce heterozygous-Gq-DREADDxFosTRAP2 transgenic mice and their null-DREADDxFosTRAP2 littermates. We captured brain-wide-activated neural ensembles during isoflurane exposure, resulting in DREADD expression in isoflurane-activated cells in the Gq-DREADD-positive mice (TRAP). We tested if chemogenetic modulation of these neural ensembles would produce anesthetic-like effects using a within-between study design. We administered either the DREADD agonist clozapine-n-oxide or deschloroclozapine after TRAP and observed loss of mobility in a modified open field test, decreased coordination on a rotarod, and thermal anti-nociception in positive mice compared to null littermates. Following behavioral testing, we used a modified iDisco+ procedure to clear intact, fixed brains and immunolabel DREADD-expressing neurons with anti-HA antibody. We imaged brains using light sheet microscopy and quantified HA expression using an Ilastik and ClearMap-based pipeline. We observed significant isoflurane-activated HA-tagged-DREADD expression in hippocampal and subcortical brain regions. In ongoing studies, we continue to investigate identified neuronal populations with region-specific targeted chemogenetic manipulations to elucidate circuit-specific mechanisms of GA.

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Poster 13

Identifying and decoding pain mediated shifts in volitional social behavior following neuropathic injury

Carlee Toddes, Kevin Bai, Isabel Halperin, Mitra Heshmati, Sam Golden

While the peripheral detection of pain is well-characterized, the brain mechanisms that transform nociception into affective pain states—and their influence on social behavior—remain unclear. Further, the role of the endogenous opioid family, which is known to modulate both pain and social interaction via modulation of nucleus accumbens medium spiny and interneuron cellular populations, has yet to be elucidated. To investigate the intersectional dynamics of pain and social behavior, we used an operant social self-administration paradigm in male and female mice paired with the spared nerve injury (SNI) model. Following 8 days of operant training where lever presses were rewarded with the introduction of an affiliative social partner, mice received SNI and were reintroduced to the task either 1 day (immediate) or 5 days (delayed) following surgery. Single-cell RNA sequencing of the nucleus accumbens was performed at the completion of behavioral testing to examine transcriptional changes in specific cellular populations resulting from SNI alone and from operant social intervention for SNI.

We found that access to social interaction significantly shaped behavioral and nociceptive outcomes. Mice with immediate post-injury social access exhibited reduced mechanical allodynia and higher operant social responding compared to those given delayed access. In contrast, delayed-access mice displayed increased social withdrawal and enhanced allodynia. These effects were sex-dependent, with females showing stronger behavioral sensitivity to both pain and social disruption. Transcriptomic analysis revealed broad gene expression shifts, including differential regulation of kappa and mu opioid receptor genes specifically in pair-housed SNI mice who were not given access to the social operant task.

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Identifying the mechanisms of noradrenergic modulation of BLA-mediated avoidance behaviors

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

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

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Neuropeptidergic control of striatal Acetylcholine release during reward learning

Michael J. Seibert, Michael R. Bruchas

The striatum integrates associative, sensory, and motor inputs to guide reward-driven actions, with dopaminergic inputs conveying reward prediction error signals and cholinergic interneurons (CINs) providing powerful local modulation through acetylcholine (ACh) release. Dopamine (DA) shapes striatal plasticity in part through its interactions with CIN activity, while ACh can regulate dopamine release. Although CIN activity is known to influence striatal plasticity and behavior, the mechanisms governing ACh release and its coordination with DA dynamics during reward learning remain unclear. Substance P (SP), an abundant striatal neuropeptide, has been shown in vitro to modulate CIN excitability, but its role in regulating striatal neuromodulatory balance in vivo has not been defined. We hypothesize that SP signaling enhances CIN activity and ACh release, thereby shaping dopamine-acetylcholine interactions to promote reward learning. Using RNAscope, we confirmed that the cognate receptor for SP, the neurokinin 1 receptor (NK1R), is highly expressed in striatal CINs. In vivo fiber photometry experiments monitoring striatal ACh and DA dynamics with the gACh3.0 and rDA3m sensors, respectively, revealed that Tac1 KO mice exhibit disrupted ACh and DA release in the dorsomedial striatum and nucleus accumbens during Pavlovian reward tasks. These changes in Ach and DA dynamics suggest that loss of SP alters cue-evoked neuromodulatory signaling and the temporal coordination between dopamine and ACh. By integrating peptidergic signaling with canonical dopaminergic and cholinergic mechanisms, this work advances understanding of how striatal circuits encode reward and highlight new avenues for targeting maladaptive learning processes in addiction and related disorders.

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Poster 16

Role of opioid receptors on fentanyl-seeking and neuronal activity in the Ventral Tegmental Area

Pradumna Sachdeva, Catalina Zamorano, Michael Bruchas

Opioid addiction is a major public health crisis, yet the endogenous mechanisms that drive opioid-seeking behavior remain poorly understood. Opioids such as fentanyl act through the μ-opioid receptor (MOR), which is expressed on inhibitory GABA neurons in the Ventral Tegmental Area (VTA), a brain region critical for reward and motivation. Activation of MORs is thought to suppress GABA neuron activity, leading to increased dopamine signaling and reinforcement. However, whether MORs on VTA GABA neurons are necessary for opioid drug seeking is unknown. We hypothesize that mice lacking MORs in the VTA will show reduced fentanyl-seeking behavior and altered GABA neuron activity. To test this, we knocked out MORs in the VTA and assessed how VTA GABA activity was altered during an oral fentanyl-seeking paradigm. I quantified fentanyl consumption, licks, active/inactive nose pokes, and sipper engagement time while recording VTA GABA activity using fiber photometry to assess how MOR knockout alters GABA activity during fentanyl-seeking behavior. By understanding how the brain's opioid circuitry is hijacked by drugs of abuse, we can contribute to the development of more effective treatments for substance use disorders, shape public health strategies, and ultimately, improve people's lives. This work aims to improve our understanding of opioid reward circuitry and inform future therapeutic approaches for substance use disorders.

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Using in vivo 2-photon calcium imaging to characterize the function of locus coeruleus activity during avoidance behavior

Madison M Martin, Sean C Piantadosi, Bailey A Wells, Sayaka Kenmochi, Marta Trzeciak, Garret D Stuber, 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 and 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 these local projections contribute to LC function in vivo. Using 2-photon imaging to record 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. These changes in activity corresponded with stimulus-induced behavioral changes. To determine how inhibitory peri-LC neurons modulate LC activity in vivo, we chemogenetically activated peri-LC neurons while recording LC calcium activity and observed a decrease in stressor-induced tonic LC activity. 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.

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Accumbens mShell Pdyn neurons' control over dopamine dynamics

Marcelina Węzik, Raajaram Gowrishankar , Carina Soares-Cunha, Ana João Rodrigues & Michael R. Bruchas

Dopaminergic neurons in the ventral tegmental area (VTA) and their projections to the nucleus accumbens (NAc) are central to associative learning. This system is modulated by dynorphin, produced by D1-type medium spiny neurons ($Pdyn^+$ neurons), acting via kappa opioid receptors (KORs). Dysregulation of dynorphin and dopamine signaling contributes to neuropsychiatric disorders, including depression and addiction. However, how dynorphin shapes dopamine dynamics during learning, particularly across dorsal and ventral NAc shell, remains unclear.

Using the kLight1.3 sensor in KOR-Cre mice, we observed reward-evoked dynorphin release in both NAc shell subregions during Pavlovian conditioning. To assess effects on dopamine, we expressed Chrimson and the GRAB-DA sensor in Pdyn-Cre mice and performed fiber photometry. In open-field recordings, optogenetic stimulation of Pdyn neurons increased dopamine in the dorsal shell but had no effect in the ventral shell, despite intact cocaine-evoked responses in both. During Pavlovian conditioning, however, stimulation in either subregion enhanced cue-evoked dopamine while reducing anticipatory behavior. Stimulation of Pdyn terminals in the VTA similarly increased cue-evoked dopamine, indicating that NAc→VTA projections are sufficient. KOR manipulations produced distinct effects depending on whether stimulation occurred locally or at terminals.

These findings show that dynorphin is released during reward processing and can enhance, rather than suppress, cue-evoked dopamine. This effect likely reflects circuit-level mechanisms such as disinhibition, potentially involving GABA co-release. Regional differences further suggest subregion-specific circuit engagement.

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In vivo testing of fluorescent μ-opioid sensor variants in pain and drug administration assays

Mary Loveless, Lily Torp, Yuxuan Wang, Sarah Wait, Catalina Zamorano, Kasey Girven, Andre Berndt, Marta Soden, Michael Bruchas

Endogenous opioids are essential signaling molecules in the central nervous system that play a role in substance use disorders, pain, and threat processing. However, the spatiotemporal dynamics of opioid signaling in vivo remain poorly understood, largely due to the lack of effective fluorescent sensors. To address this gap, our collaborators have developed a series of fluorescent opioid sensors based on the mu-opioid receptor (MOR). The top performing variants were tested in HEK cells and then the most promising candidates in vitro were recommended for in vivo testing. We injected two key variants, the original μMass construct and a new variant (μMass1.7), intracranially in adult mice in the periaqueductal gray, a region with high levels of endogenous opioid signaling. We then tested the efficacy of these sensors in detecting both endogenous enkephalin release (evoked with a noxious stimulus) and intraperitoneal injection of fentanyl. We found that both the original μMass and μMass1.7 variants show robust fluorescence changes to endogenous opioid release in a brief pain induction assay and this response is successfully blocked by naloxone, showing successful MOR targeting. Furthermore, both μMass and μMass1.7 respond to intraperitoneal injection of fentanyl, demonstrating their utility in sensing exogenous opioid binding. With both endogenous and exogenous ligands, μMass1.7 exhibits enhanced signal amplitude and improved signal-to-noise ratio than the original construct, indicative of improved sensitivity and dynamic range. These findings represent an important advance in the development of genetically encoded neuropeptide sensors, extending optical measurement capabilities to endogenous opioid signaling with receptor-level specificity.

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Imaging the neural encoding dynamics of chronic pain transition in the ventrolateral periaqueductal gray

Madalyn Critz, Kaylin Ellioff, Sean Piantadosi, Benjamin B. Land, Michael R. 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 as better understanding of the neurobiological and pharmacological mechanisms governing the development of chronic pain. The ventrolateral periaqueductal gray (vlPAG) is an important hub for the descending modulation of pain signals, with both excitatory (vGlut2) and inhibitory (vGAT) neurons bidirectionally regulating “on and off” pain responses. Prior research efforts have shown that pain neurons in the vlPAG are sensitive to pain and stress state. However, these efforts have not been able to track individual neurons in abundance during transition to chronic pain, to investigate how pain state reorganizes neuronal ensembles in the vlPAG. Here, we used in vivo 2-photon resonance microscopy, coupled to microprism technology to observe large scale calcium dynamics of thousands of vlPAG neurons in real time during the development of chronic pain in a partial sciatic nerve ligation model. Concurrently, we used a head-fixed running wheel, with time-locked nociceptive stimuli to characterize pain and escape behaviors across chronic pain development. We show that vlPAG GABAergic neurons respond dynamically to noxious stimuli, and that chronic pain state increases GABAergic activity in the vlPAG. These studies inform how the vlPAG encodes pain to confer analgesia and how these neurons transition in pain states.

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Characterizing the effects of select phytocannabinoids and terpenes for pain relief within the BLA

Kaylin J. Ellioff, Anthony English, Sean C. Piantadosi, Keming Qiu, Jessica Hart, Zoe Feit, Nephi Stella, Michael R. Bruchas, Benjamin B. Land

Chronic pain affects nearly 100 million people in the U.S., and current analgesics carry limitations, including the risk of opioid use disorder and overdose. This underscores the need for safer, effective alternatives. Cannabinoid compounds from Cannabis, particularly cannabidiol (CBD), Δ^9-tetrahydrocannabinol (THC), and terpenes, act on the endocannabinoid system and may offer therapeutic potential, especially if they work synergistically. The basolateral amygdala (BLA), which regulates pain processing through glutamatergic nocifensive ensembles, is altered in chronic pain, but the roles of terpenes and their interaction with cannabinoids remain unclear. Using a voluntary oral gelatin model alongside behavioral assays (von Frey, hot plate) and machine learning-based tracking (SLEAP), we characterized neuropathic pain phenotypes and compared chronic oral versus acute intraperitoneal delivery. We also performed bilateral BLA cannulations for local THC infusion and used in vivo 2-photon imaging and dual-color fiber photometry to measure neuronal activity and endocannabinoid signaling. Mice reliably consumed gelatin containing CBD and/or a terpene mix (α-pinene, β-myrcene, linalool, β-caryophyllene). Chronic CBD plus terpene treatment significantly reduced mechanical allodynia and normalized naturalistic behaviors for weeks. In contrast, intra-BLA THC had minimal behavioral effects, though it modestly increased withdrawal thresholds. At the circuit level, evoked BLA calcium and endocannabinoid signals were elevated, but stimulus-driven endocannabinoid responses were blunted in chronic pain and restored by THC. CBD plus terpenes also modulated BLA activity during mechanical stimulation. Overall, chronic CBD and terpene co-administration produces sustained analgesia, while THC primarily normalizes disrupted BLA signaling, supporting cannabinoid-based, non-opioid pain therapies.

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JWT-101: a novel long-lasting KOR antagonist

Kayla Kittrell, Micaela V. Ruiz, Benjamin Land

Kappa opioid receptor (KOR) ligands have been explored for anti-depressive and substance use disorder therapeutics. These therapeutic effects are partly due to biased signaling through the cJun N-terminal Kinase (JNK) pathway, which involves complex molecular interactions and downstream effects that inactivate KOR by producing reactive oxygen species (ROS). JWT-101 has shown therapeutic effects in these conditions, and we hypothesize that its mechanism of action is through KOR antagonism. I previously assessed KOR antagonistic effects using in-vivo fiber photometry with the novel peroxide sensor AAV oROS-Gr, revealing that JWT-101 significantly increases ROS production in KOR-expressing cells. Injection of 15 mg/kg JWT-101 increased oROS fluorescence compared to control post-injection. Pretreatment with either JNK-selective antagonist Aticaprant and PRDX6 inhibitor MJ33 prior to JWT-101 administration blocked oROS fluorescence, suggesting that JWT-101's activity is mediated by KOR in a JNK/PRDX6-dependent manner.

To better understand neuronal activity in the PFC, I used 2-photon microscopy to observe JWT-101 in ex-vivo live-cell imaging. After washing slices with 10 uM JWT-101, there was increased oROS fluorescence in individual cell bodies and single regions of interest (ROIs) compared to buffer. This effect was blocked by both the JNK inhibitor JN8 and the PRDX6 inhibitor MJ33, indicating that this ROS production is mediated by the JNK/PRDX6 pathway.

Both in-vivo fiber and ex-vivo live cell imaging show an increase in oROS fluorescence in the presence of JWT-101, consistent with a KOR/JNK mechanism. Exploring the novel mechanism of action of JWT-101 may provide greater insight into how its therapeutic effects work.

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Uncovering phenotypic diversity and cell-type-specific prefrontal cortex calcium dynamics in rat fentanyl self-administration

Alex Whitebirch, **Loveleen Tripathi**, **Shawn Panh**, Dayna Suess, Susan Ferguson

The proliferation of the potent synthetic opioid fentanyl has exacerbated the ongoing crisis of substance use disorder and associated overdose deaths, yet the neurobiological mechanisms that underlie individual vulnerability to addiction and relapse remain poorly understood, particularly in the context of fentanyl use. The prefrontal cortex (PFC) has been identified as a key brain structure important for cognitive functions impacted in addiction, including inhibitory control of behavior and association of drug experience with specific cues, contexts, or actions. Although the heterogenous neuronal composition of the PFC complicates attribution of addiction-related behavioral regulation to specific cortical cell types and circuits, application of cell-type-specific methods in translationally relevant rodent models have begun to elucidate the key neural substrates of opioid addiction. We used an intermittent access fentanyl self-administration (IntA SA) model to characterize individual variation and sex differences in addiction vulnerability in male and female rats. Longitudinal wireless fiber photometry recording was used to track calcium activity patterns in intratelencephalic (IT) neurons of the prelimbic cortex across acquisition of self-administration, escalation of fentanyl intake, extinction training, and cue-induced reinstatement of fentanyl seeking. We found that our fentanyl IntA SA paradigm produces distinct low- and high-risk addiction severity phenotypes and that female rats exhibited a greater propensity for high-risk classification, which was characterized by abundant and consistent fentanyl intake, robust responsiveness to conditioned and discriminative fentanyl-associated cues, and high levels of fentanyl-seeking during periods of drug unavailability, extinction training, and a cue-induced reinstatement test. Fiber photometry recordings revealed dynamic encoding of fentanyl-associated stimuli by prelimbic IT neurons across the IntA SA paradigm with event-related calcium transients observed in association with lever presses, fentanyl infusions, and presentation of conditioned and discriminative cues. Our data indicate that fentanyl IntA SA is a translationally relevant paradigm that enables investigation of phenotypic diversity and the role of sex in fentanyl addiction. Longitudinal cell-type-selective calcium recordings revealed dynamic representation of fentanyl-associated stimuli by IT neurons of the prelimbic cortex consistent with a role for this cortical subpopulation in addiction-related behaviors.

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Serotonin encoding of reward value and HTR2A-dependent modulation of behavior in the Central Amygdala

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

Serotonin (5-HT) neurons in the dorsal raphe nucleus (DRN) are known to encode reward-related signals, including responses to food reward and ramping activity during reward anticipation. The central amygdala (CeA), a key downstream target of DRN serotonergic projections, has been implicated in reward processing, yet the dynamics and functional role of serotonin release in this region remain unclear.
We investigated 5-HT dynamics in the CeA using a genetically encoded serotonin sensor during reward-based tasks. We find that serotonin release in the CeA ramps during the waiting period preceding reward delivery, mirroring anticipatory signals observed in the DRN serotonin neurons. Interestingly, this ramping signal is sensitive to changes in reward value; Serotonin dynamics during waiting are attenuated following reward devaluation, induced either by satiety or by pairing the reward with an aversive foot shock. Similarly, serotonin signals decrease under conditions of reduced expected value, including delay discounting and probabilistic reward omission, indicating sensitivity to both diminished and uncertain future rewards.
To test the causal role of CeA serotonin receptors, we used CRISPR-mediated mutagenesis of HTR2A receptors, which have been previously implicated in reward palatability. HTR2A knockout mice exhibited heightened sensitivity to satiety induced and shock-paired reward devaluation and a trend toward reduced discounting in delay discounting and probabilistic omission paradigms.
Together these findings demonstrate that serotonin release in the CeA dynamically tracks anticipated reward value and that HTR2A-expressing neurons contribute to the behavioral regulation of reward valuation.

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High-throughput engineering of genetically encoded μ-Opioid sensors with enhanced sensitivity and neuronal expression for detecting opioids in vivo

Y. Wang, L. Torp, S. Wait, L. Jin, M. Gargantiel, C. Zamorano, M. Loveless, M. Soden, M. Bruchas, and A. Berndt.

Genetically encoded fluorescent indicators (GEFIs) are powerful tools for real-time monitoring of neural activities, enabling 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 of large G protein-coupled receptors (GPCRs) like the μ-opioid receptor (μOR). To address this problem, the Berndt Lab developed the optogenetic microwell array screening system (Opto-MASS), a high-throughput screening platform capable of screening thousands of μOR-based GEFIs in a single day. Using Opto-MASS, we previously leveraged to identify an improved opioid sensor μMASS, which displays an increased fluorescence response to endogenous (met-enkephalin) and exogenous (fentanyl) opioid ligands. To further improve neuronal expression, we systematically tested membrane localization and trafficking tags. Incorporating these motifs resulted in an increase in fluorescence and enhanced membrane expression in neurons compared to μMASS, with μMASS2.0 emerging as the top-performing variant for in vivo neural applications. These advancements establish a framework for engineering next-generation opioid sensors with improved sensitivity and specificity. Taken together, we leveraged the high-throughput capabilities of Opto-MASS to engineer novel tools to detect opioids in real-time. We are currently applying these sensors in vivo to explore the spatial and temporal regulation of opioid peptides in freely behaving animals, providing critical insights into the neuromodulatory roles of endogenous opioid signaling across different brain regions.

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Multi-modal physiological and behavioral modeling to assess individualized opioid withdrawal severity in mice

Anirudh G. Ramadurai, Nicole C. Miranda, Li Li

The opioid crisis claims over 80,000 lives annually in the United States, with withdrawal severity strongly predicting relapse risk. Clinical assessments rely on subjective reports, while rodent models emphasize behavior and often omit physiological signals such as heart rate and respiration. Integrating autonomic physiology may improve translational relevance, as cardiovascular dynamics are closely linked to brain circuits governing anxiety and aversion. We hypothesized that combining physiological monitoring with machine learning-based behavioral analysis would reveal individual variability in opioid withdrawal.

We developed a multimodal platform combining whole-body plethysmography (respiration), wireless telemetry (heart rate, temperature, activity), and high-speed video in mice undergoing morphine withdrawal. Behavioral analysis used DeepLabCut to track dorsal keypoints and an unsupervised clustering approach to identify withdrawal-related behavioral modules. We then constructed a composite withdrawal severity score weighting respiratory rate, heart rate variability, withdrawal-enriched behavioral motif frequency, temperature dysregulation, and stereotyped movements such as wet-dog shakes, with components informed by clinical and preclinical literature on opioid withdrawal.

Preliminary data from morphine-treated mice reveal marked inter-individual differences across physiological and behavioral domains. Some animals show pronounced cardiovascular dysregulation with relatively modest behavioral disruption, whereas others exhibit severe behavioral suppression and increased respiratory disturbance, yielding composite scores spanning moderate to severe withdrawal. These findings demonstrate that multimodal physiological-behavioral monitoring can capture quantifiable phenotypic heterogeneity in opioid withdrawal. This framework provides an objective basis for identifying candidate biomarkers and may inform personalized treatment strategies in opioid use disorder as ongoing work expands cohort size and refines integrated models.

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Impaired Noradrenergic signaling in ketamine-induced dissociation in mice

Iris Xu, Nicole Miranda, Anirudh G. Ramadurai, Myesa Travis, Abigail Gao, Zachary Glovak, Jan Ramirez, Li Li

At subanesthetic doses, ketamine (KET) can induce a dissociated state in which core mental functions are disconnected. Recent studies have shown a shift in the brain's cortical activity in KET-induced dissociation, but the contribution of subcortical activity to dissociation remains unclear. An improved understanding of the subcortical circuit mechanism of dissociation may be useful in developing more precise dissociative anesthetics and new avenues in pain treatments. The locus coeruleus (LC) is a noradrenergic (NE) nucleus that plays an important role in stress and aversion. Here, we examine how LC-NE signaling may be impaired by KET, contributing to affective dissociation in response to aversive stimuli. We used tail suspension in mice as a model of KET-induced dissociation, as done previously. We examined LC-NE activity by expressing jGCaMP8f, a fluorescent calcium sensor, in Dbh-cre mice (n=5). We treated mice with i.p. SAL or KET (50 mg/kg) and performed a 30-s tail suspension before and 20 min after injection. We also expressed GRAB_NE2m, a fluorescent NE sensor, in the medial thalamus of wild-type mice (n=9) and performed photometry measurements as before. Our normalized photometry measurement of LC-NE activity showed a significantly decreased LC response to tail suspension in the KET-dissociated group compared to SAL (ΔAUC SAL: 82.9±21.3, KET 10.3±4.0, $p = 0.0420$). We also found significantly decreased thalamic NE release during tail suspension in the KET-dissociated group compared to SAL (ΔAUC SAL: 74.9±4.9, KET 22.5±4.3, $p< 0.0001$). Our findings suggest LC-NE signaling is impaired under KET-induced dissociation.

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Posterior paraventricular thalamus regulates defensive aggression amidst social uncertainty

Brandy Briones, Nico Masputra, Dechen Sakya, Alondra E. Torres, Raihana Oien, Adam Gordon, Marta Trzeciak, Cassidy T. Burke, Zoe Garret, Kentaro Ishii, Prabhat R. Aluri, Marissa Borrego, Garret D. Stuber

Disruptions in threat detection and maladaptive aggression are hallmark features of psychiatric disorders such as anxiety and PTSD, where heightened vigilance toward perceived threats can lead to inappropriate aggression or avoidance. Even in healthy brains, familiarity shapes social threat assessment, producing an "out-group negativity bias" or the tendency to perceive unfamiliarity as a greater potential threat. While this phenomenon is well characterized behaviorally, its underlying neural substrates remain unclear. Here, we establish a social threat bias paradigm in mice and identify the posterior paraventricular thalamus (pPVT) as a critical regulator of out-group attack bias. When reared in genetically homogeneous environments (monotypic rearing) to control which phenotypic traits are familiar (in-group) and unfamiliar (out-group), resident mice displayed increased initiated attacks toward novel out-group intruders compared with in-group. This attack bias was abolished when mice were reared in genetically heterogeneous environments (polytypic rearing), revealing experience-dependent shaping of this behavior. Fiber photometry calcium recordings showed that pPVT activity dynamically tracked social investigation and attack onset, with greater signal observed during out-group intruder contexts. Optogenetic activation of pPVT Esr1+ neurons enhanced out-group attacks, whereas reduced excitability via a conditional pPVT Esr1 knockout suppressed out-group aggression. Together, these findings indicate that pPVT neurons integrate social and contextual information to bias aggressive decisions under uncertainty and provide a neural framework for understanding how trait familiarity shapes aggression and how these processes may become dysregulated in psychiatric conditions characterized by neophobia and aggression.

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Multimodal interrogation of cell type-specific transcriptional states and brain-wide activity profiles in maladaptive opioid use

Cassidy Burke, Nailyam Nasirova, Megan Melavic, David Ottenheimer, Selena Schattauer, Larry Zweifel, Susan Ferguson, Garret Stuber

Opioid use disorder is thought to arise from persistently dysregulated neural activity in brain regions associated with reward and motivation, but the precise molecular and circuit mechanisms underlying susceptibility to escalated opioid use are not well understood. One central node in opioid reward circuitry is the nucleus accumbens, which integrates glutamatergic input from diverse brain regions with dopaminergic input from the ventral tegmental area. To characterize the transcriptional state of neurons throughout this circuit, we conducted snRNAseq in prelimbic cortex (PL), NAc, and VP in rats, after IV heroin self-administration and protracted withdrawal. Analysis of each neuronal subtype revealed significant transcriptional alterations in multiple NAc D1 and D2 expressing MSN sub-clusters, as well as L2/3 and L4/5 IT neurons in PL, including a conserved response in genes associated with postsynaptic densities across these distinct cell types. In contrast, VP neurons were largely unchanged by heroin exposure, despite high expression of the μ-opioid receptor in VP. One specific gene of interest was the metabotropic glutamate receptor mGluR5, which acts postsynaptically to modulate neuronal excitability. Using an intersectional CRISPR/Cas9 viral strategy, we knocked out mGluR5 from D1 MSNs in the NAc, which are known to play an outsize role in regulating both opioid reward and negative symptoms during opioid withdrawal. This intervention was sufficient to alter likelihood of future heroin escalation, but outcomes depended on addiction risk status, suggesting that the cell-type specific role of NAc glutamate changes as individuals move through cycles of drug use, abstinence, and relapse.

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Diversity of GABAergic cell types in the mesolimbic system

Abigail J. Elerding, Kentaro Ishii, Adam Gordon-Fennell, Josef Dostal, Eleanor Mauk, Kayla S. Buechler, Marta E. Soden, Richard D. Palmiter, Larry S. Zweifel & Garret D. Stuber

The ventral tegmental area (VTA) is a heterogeneous brain area that plays a significant role in reward, motivation, and aversion. While dopamine (DA) neurons are a major output of the VTA and have been extensively studied for their role in reward learning, they are tightly regulated by local GABAergic circuitry. VTA GABA neurons modulate DA neuron activity through both inhibitory and disinhibitory mechanisms. Beyond this, VTA GABA neurons are critical mediators of reward, aversion, and learning. However, while functional specialization across subpopulations of DA neurons is well established, it remains unclear whether distinct GABAergic subtypes within the VTA serve specialized roles in behavior. Recent single-nucleus RNA sequencing has revealed genetically distinct GABAergic subpopulations within the VTA, but the functional contributions of these subtypes is not yet 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. We utilized intersect strategies in mice to isolate GABA neurons that express Pnoc, Crhbp, or Cbln4. Each have a unique set of responses to rewards and aversive stimuli during head-fixed behavior. Remarkably, responses to aversive stimuli were almost entirely absent in the Crhbp+ subtype, which instead displayed responses to reward consumption. In addition, preliminary results suggest that direct activation of VTA GABA subtypes differentially contribute to appetitive and aversive behavior. Further resolution of the function and diversity of VTA GABA neurons will yield deeper insights into this important class of neurons and how they coordinately regulate the mesolimbic dopamine system.

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Dopamine modulation of Nucleus Accumbens dynamics across cue-reward learning

Lucy Tian, Nirupama Niranjan, Yumi Lee, Garret Stuber

Learning to associate predictive cues with rewarding outcomes relies on mesolimbic dopamine signaling and its downstream influence within the nucleus accumbens (NAc). The NAc is composed primarily of D1- and D2-expressing medium spiny neurons (MSNs), which are traditionally linked to action initiation and suppression, yet how these populations exhibit heterogeneous changes in their encoding of task variables across cue-reward learning remains unclear. Using a head-fixed odor Pavlovian conditioning paradigm combined with large-scale, cell-type-specific two-photon calcium imaging through implanted microprisms, we longitudinally tracked thousands of individually identified D1 and D2 MSNs across the course of learning. Results show that although individual D1 and D2 MSNs display highly heterogeneous response patterns to cues, reward delivery, and licking, both populations form stable ensemble representations of reward. Cue-related activity grows over training, with D1 MSNs increasingly responsive during reward-predictive CS+ trials, while D2 MSNs show patterns consistent with action suppression and increased responding during reward absent CS- trials. Dual-color fiber photometry recordings of MSN activity and dopamine further show that population responses in D1 and D2 cells become more correlated with dopamine release during CS+ trials as learning progresses, but less correlated during CS- trials. Together, these findings indicate that dopamine refines population-level coding despite substantial variability at the single-cell level. Ongoing work will combine two-photon MSN recordings with dopamine measurements to test whether spatial differences in dopamine release within the same imaging field predict local variation in D1 and D2 recruitment, providing a mechanistic link between dopamine dynamics and evolving accumbens circuit organization.

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A role for central amygdala acetylcholine in conditioned taste aversion

Weston Fleming, Jordan Pauli, Marta Trzeciak, Ken Ishii, Richard Palmiter, Garret Stuber

When an animal consumes a new food and consequently feels ill, it immediately and robustly learns to avoid this food in the future, a form of basic aversive learning termed conditioned taste aversion. Postingestive malaise often occurs minutes to hours following novel food consumption, likely necessitating a neural mechanism that can facilitate re-activation of novel food-response neurons during malaise. Neuromodulators, acting through G-protein-coupled receptors to influence neuronal excitability on an extended timescale, may facilitate this process. Parabrachial nucleus Calca neurons and their projection to the central amygdala are required for formation of conditioned taste aversion (CTA). Here, we demonstrate that these neurons are part of a cholinergic parabrachial population which releases acetylcholine in the central amygdala. Acetylcholine release is observed in central amygdala in response to consumption of a novel solution and during visceral malaise, implying a role in the acquisition of conditioned taste aversion. Using 2-photon calcium imaging in brain slices, we show that acetylcholine widely activates central amygdala neurons on an extended timescale and enhances glutamatergic responsivity on a timescale consistent with CTA learning. We then use a CRISPR-Cas9 viral system to show that ACh from PBN neurons is required for normal conditioned taste aversion behavior. Optogenetic experiments confirm these cholinergic neurons contribute to learning at novel solution consumption and malaise. Finally, we perform large-scale neuronal recordings in central amygdala and combine this method with our CRISPR approach to show that, in absence of ACh from PBN, central amygdala neurons are less responsive to a novel solution, less synchronous during visceral malaise, and less discriminative between the conditioned solution and water. Together, our data point to the cholinergic input from PBN to central amygdala as a key neuromodulatory signal that contributes to neuronal activation during novel solution consumption, re-activation during visceral malaise, and plasticity required for later avoidance of dangerous solutions.

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Modulation of mGluR5 alters heroin self-administration behavior

Megan Melavic, Cassidy Burke, Susan Ferguson, Garret Stuber

Opioid use disorder is characterized by persistent drug-seeking behavior and long-lasting neuroadaptations following repeated drug exposure. Glutamatergic signaling, specifically through the metabotropic glutamate receptor 5 (mGluR5), has been implicated in synaptic plasticity associated with substance use disorders and protracted withdrawal. However, the role of mGluR5 in heroin self-administration remains incompletely understood.

To investigate the contribution of mGluR5 in heroin reinforcement and associated neuroadaptations, rats were trained in intravenous heroin self-administration paradigms. Pharmacological manipulation of mGluR5 signaling was performed after training where the rats could be categorized as low or high takers. Behavioral measures included acquisition and maintenance of intermittent self-administration, extinction, and cue-induced reinstatement. Additional analyses were conducted to assess markers of plasticity, cell-type specific D1 and D2 neurons in the nucleus accumbens, and spatial transcriptomics. Modulation of mGluR5 signaling significantly altered heroin self-administration behavior, indicating a role for this receptor in regulating opioid reinforcement.

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Impact of fentanyl on post-mTBI behavior

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

Mild Traumatic Brain Injuries (mTBIs) are a prevalent condition that veterans experience during training and during their time in the field. Due to pain associated with this type of injury, opioid prescriptions are common, and this can open a pathway to opioid use disorder (OUD). Understanding the behavioral effects of repeated exposure to fentanyl after mTBIs could be a crucial step in understanding OUD. We hypothesize mice that undergo chronic fentanyl treatment after blast induced mTBI will develop greater hyperalgesia during withdrawal, increased anxiety-like behaviors following fentanyl administration, diminished motivational state, and increased fentanyl preference. This was modeled in mice that experienced multiple overpressure blast exposures that mimic fully body blast injuries that veterans may experience within the field. Shortly after inducing the mTBIs, minipump implants allowed a slow month-long administration of fentanyl treatment. One month after fentanyl exposure, an open field test and novelty-suppressed feeding assay were administered to see the long-term withdrawal effects on anxiety-like behaviors and motivational states. Throughout this experimental paradigm, there were many timepoints for tail flick tests to examine how the levels of pain sensitivity changed with each condition. The last test conducted was a two-bottle choice assay, which examines fentanyl seeking by allowing the mouse free access to a water bottle and fentanyl water bottle during the dark cycle over 11 nights. The results are currently being analyzed. These results can contribute to finding novel and more effective interventions for OUD.

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Identifying and validating neuronal PKA inhibitors as targets of inflammatory pain treatment

Christina Gao, Veselina Petrova, Shannon Zhen, Clifford Woolf, Aakanksha Jain

Nociceptors are specialized sensory neurons that detect potentially damaging stimuli. In healthy conditions, nociceptors respond exclusively to noxious stimuli such as extreme pressure or heat. During inflammation, however, immune ligands can reduce their activation thresholds, enabling pain to otherwise non-damaging stimuli such as gentle touch or warm temperature. This process of peripheral sensitization underlies inflammatory pain. To treat inflammatory pain, it is ideal to target immune-mediated peripheral sensitization without disrupting protective pain responses. One key pathway driving peripheral sensitization involves PKA activation. PKA inhibition is therefore a promising strategy to safely and effectively alleviate inflammatory pain. We set out to identify inhibitors of PKA in sensory neurons by performing a high-throughput screen on human induced pluripotent stem cell-derived sensory neurons using a fluorescent-based PKA sensor. Following treatment with forskolin (FSK), an established PKA activator, we saw a significant increase in PKA activity. We then screened 1,119 small molecules to identify candidates that could inhibit PKA activity. We identified 11 neuronal PKA inhibitors, 7 of which showed dose-dependent effects. These included H89, a known PKA inhibitor. To validate these candidates in vivo, we developed a mouse model of FSK-induced pain. Using the Hargreaves assay, we found that intraplantar injection of FSK (50 nmol in 10 µL solution) induces thermal hypersensitivity at 4 hours, which begins to resolve at 24 hours. Ongoing experiments are testing whether treatment with candidate molecules would attenuate FSK-mediated hypersensitivity. In the long term, we aim to develop PKA inhibitors as potential therapeutics for inflammatory pain.

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Engineering antigen-dependent stability in nanobodies: A bioinformatics tool for tuning intracellular nanobody stability.

Alvin Fu, Rohith Leeladharan, Junhee Park, Shriya Kapila, Liuhan Ke, Amina Mohamed, Tanaka Chipinda, Thien Tran, Cynthia Zhao, Radha Manohar Kapgate, Anh Leith, Jonathan Tang

Nanobodies, derived from camelid antibodies, are highly prized reagents due to their ability to bind their antigen with high affinity, specificity and due to their ease of genetic expression inside cells. Conditionally stable nanobodies, nanobody variants containing mutations in their sequence, are stable only upon antigen binding, making them ideal biosensors. However, the generalizability of conditionally stable nanobodies depends on contextual interactions between nanobody sequences as well as with their fusion protein sequences. To account for contextual effects and thereby creating an individualized way of engineering nanobody for desired stability modulations, we developed a novel bioinformatics tool based on large language model (LLM) that takes an input nanobody sequence and makes predictions about its intracellular stability. The predictions integrate information about nanobody binding to avoid disruptive mutations, guided by a dataset of 389 nanobody-target interfaces. The LLM was first pre-trained on 1.4 million camelid antibody sequences, then fine-tuned to perform binary classification of intracellular stability, producing an F1 score of 0.8. Generative models were developed using a form of Masked Language Modeling (MLM). Models' predictions were validated using reporter assays and western blot in human cell culture across multiple tissue types. The tool integrates concepts from LLM, nanobody bidirectional stability modulation, nanobody interface classifications, and web integration of modalities to create a tool that can facilitate biomedical and cancer endeavors in the creation of biosensors against any target intracellular proteins for which nanobodies can be selected. This tool has the potential to greatly speed up biosensor engineering, promoting diverse applications in biomedicine.

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Protein based therapeutics for opioid use disorders and overdose

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Poster 38

Noradrenergic Modulation of Breathing: Implications for Opioid-Induced Respiratory Depression

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Opioid overdose caused over 80,000 deaths in the United States in 2022, largely due to sedation and respiratory depression. Understanding neural circuits involved in arousal and respiratory control is critical for identifying therapeutic targets. The locus coeruleus (LC), a major noradrenergic nucleus regulating arousal and autonomic function, may also modulate respiration, although its role remains unclear. Here, we investigated the role of the LC–norepinephrine (NE) system in respiratory control and evaluated a wireless physiological monitoring system (nVital, NeuroLux) for assessing respiration in freely moving mice.

Anterograde viral tracing in Dbh-cre mice (n=6) revealed direct LC projections to the preBötzinger Complex (preBötC), supporting an anatomical pathway for respiratory modulation. In parallel, wildtype mice implanted with nVital devices showed respiratory frequencies closely matching whole-body plethysmography under baseline conditions (192±6 vs. 191±5 BPM; p=0.96) and during escalating morphine exposure (10–50 mg/kg), with both systems detecting dose-dependent respiratory depression.

To assess LC–NE function, Dbh-cre mice expressing ChrimsonR in LC neurons underwent optogenetic stimulation during respiratory recordings. Preliminary testing showed that low-frequency stimulation (3–5 Hz) increased respiratory rate, whereas 15 Hz had no effect. During baseline conditions, 3 Hz stimulation significantly increased respiratory frequency (Pleth: 191±6 vs. 218±12 BPM; nVital: 190±5 vs. 219±9 BPM; $p<0.0001$). However, LC stimulation failed to rescue morphine-induced respiratory depression.

These findings demonstrate that LC–NE neurons modulate breathing under basal conditions but may be insufficient to overcome opioid-induced respiratory depression, suggesting state-dependent respiratory control.

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Support

The NAPE Center is supported by a NIDA P30 grant: P30DA048736
This event was made possible with the generous support of Dr. Richard Palmiter.