Illustrations

Cover images by *Poorna Dharmasri*

Special Thanks

Pre-doctoral Neuroscience Training Program
(PI: Jessica Mong)

And, the continued support of the School of Medicine, the Dental School, and the Graduate School.

About the Program in Neuroscience

The Program in Neuroscience at the University of Maryland offers research training in a wide range of brain sciences, including cellular, molecular and integrative neuroscience. Program faculty consists of more than 100 neuroscientists with laboratories located in the Medical, Dental, Nursing and Pharmacy Schools, and the Maryland Psychiatric Research Center. The program is affiliated with the Graduate Program in Life Sciences in the School of Medicine. Investigators utilize a wide variety of state-of-the-art techniques to investigate topics whose scope ranges from the single molecule to the human brain.

The University of Maryland campus is located in the heart of historic, downtown Baltimore, offering all the amenities of city life while maintaining easy access to the countryside and the irresistible appeal of the largest estuary system in the world, the Chesapeake Bay.

To learn more about our program and to keep updated on upcoming seminars, retreats and other exciting program events, please visit:

http://neuroscience.umaryland.edu

PIN Gratefully Acknowledges

- Jessica Mong for her outstanding service as the outgoing Director of Graduate Education

- Mary Kay Lobo for her outstanding service as the new Director of Graduate Education

- Brian Mathur for chairing the Retreat Committee and coordinating Neuroscience Journal Club

- Norbert Myslinski for his work on the International and National Brain Bee

- Todd Gould for chairing the PIN Seminar Committee

- Poorna Dharmasri for serving as student representative on the PIN Training Committee and chairing the PIN Student Training Committee.

- Ashley Marquardt for serving as the student representative for the PIN Seminar Committee and the voice behind the new PIN Twitter feed.
  Follow us at **@UMMedNeuro**

- Quinton Banks, Sam Bacharach, Amanda Labuza and Ashley Marquardt for serving on the GSA

- Kasey Girven for serving as President of NOVA (Neuroscience Outreach and Volunteer Association)

- Special thanks to all Faculty, Students and Staff whose time and effort ensured the recruitment of an outstanding group of new PIN students

- Dudley Strickland and Sharron Graves for leadership and service to GPILS

- The continued support of The School of Medicine, The Dental School, and The Graduate School

- PIN's graduate students and postdocs - ***the point of it all*!**

And to the Program in Neuroscience Standing Committees:

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Todd Gould
David Seminowicz
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*Kara Cover
*Austin Ramsey
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21st Annual Neuroscience Retreat

Friday, June 8th, 2017

Notre Dame of Maryland University

Baltimore, MD 21210

Schedule

8:30-9:00	**Registration** (Coffee provided)
9:00-9:30	**Introductory Remarks (Michael Shipley)** **Awarding of J. Tyson Tildon for a Promising Neuroscientist and Community Servant**
9:30-10:30	**Research Talks:**
	Tara LeGates "Reward behavior is regulated by the strength of hippocampus-nucleus accumbens excitatory synapses"
	Polymnia Georgiou "Human experimenter sex modulates mouse behavioral responses to stress and to the antidepressant ketamine"
	Michael White "The Claustrum: from Top to Bottom"
10:30-11:15	**Grant Proposal Challenge**
11:15-12:15	**PIN Challenge**
12:30-1:30	**Lunch**
1:30-1:45	**1st Year Students' Video**
1:45-2:45	**Keynote Address:** ***"Understanding basal ganglia function through presynaptic modulomics"*** **David M. Lovinger, PhD** **Deputy Scientific Director NIAAA/NIH** **Senior Investigator and Chief of the Laboratory of Integrative Neuroscience**
2:45-4:15	**Poster Session** **(**Beverages & snacks provided)
4:15-4:45	**Grant Proposal Presentations**
4:45-5:00	**Awards**

J. Tyson Tildon Award for a Promising Neuroscientist and Community Servant

In memory of J. Tyson Tildon and his commitment both to neuroscience research and service to his community, the J. Tyson Tildon Award for a Promising Neuroscientist and Community Servant will be given, **periodically**, to a dynamic postdoctoral fellow who has demonstrated community service. This is a cash award to help the recipient launch her/his career.

Dr. Tildon was the founder and head of the Division of Pediatric Research and first Associate Dean for Research for the University of Maryland School of Medicine. Dr. Tildon also served on boards for the Baltimore City Public Schools, United Way of Maryland, Associated Black Charities, WYPR radio, the Civil Services Commission of Baltimore, the Maryland Academy of Sciences, the Enoch Pratt Free Library and the American Red Cross.

Nominee Eligibility Criteria
The postdoctoral fellow chosen to receive this award will have demonstrated a history of critical thinking and a passion for continued learning in neuroscience. Additionally, the fellow will have a proven commitment to helping others in the scientific community or community at large, reflecting the spirit of Dr. Tildon's dedication to the pursuit of research and exceptional service.

Nomination process
To be nominated for this award, the mentor must have known the postdoctoral fellow for at least one year in order to gauge her/his character, ability, and commitment to service. The mentor must submit a nomination letter, the nominee's CV, a brief personal statement written by the nominee (no more than one page), and 1-2 other supporting letters. At least one supporting letter must be from someone who can directly address the fellow's service contributions.

Nomination deadline
All materials should be submitted to the Director of the Neuroscience Program by April 15.

Award announcement
The award will be presented at the Annual Program in Neuroscience Retreat the following June.

Award amount
Cash in the amount of $ 3,000.00-5,000.00

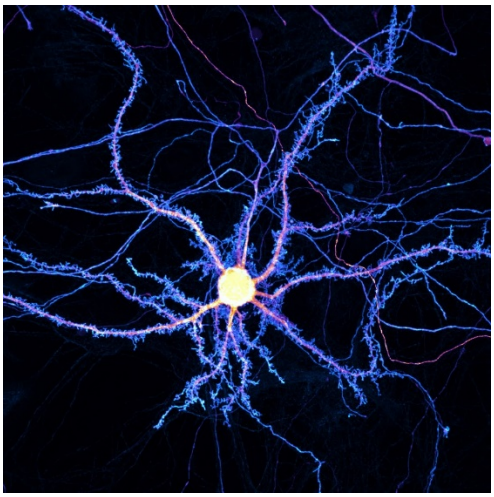
Award conditions
This award is to help the recipient launch her/his career. For example, it can be used by the recipient to travel to a conference to present her/his research and gain valuable networking, or to train in specific methodology by working in the expert in the field

After one year, the awardee will be expected to submit a brief statement of how the award was used to benefit her/his career.

KEYNOTE SPEAKER:

David M. Lovinger, Ph.D., Senior Investigator and Chief of the Laboratory of Integrative Neuroscience at the National Institute on Alcohol Abuse and Alcoholism (NIAAA)

David Lovinger, PhD, is a Senior Investigator and Chief of the Laboratory of Integrative Neuroscience at NIAAA. Dr. Lovinger received a B.A. in Psychology from the University of Arizona in 1981 and a Ph.D in Psychology from Northwestern University in 1987. At Northwestern, he worked with Dr. Aryeh Routtenberg studying the roles of Protein Kinase C and its substrate, the GAP-43/F1 protein, in hippocampal long term potentiation. His postdoctoral research at the NIAAA focused on the effects of alcohol on ligand-gated ion channels. In 1991 Dr. Lovinger moved to the Vanderbilt University School of Medicine as an Assistant Professor, where in 1998 he rose to the rank of Professor. At Vanderbilt, he was also the Deputy Director for Biomedical Science and the Director of the Neuroscience Core within the Kennedy Center. Dr. Lovinger joined the NIAAA in 2001 as a Senior Investigator and Chief of the Laboratory of Integrative Neuroscience. His laboratory is currently studying the modulation and plasticity of synaptic transmission at corticostriatal synapses and the mechanisms by which abused substances effect synaptic transmission.



Cultured rat dissociated hippocampal neuron expressing a cell-fill fluorescent protein, by Poorna Dharmasri, Doctoral Candidate, Blanpied Lab.

Research Talks

Reward behavior is regulated by the strength of hippocampus-nucleus accumbens excitatory synapses

Tara LeGates

Reward drives motivated behaviors and is key for survival, resulting in strong evolutionary pressure to retain contextual information regarding rewarding stimuli. This drive may be abnormally strong, such as in addiction, or weak, such as in depression, in which anhedonia, or loss of pleasure to rewarding stimuli, is a prominent symptom. Optogenetic and traditional approaches have allowed the mapping of key brain circuits underlying the perception and processing of rewarding stimuli. However, the nature of the critical synapses and neurophysiological changes that underlie the encoding of contextual information within these reward processing circuits remain unknown. The hippocampus, critical for processing and storing spatial information, innervates the shell of the nucleus accumbens (NAc), a key structure that integrates the sensation and recognition of rewarding stimuli. This input is important for driving NAc activity, and modulation of its strength may play a role in the proper regulation of goal-directed behaviors. Hippocampal activity is altered by changes in contextual features of a situation such as the presence of a reward, however there are few robust descriptions of the mechanisms underlying induction or expression of long-term potentiation (LTP) and no evidence whether plasticity at these synapses contributes to reward-related behavior. We have defined the mechanisms underlying activity-dependent potentiation at the hippocampus-NAc synapse and demonstrate that induction of canonical LTP of this synapse *in vivo* is sufficient to drive a behavioral preference for a neutral place. Conversely, chronic stress, which induces anhedonia, decreases the strength of this synapse and impairs LTP, whereas antidepressant treatment, sufficient to reverse anhedonia, is accompanied by a reversal of these stress-induced changes. We conclude that hippocampal-NAc synapses display activity-dependent plasticity, and their strength is a critical determinant of contextual reward behavior. This work defines a specified neuronal circuit responsible for regulating contextual reward behavior and furthers our understanding of excitatory synaptic strength as a critical mediator of this process. Understanding the neuronal changes that underlie depression and antidepressant response will provide key insight into developing new, more effective treatments for this disorder.

Human experimenter sex modulates mouse behavioral responses to stress and to the antidepressant ketamine

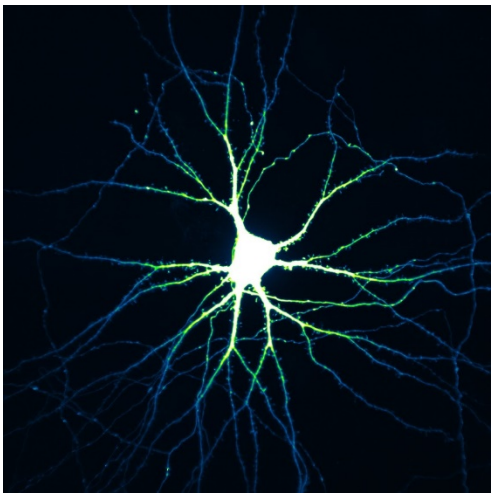
Polymnia Georgiou

Rodent differentiation of the sex of human experimenters may influence physiological and behavioral responses. We show that mice manifest aversion to male odors, and attraction to female odors, while showing increased susceptibility to stress responses when handled by a male. Administration of the antidepressant drug ketamine to mice by male experimenters reversed stress-mediated behavioral responses, while such responses were absent following injection of the drug by a female-experimenter. Similar experimenter sex-dependent effects were identified with the ketamine metabolite (*2R,6R*)-hydroxynorketamine, but not with other mechanistically distinct antidepressants. Non-antidepressant behavioral actions of ketamine where present regardless of the gender of the experimenter. We establish that male scent is necessary to induce ketamine's antidepressant effects in our mouse models; however female scent dominantly prevents such an effect in a manner dependent upon adrenal-mediated production of stress hormones. Moreover, administration of corticotropin-releasing factor 1 antagonist prior to ketamine administration by male experimenters inhibited ketamine's antidepressant effects. Ketamine administration increased GluA1 AMPA receptor subunit in the ventral hippocampus when injected by male but not female experimenters. Overall, these findings demonstrate that experimenter sex influences the outcome of behavioral and pharmacological assessments, impacting replicability, and arguing that experimenter sex should be considered as a relevant experimental variable.

The Claustrum: from Top to Bottom

Michael White

Despite much speculation, the function of the claustrum, a thin telencephalic nucleus, remains largely unknown. The claustrum is reciprocally connected with seemingly the entire cortical mantle, which motivates hypotheses of claustrum function that include multi-sensory integration and top-down/bottom-up attention. Support for any functional hypothesis requires an understanding of a pattern of claustrum connectivity with cortex and an understanding of the cortical inputs that drive claustrum activity. As such, we use neuronal tract tracing and slice electrophysiology in mice and find that the claustrum is well-connected with executive areas of cortex, such as anterior cingulate cortex (ACC), and highly responsive to stimulation of ACC inputs. In contrast, the claustrum is less connected with and less responsive to inputs from hierarchically lower cortices. These findings suggest that claustrum mediates executive function, which we subsequently test using a five-choice response assay and *in vivo* optogenetics. We find that claustrum circuits are critical for top-down cognitive control relative to other basic brain functions, such as motor control and stimulus-response action strategy. These results provide novel functional insight into the claustrum, a brain structure that is well-positioned to orchestrate cortical activity to mediate executive function.



Cultured rat dissociated hippocampal neuron expressing a cell-fill fluorescent protein, by Poorna Dharmasri, Doctoral Candidate, Blanpied Lab

Poster Presentations

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1. Characterization of cerebrovasculature in mice with selective deletion of LRP1 in smooth muscle cells

Allison L. Arai, Brittany O. Aicher, Rebeca Galisteo, Selen M. Catania, Dudley K. Strickland

Center for Vascular and Inflammatory Diseases, University of Maryland School of Medicine

Low-density lipoprotein receptor-related protein-1 (LRP1) is an endocytic receptor that traffics numerous ligands. Genetic segregation studies suggest enrichment of certain alleles of LRP1 in patients with abdominal aortic aneurysms. However, the mechanism(s) by which LRP1 might be involved in aneurysm formation is unknown. Our lab produced a sm22 promoter-driven knock-out of LRP1 (smLRP1$^{-/-}$) in smooth muscle cells to explore the role of LRP1 in the vasculature. Using high resolution contrast-enhanced micro-computed tomography following radio-opaque vascular casting to measure vascular dilatation, we observed significant dilatation of all regions of the aorta (ascending, descending thoracic, and abdominal) as well as other vascular beds (eg. superior mesenteric artery) as the mice aged ($p<0.001$ by two-way ANOVA, post-hoc: Sidak's multiple comparison test). After these results, we were further interested in the impact of smLRP1$^{-/-}$ on the cerebrovasculature. We employed the same casting method to the cerebrovasculature in adult (16 weeks), mid-age (1.5 years), and aged (2 years+) mice to detect intracranial aneurysm formation. There was no significant difference in spontaneous intracranial aneurysm formation in smLRP1$^{-/-}$ compared to wild-type. Measurements of vessel diameter will determine if smLRP1-/- mice have general cerebrovasculature dilatation as seen in the aorta. Cerebral blood flow will be assessed by laser doppler imaging. In future studies, we will induce aneurysm formation with a high salt diet to test if the cerebrovasculature is susceptible to intracranial aneurysm under sustained blood pressure elevation. Our study will provide insight into the susceptibility of the cerebrovasculature to LRP1-related aneurysm formation and vessel dilatation.

2. Binge ethanol drinking and the central amygdala: a possible role for a unique population of corticotropin-releasing factor neurons

Sonia Aroni[*,1], James M. Irving[*,1], Houman Qadir[1], Dennis R. Sparta[1]

[1]*Department of Anatomy and Neurobiology, University of Maryland School of Medicine*

Binge alcohol drinking is a severe public health problem that is considered a critical first step in the development of alcoholism and is linked to long-term alteration in brain stress and reward circuitry. Corticotropin-releasing factor (CRF) neurons in the central amygdala (CeA) appear to be critically involved in the escalation of binge drinking, but their neural activity during acute and chronic binge drinking remains poorly understood.

Here we combined *in vivo* electrophysiology with optogenetics to identify and record CeA-CRF neurons in a rodent model of binge drinking. We found that CeA-CRF units had more regular and higher overall firing rate vs. non-CRF units. Furthermore, we identified four sub-types of CRF units based on their encoding of licks for ethanol: lick-excited (CRF-E), lick-inhibited (CRF-I), lick-predictive (CRF-P), and no response (CRF-NR). We focused our analysis on CRF-NR and CRF-P units, due to their higher prevalence. We observed that CRF-P units had a significantly higher firing rate, as well as the percentage of spikes in bursts compared to CRF-NR units, making them uniquely responsive to alcohol consumption. We then further investigated if the firing and bursting activity of CRF units change over repeated sessions of drinking. We found that CRF-P units significantly increased firing rates and bursts after repeated ethanol sessions, suggesting alcohol-induced plasticity.

Altogether, our data show an important role for a subclass of CeA-CRF neurons in binge drinking and could lead to novel, circuit-specific strategies for therapeutic intervention for alcohol use disorder.

3. Transcranial Direct Current Stimulation (tDCS) for the Nicotine Withdrawal Syndrome

Sarah Aronson[1], Betty Jo Salmeron[2], Thomas J. Ross[2], Elliot A. Stein[2]

Medical Scientist Training Program, University of Maryland School of Medicine
Neuroimaging Research Branch, National Institute on Drug Abuse

Symptoms of nicotine withdrawal remain a major impediment for smokers trying to quit; most attempts fail within the first week of abstinence. Resting state functional connectivity studies have revealed that the cognitive and affective disturbances of Nicotine Withdrawal Syndrome may be attributed to dysregulation within and between 3 large-scale brain networks: reduced connectivity within the Executive Control Network (ECN), and between the ECN & Salience Network (SN); along with increased connectivity within the Default Mode Network (DMN), and between the DMN & SN. Transcranial Direct Current Stimulation (tDCS) has the potential to modify these neuronal circuits by producing a subthreshold conductive current through the scalp. Two potential targets for tDCS as a smoking cessation aid are the dorsolateral pre-frontal cortex (dlPFC), a node of the ECN, and the ventromedial prefrontal cortex (vmPFC), a node of the DMN. It is hypothesized that connectivity of the ECN, DMN, & SN will be modified by acute application of tDCS to cortical nodes of ECN and DMN networks. Non-smokers and matched smokers (in both nicotine deprived and sated conditions) will serve in a randomized, sham-controlled, double-blind, tDCS crossover design with 3 stimulation conditions: (1) anodal left-dlPFC + cathodal right-vmPFC; (2) cathodal left-dlPFC + anodal right-vmPFC, and (3) sham. The effects of 25 min, 2mA tDCS will be measured with tasks probing relevant cognitive constructs (the Parametric Flanker Task, N-back Task, and Amygdala Task), and simultaneous functional MRI, allowing greater insight into the neurophysiological effects of tDCS. This study will explore the ability for tDCS to alter cognitive constructs and neuronal activity previously implicated in nicotine dependence, and thus the device's potential serve as a smoking cessation tool.

4. Compositional changes in gut microbiome of rats with stress-induced comorbid visceral pain

Jamila Asgar, Jiale Yang, Richard Traub, Jacques Ravel*, Wei Guo, Shipping Zou, Ronald Dubner, Ke Ren and Feng Wei

*Department of Neural and Pain Sciences, School of Dentistry; & Program in Neuroscience, *Institute for Genome Sciences*

Although the etiology of chronic overlapping pain conditions (COPCs) is unclear, they are highly prevalent in women and often impacted by psychological stress. Our previous study showed that in female rats with existing orofacial neuropathic pain, 3-day repeated forced swimming stress leads to prolonged visceral hypersensitivity including referred hyperalgesia in the lower back area. In rats with such comorbid pain, 5-HT3A receptor upregulation occurrs in the lumbosacral spinal cord and dorsal root ganglia. Intrathecal injection of 5-HT3R antagonist transiently attenuates stress-induced lower back pain, suggesting 5-HT3R-mediated sensitization of primary afferents from the gut. However, the underlying mechanisms in the gut remain poorly understood. Based on recent literature, psychological stress can lead to gut microbial dysregulation in animal models; and gut microbial dysbiosis is a common finding among IBS patients. In the present study, we examined microbiota composition in the gut of rats with orofacial pain before and after stress. We revealed extensive compositional changes in fecal microbiota of female rats with orofacial pain after stress by using 16S rRNA-based analysis. Compared with nerve injury or stress alone, rats with comorbid pain showed an increase in relative abundance of bacterial species from phylum Firmicutes, including families *Clostridiaceae*, *Ruminococcaceae*, *Eubacteriaceae*, and *Erysipelotrichaceae*, and a reduction in species from phyla Bacteroidetes, Actinobacteria, and Verrucomicrobia. Our results suggest that female rats with comorbid pain share multiple features of gut dysbiosis with IBS patients. Understanding gut mechanisms of stress-induced comorbid pain will promote novel therapeutic strategies for managing COPCs including IBS.

5. Role of Cannabinoid-1 receptor signaling in sign-tracking conditioned reinforcement

Sam Z. Bacharach[1,2], Helen M. Nasser[1], Natalie E. Zlebnik[1], Hannah M. Dantrassy[1], Daniel E. Kochli[1] , Utsav Gyawali[1,2] , Joseph F. Cheer[1,2,3], Donna J. Calu[1,2]

[1]*Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD*
[2]*Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD*
[3]*Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD*

Endocannabinoids (eCBs) are critical gatekeepers of dopaminergic signaling and disrupting cannabinoid-1 (CB1) receptor signaling alters DA dynamics to attenuate cue-motivated behaviors. Prior studies suggest that dopamine (DA) release plays a critical role in driving sign-tracking. Here, we determine whether systemic injections of rimonabant, a CB1 receptor inverse agonist, during Pavlovian lever autoshaping impairs the expression of sign-tracking. We then examined whether rimonabant blocks the reinforcing properties of the Pavlovian lever cue in a conditioned reinforcement test. We find that rimonabant dose-dependently decreased lever contact and probability, and increased sign-tracker's latency to approach the lever cue early in Pavlovian training. With extended training, many previously goal-tracking and intermediate rats shifted to lever approach, which remained dose-dependently sensitive to rimonabant. Rimonabant attenuated cue- evoked food-cup approach early, but not late, in conditioning, and did not affect pellet retrieval, baseline motor behavior or pellet consumption. Additionally, the inserted lever cue served as a robust conditioned reinforcer after Pavlovian lever autoshaping, and 1 mg/kg rimonabant blocked conditioned reinforcement. Together, our results suggest that CB1 signaling mediates two critical properties of incentive stimuli; their ability to attract and their ability to reinforce behavior.

6. Optical Recordings of Action Potential Initiation and Propagation in Mouse Skeletal Muscle Fibers

Quinton Banks, Stephen Pratt, Shama Iyer, Richard Lovering, Erick Hernández-Ochoa, and Martin Schneider

Department of Biochemistry, University of Maryland School of Medicine

Individual skeletal muscle fibers have been used to examine a wide variety of cellular functions and pathologies. Among other parameters, skeletal muscle action potential propagation has been measured to assess the integrity and function of skeletal muscle. In this paper, we utilize Di-8-ANEPPS, a potentiometric dye and mag-fluo-4, a low-affinity intracellular calcium indicator to non-invasively and reliably measure action potential conduction velocity in skeletal muscle. We used an extracellular bipolar electrode to generate an electric field that will initiate an action potential at one end of the fiber or the other. Using enzymatically dissociated flexor digitorum brevis (FDB) fibers, we demonstrate the strength and applicability of this technique. Using high-speed line scans, we estimate the conduction velocity to be approximately 0.4 m/s. In addition to measuring the conduction velocity, we can also measure the passive electrotonic potentials elicited by pulses by either applying tetrodotoxin (TTX) or reducing the bath sodium levels. We applied these methodologies to FDB fibers under elevated extracellular potassium conditions, and found that the conduction velocity is significantly reduced compared to our control concentration. Lastly, we have constructed a circuit model of a skeletal muscle in order to predict passive polarization of the fiber by the field stimuli. Our predictions from the model fiber closely resemble the recordings acquired from *in vitro* assays. With these techniques, we can examine how many different pathologies and mutations affect skeletal muscle action potential propagation. Our work demonstrates the utility of using Di-8-ANEPPS or mag-fluo-4 to non-invasively measure action potential conduction velocity.

7. Receptor density and distribution of 5-HT_2 receptors in the cingulate cortex in autism: A multiple concentration saturation binding study in children and adults

Brandenburg, C. and Blatt, G.J.

Autism Neurocircuitry Laboratory, Program in Neurosciences, The Hussman Institute for Autism, Baltimore, MD

Although SSRIs are among the most commonly prescribed medications in autism, several studies show variable efficacy with SSRI use. Some of this variability may be a result of differential expression of serotonin (5-HT) receptors across individuals. 5-HT_2 receptors are G-protein coupled receptors that are located mostly postsynaptically in limbic regions such as the anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC). The objective of this study was to determine differences in 5-HT_2 receptor density and/or affinity between autism and neurotypical individuals through a saturation binding assay. Postmortem brain tissue from the ACC and PCC (n=16-19 autism, n=18-19 controls) were incubated with ^{3}H ketanserin (Perkin Elmer) at concentrations of 120, 90, 30, 9, 3, 1.5 and 0.5 nM then loaded into X-ray cassettes with tritium standards and tritium-sensitive hyperfilm. Non-specific binding was determined with a competitive displacer (Ritanserin 100µM). After exposure, films were developed and digitized to quantify ligand binding in femtomoles per milligram of tissue in superficial and deep layers of each region. A Welch's t-test was utilized for statistical analysis. 5HT_2 receptor density and affinity in the ACC and PCC did not have statistically significant differences between total autism and control cases. However, when the cases were grouped into children (≤16) (n=8-9) and adults (>16) (n=9) the superficial and deep layers of the adult ACC showed a significantly lower B_{max} in the autism cases (p=0.040 and p=0.050). No differences were seen in the PCC.

8. Cocaine-induced histone methylation on Egr3 and Nab2 promoters

R. Chandra[1], B. Evans[1], M. Mcglincy[1], A. Chow[1], K. K. Cover[2], M. Engeln[1], M.K. Lobo[1]

[1]Anatomy and Neurobiology, Univ. of Maryland Sch. of Med., Baltimore, MD;
[2]Department of Pharmacology, University of Maryland School of Medicine, Baltimore, MD, USA.

The nucleus accumbens (NAc) is a critical brain region, which mediates motivation for drugs of abuse. The NAc is composed of two types of medium spiny neurons (MSNs), which are differentiated by their enrichment of dopamine D1 vs. D2 receptors. We previously demonstrated that the transcription factor, Egr3, is upregulated in D1-MSNs and down-regulated in D2-MSNs after repeated cocaine exposure. It is postulated that Egr3 regulates its co-repressor, Nab2, and they both act together as a feedback mechanism to repress Egr3 transcription. Consistent with this, we observe a reduction of Nab2 in D1-MSNs and an increase of Nab2 in D2-MSNs after repeated cocaine exposure. Interestingly Egr3 also targets many histone methyltransferase and demethylase enzymes. Our previous work demonstrates that Egr3 transcriptionally regulates a histone lysine methylation enzyme in NAc after repeated cocaine exposure and we are currently investigating Egr3 binding on promoters of histone demethylase enzymes including lysine specific histone demethylase 1A, KDM1A, under these same conditions. In parallel we have examined mRNA levels of KDM1A in NAc D1-MSNs and D2-MSNs after repeated cocaine. Similar to Egr3, we observe an enrichment of KDM1A mRNA in D1-MSNs and a reduction in D2-MSNs in the cocaine group compared to the saline control group. We are also examining KDM1A binding and associated histone methylation marks at Egr3 and Nab2 promoters after repeated cocaine exposure. Using chromatin immunoprecipitation (ChIP) we observe altered KDM1A binding, as well as altered H3K4me3 and H3K9me2 on Egr3 and Nab2 promoters in NAc in the cocaine group compared to saline controls. Overall our studies are providing new information into the effects of cocaine on histone demethylation and its potential regulation of Egr3 and Nab2 transcription in MSN subtypes.

9. Rostral thalamic intralaminar nuclei modulation of striatal microcircuitry and action.

Kara K. Cover, Utsav Gyawali, Ashley E. Marquardt, Chaoqi Mu, Mary H. Patton, Michael G. White, Bradley M. Roberts and Brian N. Mathur.

Department of Pharmacology, University of Maryland School of Medicine

Survival in a rapidly-changing environment requires attention to sensory stimuli in order to optimize action for reward acquisition. How specific neural circuits subserve this complex function is unclear. The thalamic intralaminar nuclei (ILN) are positioned to receive salient sensory stimuli and to directly modulate actions through a direct projection to the dorsal striatum, a critical node for action selection. The rodent ILN are distributed in two groupings: whereas the caudal rILN has been examined for its role in sensory-cued action encoding, the rostral ILN has been largely ignored. Using optogenetics and fast-scan cyclic voltammetry in mice, we identified a unique microcircuit by which the rILN innervate the striatum to stably elicit dopamine release. In vivo, we found that mice lever-press for 5Hz optogenetic stimulation of rILN axons in the striatum and demonstrate a real-time place preference for rILN axon activation that was abolished by D1 receptor antagonism (0.50mg/kg SCH23390). Chemogenetic suppression of the rILN significantly decreases both locomotion and dopaminergic axon activity in the dorsal striatum as measured by fiber photometry. On a sensory-cued lever-pressing task, we found that rILN inputs to the striatum encode an expectation signal, suggesting that the rILN may be involved in the timing of action initiation in response to an expected, salient cue. Together, our results provide insight into how the thalamus may contribute to salience-informed action selection, which has implications for several disorders of action including Parkinson’s disease and addiction.

10. Acute glucocorticoid administration rapidly modulates hippocampal local field potentials

Anthony Cole[1,2], Scott M Thompson[1]

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Glucocorticoids have canonically been described as mediating late stress actions including immunosuppression and regulation of blood glucose levels via nuclear receptors. Recent evidence suggests glucocorticoid receptors can also act rapidly via non-genomic pathways. The high expression of these glucocorticoid receptors in brain regions such as the hippocampus has profound implications for our understanding of glucocorticoid activity in the brain during stress. Our lab has hypothesized that glucocorticoids in the brain are able to modulate hippocampal neuronal activity via these glucocorticoid receptors, with potential implications for regulation of the HPA axis and depression. We have tested this hypothesis by recording local field potentials in anesthetized rat hippocampus *in vivo* in response to administration of the synthetic glucocorticoid dexamethasone. Initial results demonstrate that acute administration of dexamethasone is able to alter hippocampal field potentials rapidly and drastically. We characterized this rapid response and found it to mimic burst suppression firing, a characteristic pattern seen in electroencephalography. Our work will further explore the modulation of these firing patterns by glucocorticoids, potential glucocorticoid interaction with anesthetics, and the role of this signaling in chronic stress models.

11. Glutamate Receptors as Directors of Subsynaptic Nanostructure

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Our lab recently reported a novel coordination of synaptic organization between nanoclusters of proteins that establish presynaptic sites of action potential-evoked neurotransmitter release (RIM1/2) and postsynaptic nanoclusters of glutamate receptors and scaffold proteins (PSD-95). This aligned, trans-synaptic 'nanocolumn' is expected to increase synaptic strength by enhancing the probability of receptor activation during synaptic transmission, and may reveal a modular structure important for synaptic plasticity. *Crucially, the mechanism by which this alignment is established is unknown.* One unexplored explanation involves the ionotropic glutamate receptors. AMPARs and NMDARs are arranged in nanoclusters, are enriched within the nanocolumn, interact with PSD-95, and their large extracellular domains bind numerous trans-synaptic proteins found in the synaptic cleft. **We therefore hypothesize that glutamate receptors mediate trans-synaptic alignment of RIM1/2 and PSD-95 nanoclusters.** We will start by testing the effect of AMPAR and NMDAR knockdown on nanostructure. Currently, we are leveraging whole-cell patch clamp electrophysiology to validate the loss of AMPAR and NMDAR currents in miRNA-treated cultured primary dissociated hippocampal neurons. We are also testing the effect of GluA2, GluA1, and GluN1 overexpression on nanostructure. We will test the sufficiency of subsynaptic receptor positioning in dictating presynaptic protein organization by optically recruiting glutamate receptors to both central and peripheral sites within the postsynaptic density. We predict alignment of RIM1/2 nanoclusters will reflect the positioning of recruited receptors. Glutamate receptors are thought to be passive entities within the synapse; this project tests the view that they actively shape the structure of synapses and thus control their own activation.

12. Juvenile onset of stereotypy with loss of BDNF signaling in D1R expressing striatal neurons is associated with altered neuronal morphology

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Imbalance between D1- vs. D2-receptor containing medium spiny neuron (MSN) basal ganglia output-pathways is implicated in stereotyped disorders such as Tourette Syndrome (TS) and obsessive-compulsive disorder (OCD). Surprisingly, there is little information on the molecular role of MSN subtypes in stereotypy disorders. We have a mouse model with a deletion of TrkB (the BDNF receptor) in D1-MSNs (D1-Cre-flTrkB mice), in which a subset of mice display involuntary stereotypic behaviors beginning around 3 weeks of age. We first characterized repetitive behaviors in D1-Cre-flTrkB mice with stereotypy (S), or with no stereotypy (NS), and D1-Cre control mice. Complete turns, head tics, rearing, and grooming are assessed weekly from ages 3 to 8 weeks. We found that D1-Cre-flTrkB-S mice display more complete turns at all ages compared to D1-Cre-flTrkB-NS and control mice. D1-Cre-flTrkB-S mice exclusively display head tics, which decline from juvenile to adult ages. We then selectively inhibited dorsal striatum D1-MSNs using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs; AAV-DIO-HM4D(Gi)-mCherry) to evaluate if abnormal D1-MSN activity is responsible for stereotypic behaviors. Our data suggest that selective inhibition of D1-MSNs with DREADDs reduces circling behavior. To get further insight into the molecular alterations in D1-MSNs of mice with stereotypy, we crossed D1-Cre-flTrkB mice with flRiboTag (RT) mice to extract cell-type specific mRNA from D1-MSNs. We then conducted cell-type specific RNA-sequencing on D1-Cre-flTrkB-RT-NS, D1-Cre-flTrkB-RT-S and D1-Cre-RT mice. Transcriptome analysis revealed differential gene expression between all groups and gene ontology analysis highlighted significant changes in gene expression between mice with and without stereotypy for genes involved in dendritic development and synaptic function. These results led us to evaluate D1-MSN morphology. Our cell-reconstruction studies reveal decreased dendritic length and reduced spine density in mice displaying stereotypy. These alterations could support dysfunctional activity in D1-MSNs. Our ongoing work can provide novel insight into the cell subtypes and molecular mechanisms underlying stereotypy disorders.

13. Dendritic remodeling of D1 neurons by RhoA/Rho-kinase mediates depression-like behavior

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Depression alters the structure and function of brain reward circuitry. Preclinical evidence suggests that medium spiny neurons (MSNs) in the nucleus accumbens (NAc) undergo structural plasticity, however the molecular mechanism and behavioral significance is poorly understood. Here we report that atrophy of D1, but not D2 receptor containing MSNs is strongly associated with social avoidance in mice subject to social defeat stress. D1-MSN atrophy is caused by cell-type specific upregulation of the GTPase RhoA and its effector Rho-kinase. Pharmacologic and genetic reduction of activated RhoA prevents depressive outcomes to stress by preventing loss of D1-MSN dendritic arbor. Pharmacologic and genetic promotion of activated RhoA enhances depressive outcomes by reducing D1-MSN dendritic arbor and is sufficient to promote depressive-like behaviors in the absence of stress. Chronic treatment with Rho-kinase inhibitor Y-27632 after chronic social defeat stress reverses depression-like behaviors by restoring D1-MSN dendritic complexity. Taken together, our data indicate functional roles for RhoA and Rho-kinase in mediating depression-like behaviors via dendritic remodeling of NAc D1-MSNs and may prove a useful target for new depression therapeutics.

14. Elucidating the role of the IC-vBNST projection in cue-reward associations

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The National Institute on Drug Abuse cites that drug relapse occurs in 40-60% of addicts. An addict's surrounding environment is littered with salient cues leading to intense drug cravings increasing the likelihood of renewed drug taking. Neuronal activity within the insular cortex (IC) is implicated in processing cues associated with drug delivery making this site a potential target for addiction research. Although the insula is connected to numerous brain regions, its projection to the bed nucleus of the stria terminalis (BNST) remains particularly interesting due to the BNST's connectivity with brain reward centers. For example, the BNST acts as a critical relay station for the ventral tegmental area (VTA), by increasing dopaminergic activity through drive on GABAergic neurons within the VTA resulting in the disinhibition of dopamine neuron activity and enhanced release. My preliminary findings indicate that the IC synapses onto ventral BNST neurons, which when optically stimulated in vivo, results in reward and reinforcement. Importantly, I found that blocking dopamine transmission significantly blunts the behavior patterns observed following IC-vBNST optical stimulation, suggesting that dopamine neurotransmission is required. Therefore, I hypothesize that reward-predictive cues activate IC projections that synapse onto projection neurons in the vBNST serving to activate the mesolimbic dopamine pathway resulting in acquisition of cue-reward associations. Currently my preliminary findings indicate that the IC is necessary for the acquisition of reward-predictive cues, here mice received IC terminal inhibition in the vBNST during a Pavlovian conditioning task and had significant reductions in conditioned responding.

15. The Molecular Crosstalk between Autophagy and Neuroinflammation following Traumatic Brain

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The autophagy-lysosomal pathway serves an important role in cellular homeostasis and protection against neurodegeneration. Autophagy has been also implicated in regulation of immune and inflammatory responses. Specifically, high levels of autophagy flux – the progress of substrates through autophagic compartments leading to their delivery and degradation in the lysosomes – are generally associated with anti-inflammatory, and inhibition of flux with pro-inflammatory phenotypes. To determine if autophagy may be involved in modulation of neuroinflammation after TBI, we assessed the levels of autophagy in resident microglia and infiltrating macrophages following moderate controlled cortical impact (CCI) in *C57Bl/6* mice. Consistent with a potential function in neuroinflammation, we observed accumulation of autophagosomes and inhibition of autophagy flux specifically in the activated microglia/macrophages. Our studies using transgenic *Cx3Cr1-GFP* microglial and *Ccr2-RFP* monocyte reporter mice demonstrated that infiltrating macrophages are affected by the block of autophagy flux to a higher degree than activated resident microglia. Autophagy impairment in the activated cells of the microglia/macrophage lineage peaked at day 3 post-CCI and persisted at least through day 7. At day 3 after CCI, the cells with inhibited autophagy reveal a mixed inflammatory phenotype, characterized by expression of both pro- and anti-inflammatory polarization markers. We hypothesize that inhibition of autophagy within these cells could promote their pro-inflammatory polarization after TBI. This is supported by our *in vitro* experiments demonstrating that inhibition of autophagy can potentiate pro-inflammatory activation induced by LPS treatment of BV2 microglial cells and *in vivo* data showing altered expression of inflammatory markers in autophagy hypomorph *Becn1*$^{+/-}$ mice post-injury.

16. Cell type and pathway-specific imaging of cannabinoid receptor modulation at cholinergic terminals

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The hippocampus is well-known as a center for memory consolidation and spatial navigation, receiving input from a wide array of neuronal circuits. Acetylcholine input to the hippocampus is critical during memory formation, with abnormal activity implicated in memory-related disorders, such as Alzheimer's disease. The endocannabinoid (eCB) system, known for modulation of marijuana's psychoactive and reinforcing aspects in the brain, is a wide neurochemical network that also plays a role in memory consolidation. We recently found dense expression of the cannabinoid type 1 (CB1) receptor on medial septal cholinergic neurons innervating the hippocampus, suggesting an important role of this pathway in mnemonic processes. Harnessing this anatomical framework, we used our recently-published transgenic mouse line bearing a selective deletion of cholinergic CB1 receptors. We tested short-term spatial memory in these animals, which showed increased function in a novel object recognition (NOR) task. NOR allows for familiarization to the environment and a set of objects before introducing a novel object in an open field, measuring rodents' innate tendency to recognize novel objects from familiar objects. To further understand cholinergic signaling in the hippocampus, we employed *in vivo* calcium imaging through miniature endoscopes to visualize longitudinal patterns of activity at CA1 pyramidal neurons, which were contrasted between experimental and control animal cohorts of both sexes. These results will elucidate the extent of the eCB system's regulation of memory function within the hippocampus and may offer insight into abnormal cholinergic activity associated with memory-related symptoms during cannabis use and memory disorders.

17. Hippocampal, nucleus accumbens and prefrontal cortical activity in the awake behaving rodent

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Major depression is a debilitating illness and a leading cause of worldwide disability. In the United States alone, it is estimated that over 7% individuals ages 12 and older suffer from major depression at any given time and 12.7% of people take antidepressant medications. Despite these numbers, the pathophysiology of depression and mechanism of action of the antidepressant effect remain poorly understood. However, across human and animal studies, the hippocampus (hip), nucleus accumbens (Nac) and prefrontal cortex (pfc) have been repeatedly implicated in the etiology and treatment of depression. These 3 regions share reciprocal connections, with both pfc and hip neurons projecting to the Nac, a critical node in the mesolimbic reward circuitry. Recently, our lab has shown that a negative allosteric modulator of a specific GABA-A receptor (GABA-NAM), highly expressed in the hip and pfc, has a rapid acting antidepressant effect in the stress based rodent model of anhedonia. I hypothesize that this GABA-NAM exerts its antidepressant effect by relieving inhibition in the hip and increasing power and coherence of the electrical oscillations in the hip and pfc projecting to the Nac. To test this hypothesis, I will record local field potentials (LFPs) from the hip, pfc and Nac in awake behaving rodents at baseline, after 14 days of chronic corticosterone treatment, and following GABA-NAM treatment. The data presented here are representative baseline recordings from these 3 brain regions from under awake and behaving conditions.

18. Group II metabotropic glutamate receptor blockade promotes stress resilience

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Stress is one of the most widely recognized risk factors for the onset and recurrence of major depression. Enhancing stress resilience may therefore be a therapeutic strategy to prevent the onset of depression in at-risk populations, or the exacerbation or recurrence of its symptoms. Group II metabotropic glutamate receptor ($mGluR_2$ and $mGluR_3$) antagonists have gained attention for their rapid-acting antidepressant actions in animal models; however, the effects of modulating these receptors on stress resilience have not been extensively studied. We assessed the effects of stress on knockout mice lacking either $mGluR_2$ ($Grm_2^{-/-}$) or $mGluR_3$ ($Grm_3^{-/-}$) via forced-swimming test (FST) immobility time, stress-induced hyperthermia (SIH), inescapable shock (IES)- and corticosterone-induced escape deficits, and chronic social defeat stress (CSDS)-induced sucrose preference deficits. Additionally, we investigated the effects of pretreatment with the $mGluR_{2/3}$ antagonist LY341495 or agonist LY379268 on IES-induced escape deficits and SIH. We show that $Grm_2^{-/-}$, but not $Grm_3^{-/-}$, mice are more resilient to stress in all included behavioral tests, compared to WT controls. Further, blockade of $mGluR_{2/3}$ during stress enhances resilience (reduced IES-induced escape deficits and attenuated SIH), while activation of these receptors at the same time point increases susceptibility. Finally, we demonstrate that pretreatment with LY341495, up to 3 days before stress, prevents the onset and recurrence of IES-induced escape deficits. Using both pharmacological and genetic manipulations, our results demonstrate that the activity of $mGluR_{2/3}$ can bidirectionally modulate stress resilience. Moreover, these data demonstrate that $mGluR_2$ antagonists may be protective against stress-induced changes which underlie susceptibility to depression.

19. 7B2 chaperone knockout in APP model mice results in reduced plaque burden

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Impairment of neuronal proteostasis is a hallmark of Alzheimer's and other neurodegenerative diseases. However, the underlying molecular mechanisms leading to pathogenic protein aggregation, and the role of secretory chaperone proteins in this process, are poorly understood. We have previously shown that the neural-and endocrine-specific secretory chaperone 7B2 potently blocks *in vitro* fibrillation of Aβ42. To determine whether 7B2 can function as a chaperone *in vivo,* we measured plaque formation and performed behavioral assays in 7B2-deficient mice in an hAPPswe/PS1dE9 Alzheimer's model mouse background. Immunocytochemical analysis of cortical levels of thioflavin S- and Aβ-reactive plaques showed that APP mice with a partial or complete lack of 7B2 expression exhibited a significantly lower number and burden of thioflavin S-reactive, as well as Aβ-immunoreactive, plaques. However, 7B2 knockout did not affect total brain levels of either soluble or insoluble Aβ. While hAPP model mice performed poorly in the Morris water maze, brain 7B2 levels did not impact performance. Since 7B2 loss reduced amyloid plaque burden, we conclude that brain 7B2 can impact Aβ disposition in a manner that facilitates plaque formation. These results are reminiscent of prior findings in APP model mice lacking the ubiquitous secretory chaperone clusterin.

20. Metabolism of [1,6-^{13}C]glucose in the cerebellum of 18 day old male and female rats: Comparison with cerebral metabolism

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The cerebellum plays an integrative role in brain, supporting motor behavior and learning, cognition and affective states. We performed *ex vivo* studies of cerebellar and cortical metabolism in brains from 18 day old rats 30 minutes after i.p. injection of [1,6-^{13}C]glucose. The concentrations of creatine, myo-inositol and glutamate were higher in cerebellum than in cerebrum; whereas, concentrations of N-acetylaspartate (NAA), GABA, lactate and aspartate were lower. Incorporation of label from metabolism of [1,6-^{13}C]glucose into glutamate, GABA and glutamine were lower in cerebellum than in cerebrum. However, overall labeling of glutamine relative to glutamate was higher in cerebellum than in the cerebrum. Formation of GABA from precursors synthesized via the pyruvate carboxylase pathway in astrocytes was higher in cerebellum than cerebrum. These findings underscore the relatively high astrocyte metabolism in cerebellum at PND 18 and marked contribution of glial metabolism to GABA formation in this brain region.

21. Identification of a novel brain-permeable inhibitor of kynurenine 3-monooxygenase

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Kynurenine 3-monooxygenase (KMO) converts kynurenine to 3-hydroxykynurenine (3-HK) and thus modulates the levels of several neuroactive metabolites of the kynurenine pathway, the major catabolic route of the essential amino acid tryptophan. KMO is therefore a possible drug target for neurodegenerative and neuroinflammatory diseases, with inhibition of the enzyme believed to be therapeutically desirable (Zwilling et al., 2011). However, no brain-penetrant KMO inhibitors have been identified so far.

Using a well-established assay (Sathyasaikumar et al., 2011), the novel brain penetrant KMO inhibitor (compound 1) and its prodrug (compound 1b) were first tested for their ability to inhibit rat liver KMO activity in vitro. Next, we measured the concentration of compound 1 into the mouse brain following an intravenous injection of 1 and 1b (n=6). Finally, using the method described by Guidetti et al., 1995, we examined the ability of intravenously injected compound 1b to attenuate the neosynthesis of [3H]-3-HK from [3H]-kynurenine in the rat striatum in vivo.

Compound 1 potently inhibited rat liver KMO activity (IC50 ~100 nM), while compound 1b was approximately 20,000 times less potent (IC50 >2 mM). Next, we observed compound 1 to be poorly brain penetrant. In contrast, compound 1b crossed the blood-brain barrier quite effectively and was rapidly converted into compound 1 in the brain. The de novo production of [3H]-3-HK was significantly reduced in animals receiving compound 1b [from 3.4 ± 0.7 % in controls (n = 9) to 1.1 ± 0.3 % (n = 7) of applied [3H]-kynurenine; $p < 0.05$ by paired t-test].

Our study provides evidence for the efficacy of the first brain-penetrable KMO inhibitor. Further development of this compound may enhance the therapeutic options for patients suffering from neurodegenerative and neuroinflammatory diseases such as Alzheimer's, Parkinson's, and Huntington's disease.

22. Sensorimotor Peak Alpha Frequency relationship to regions across the brain is modulated by pain

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Objective markers of pain sensitivity represent a key tool for identifying individuals who may be at high risk for developing chronic pain. Sensorimotor peak alpha frequency (PAF), the frequency band within the 8-12 Hz alpha range, may represent one such marker; previous work from our lab has shown that EEG-recorded sensorimotor PAF could predict reported pain intensities to a painful event occurring either 45 minutes or 4 days later. Understanding how sensorimotor PAF modulates brain activity represents an important avenue for increasing understanding of the mechanisms that generate individual differences in pain sensitivity. To address this question we use combined EEG-fMRI (n=11) to probe the relationship between sensorimotor PAF and brain activity during a baseline, eyes-closed resting state scan and a capsaicin heat-pain, eyes-closed resting state scan. Following EEG artifact correction, we extracted PAF from electrodes over sensorimotor cortex and created a PAF timeseries by calculating PAF within 2 second epochs that matched the MRI TR (237 time steps for each scan). We convolved this PAF timeseries with a canonical HRF and treated it as a regressor, predicting each voxel's timeseries. At baseline, PAF positively predicted fMRI in supramarginal gyrus, sensorimotor cortex, cuneus, thalamus, and negatively in subgenual anterior cingulate cortex (ACC), precuneus, and lateral prefrontal cortices. These effects tended to be small to moderate. After inducing capsaicin heat-pain, PAF predicting a novel pattern of brain regions, with a strong increase in prediction to the intraparietal sulcus, the dorsal premotor cortex, and the dorsal ACC, all regions involved in attention and cognitive control. After capsaicin, PAF negatively predicted fMRI in angular gyrus, precuneus, and lateral prefrontal cortex with effect sizes largely equivalent to baseline. In conclusion, we provide analysis from a pilot study relating PAF to spontaneous fMRI signals across the brain and demonstrate the feasibility of this approach. Our results suggest that sensorimotor PAF coupling across the brain is altered by pain, giving a possible mechanism through which PAF affects pain sensitivity.

23. Small Ankyrin 1 may regulate SERCA activity in astrocytes but not in neurons

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Intercellular calcium (Ca^{2+}) regulation is very important for brain function. Ca^{2+} reuptake into the endoplasmic reticulum (ER) is associated with several diseases, including Alzheimer's and Huntington's diseases. Ca^{2+} reuptake into the ER in neurons and astrocytes is mediated by sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA). Our laboratory has recently shown that SERCA activity can be mediated by a small transmembrane protein, small ankyrin 1 (sAnk1), in skeletal muscle.
Here we show that sAnk1 may also regulate SERCA activity in astrocytes but not in neurons. We have used qPCR and western blot to show sAnk1 and SERCA expression in the CNS. Immunocytoshemistry (ICC) in primary hippocampal cultures (kindly provided by the Blanpied laboratory) showed no localization of sAnk1 and SERCA. This was confirmed by examining single neurons transfected to express gCamp. Neurons filled with gCamp stained for SERCA not for sAnk1 . Hippocampal cultures labeled for sAnk1, SERCA, and markers for neurons and astrocytes (NeuN and GFAP respectively) showed sAnk1 expression only in astrocytes. Therefore, sAnk1 may inhibit SERCA activity in astrocytes, showing a distinct regulation of Ca^{2+} reuptake in astrocytes compared to neurons. These findings will be tested further in studies of isolated primary neurons and astrocytes and by co-immunoprecipitation of sAnk1 and SERCA in astrocytic cultures, compared to neuronal cultures, and by Ca^{2+}-ATPase assays.

24. Elucidating the role of mitochondrial dysfunction in Amyotrophic Lateral Sclerosis pathogenesis

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Amyotrophic Lateral Sclerosis (ALS) is a debilitating neurodegenerative disorder that induces fatality through the loss of upper and lower motor neurons, which innervate vital muscles throughout the body. Mutations in UBQLN2 and TBK1 have been found to cause ALS with frontotemporal dementia. Both of these proteins are vital for maintaining proteostasis; TBK1 phosphorylates autophagy adaptors to ensure proper cargo recruitment into autophagosomes and UBQLN2 links ubiquitinated misfolded proteins to autophagic and proteasomal degradation systems. These functions help to deter the accumulation of protein aggregates, which is a hallmark of ALS pathology. But, while proteostasis is a prominent disease mechanism, ALS is a multifactorial disorder, which has been linked to alternative diseases mechanisms, such as mitochondrial dysfunction. Using a UBQLN2 ALS mouse model established in our lab, spinal cord and hippocampal tissues from WT UBQLN2 and a P497S UBQLN2 ALS mutant were used to generate a large-scale proteomics profile. Proteomic comparison of WT and mutant spinal cord tissues indicated a systematic downregulation of mitochondrial proteins in the spinal cords of the mutant. The role of mutations in UBQLN2 and TBK1 in impacting mitochondrial structure and function has yet to be elucidated. Using CRISPR-Cas9, we generated stable knock-out cell lines of TBK1 and UBQLN2. Preliminary experiments using Agilent Seahorse XF analyzers suggest that many features of mitochondrial function, such as respiration and coupling efficiency, are attenuated in the knock-outs. Future studies pairing our generated stable cell lines and isolated mitochondria from spinal cord tissues of our ALS mice with electron microscopy, mitochondrial oxidative stress tests, and biochemical protein-protein interaction experiments will help to elucidate the potential pathological interactions between mutated UBQLN2 and TBK1 and mitochondria.

25. NMDA receptor inhibition is not a determinant for the antidepressant effects of the ketamine metabolite (*2R,6R*)-hydroxynorketamine

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Preclinical studies indicate that (*2R,6R*)-hydroxynorketamine (HNK) is a fast-acting antidepressant devoid of ketamine's untoward behavioral side effects. Although NMDA glutamate receptor (NMDAR) inhibition has been proposed to underlie ketamine's antidepressant effects, its contribution to (*2R,6R*)-HNK's antidepressant effects remains to be completely elucidated. We systematically assessed the effects of (*2R,6R*)-HNK, compared to ketamine, on NMDAR activity. (*2R,6R*)-HNK concentrations following an antidepressant-relevant dose of 10 mg/kg were measured in the extracellular compartment of the hippocampus (using microdialysis), whole brain tissue, and plasma of mice. The effectiveness of ketamine or (*2R,6R*)-HNK to prevent NMDA-induced lethality in mice was examined. The effects of ketamine and (*2R,6R*)-HNK were evaluated, *in vitro*, on: (i) NMDAR-mediated field excitatory postsynaptic potentials in the mouse hippocampus, (ii) NMDAR-mediated miniature excitatory postsynaptic currents in rat CA1 pyramidal neurons, (iii) NMDA-evoked currents in CA1 pyramidal neurons, and (iv) recombinant GluN1/2A, GluN1/2B, GluN1/2C, and GluN1/2D NMDARs expressed in *Xenopus* oocytes. (2S,6S)-HNK was also tested in mouse hippocampus NMDAR-mediated fEPSPs and *Xenopus* oocytes. The antidepressant dose of (*2R,6R*)-HNK generated maximal concentrations of approximately 10 µM in all tissue. *In vivo* and *in vitro*, ketamine inhibited NMDAR-mediated responses with 10-20-fold greater potency than (*2R,6R*)-HNK, while the (*2S,6S*)-HNK was more potent than (*2R,6R*)-HNK, but less potent than ketamine. These data suggest that NMDAR inhibition is unlikely to contribute to the (*2R,6R*)-HNK's antidepressant behavioral effects. NMDAR inhibition is a major determinant of ketamine's undesirable side-effects, and thus (*2R,6R*)-HNK, and next generation drugs sharing similar pharmacodynamics, may be better tolerated than ketamine.

26. Microglial phagocytosis of newborn cells sculpts the cellular composition of the neonatal rat amygdala in a sex dependent manner

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The amygdala, a sexually dimorphic brain region, mediates a conserved male bias in juvenile social play behavior. This sex difference arises following sexual differentiation of the amygdala, in which males exhibit fewer newborn cells shortly after birth. We hypothesized that microglia may underlie this sex difference, as microglia are significantly more phagocytic in the male amygdala during this timepoint. Results from immunohistochemical analysis support this, as the majority of Iba1-labeled microglial phagocytic cups in both sexes co-label with NucRed, a DNA-binding dye, and PCNA, a marker of recently divided cells, indicating microglia actively phagocytose newborn cells in the developing amygdala. To explore how this sex difference affects the amygdala's architecture later in life, we treated pups with BrdU on postnatal day 0 (PN0) to PN4 to label newborn cells and sacrificed at PN26. In the medial amygdala (MeA), the site of masculinization of play, BrdU+ cells in both sexes predominantly co-labeled with GFAP, an astrocyte marker. Females had a higher density of BrdU+ cells including a higher density of GFAP+/BrdU+ cells in the posterodorsal MeA. We then hypothesized that microglia generate the sex difference in postnatally-born cells by phagocytosing cells fated to become astrocytes. Indeed, in the PN4 amygdala, microglial phagocytic cups were enriched for ALDH1L1, an astrocyte-specific marker, and a greater percentage of cups co-labeled with ALDH1L1 in males. Together, these data indicate that microglia produce developmental sex differences by phagocytosing newborn cells, particularly astrocytes, in the amygdala, sculpting sex differences in neural circuitry with relevance for social play.

27. Post-translational Activation of Glucokinase in Glucosensing Hypothalamic Neurons

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Glucokinase (GCK) is the rate-limiting enzyme for glucose metabolism in glucosensing tissues, including hypothalamic nuclei, and is post-translationally regulated by signaling pathways in the liver and the pancreas; yet it is unclear if similar regulation of occurs in the hypothalamus. **Using the hypothalamically-derived, glucosensing GT1-7 cell line, the existence of a ligand-stimulated intracellular signaling cascade that culminates in the S-nitrosylation and activation of GCK is demonstrated.** Glucokinase activity is assessed by either measuring NAD(P)H autofluorescence while raising extracellular glucose, or through expression of a GCK FRET biosensor. Treatment of GT1-7 cells with isoproterenol (ISP), a G_s GPCR agonist, augments a glucose-dependent rise in NAD(P)H autofluorescence, and activates the GCK biosensor. These effects of ISP are impeded by the application of the nitric oxide synthase inhibitor, L-NAME. Additionally, incorporation of an S-nitrosylation-blocking V367M mutation into the biosensor prevents its activation by ISP. In contrast, treatment of GT1-7 neurons with the nitric oxide donor, SNAP, enhances a glucose-dependent rise in NAD(P)H autofluorescence and activates the GCK biosensor. Further, the potentiation of glucose metabolism by treatment with ISP is also disrupted by the application of either 2-APB, an IP3R channel blocker, or a high concentration of Ryanodine, which blocks RyRs. Contrarily, the effect of ISP on glucose metabolism persists in the absence of extracellular calcium. **Broadly, these results provide a mechanism by which neurotransmitters might regulate hypothalamic glucosensing.**

28. Regulation of NMDA receptor activation following spontaneous glutamate release

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NMDA receptor (NMDAR) activation due to spontaneous release of neurotransmitter plays a critical role in both maintenance and modification of synaptic strength. Because Ca^{2+} influx through NMDA receptors gates multiple signaling cascades at the postsynaptic density (PSD), the mechanisms that regulate receptor activation are important to delineate. However, the sources of variability between synapses in NMDAR activation by spontaneous release are not clear. Here, we asked what NMDAR subtype mediates the majority of response to spontaneous neurotransmitter release, and whether structural aspects of the synapse contribute to inter-synaptic variability.

To isolate NMDA receptor-mediated Ca^{2+} influx, we imaged GCaMP6f in cultured hippocampal neurons (21 to 35 DIV) in the presence of TTX, 0 Mg^{2+}, ryanodine, DNQX, and thapsigargin, allowing detection of miniature spontaneous Ca^{2+} transients (mSCaTs) in single spines. The GCaMP6f response reflected the total NMDAR-mediated Ca^{2+} influx, since mSCaT amplitude was modulated bidirectionally by altering extracellular [Mg^{2+}] or [Ca^{2+}]. Amplitude and frequency of mSCaTs were remarkably variable between and within synapses. A low concentration of the high-affinity antagonist CPP strongly decreased event frequency, but prompted a much smaller reduction in amplitude. Importantly, in essentially all synapses, responses were at least partially blocked by the GluN2B-specific antagonist ifenprodil, and in many, mSCaTs were eliminated. Thus, in these cells, very few NMDARs are activated by spontaneous release, and the majority of these contain GluN2B.

We next asked whether synapse size or subcellular position contribute to the magnitude of NMDAR activation. We measured spine area, and in order to obtain a highly resolved measurement of PSD area, we performed live or post hoc correlative super-resolution imaging of PSDs following Ca^{2+} imaging of the same synapses. Neither spine area nor PSD area correlated with NMDAR activation, revealing that NMDAR activation is independent of synapse size. Additionally, we found no evidence that NMDAR activation was affected by synapse distance or number of branch points from the soma.

These data suggest that following spontaneous glutamate release, the magnitude of spine Ca^{2+} elevation is highly variable despite the low number of NMDA receptors activated per release event. This variance may arise principally from the stochastic properties of channel opening rather than the many other factors that contribute to the number of activated receptors. Additionally, this reveals a novel role of GluN2B-NMDARs in responding to spontaneous release at individual hippocampal synapses.

29. Development of a custom multi-site fiber photometry system for monitoring claustrum activity in freely behaving mice

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The claustrum is a prominent telencephalic nucleus of unknown function that is estimated, by volume, to be the most highly connected structure in the mammalian brain. Elucidating claustrum function thus represents a major challenge in neuroscience. The hurdles standing in the way of claustrum functional description are: 1) the elongated, ribbon-like shape of the claustrum and; 2) the proximity of the claustrum to white matter structures. To surmount this, a method to monitor claustrum activity is required that: 1) selectively detects claustrum activity; 2) allows for freely moving behavior and; 3) is devoid of movement artifacts. To this end, we herein describe a custom multi-site fiber photometry system that controls for motion artifacts from photometry fiber commutation, background autofluorescence, and laser power fluctuations. We employed mice expressing cre recombinase selectively in claustrum projection neurons. To test a role for the claustrum in top-down cognitive control of action, we used the cognitively-demanding five-choice response task and the simpler one-choice response task as a control while we monitored claustrum activity. Claustrum activity increased prior to an expected cue on the five-choice response task, but not on the one-choice response task. Dynamic encoding by the claustrum leading up to reward acquisition was also observed. Optogenetic claustrum inhibition prior to the expected cue decreased performance accuracy on the five-choice, but not the one-choice task. These data suggest that fiber photometry is an effective means to unlocking claustrum function and support the notion that the claustrum contributes to top-down cognitive control.

30. Reduced ventral striatum enkephalins regulate depression-like phenotype to social defeat stress and enhances vulnerability to stress in a Huntington's Disease model

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Enkephalins are primary endogenous ligands for delta opioid receptors (DORs) and enriched in D2-medium spiny neurons in the striatum. Enkephalins are highly implicated in depression but their exact role in depression is not fully investigated. To provide insight into enkephalin function we used chronic social defeat paradigm, after which the mice were either categorized as susceptible (depression-like) or resilient to stress based on their performance in a social interaction test. We also used the zQ175 knock-in mouse (zQ175), an animal model of Huntington's disease in which comorbidity with mood disorders is common. In our study, zQ175 showed enhanced susceptibility to a subthreshold defeat stress that does not induce phenotype to wildtype littermates. Compared to the controls, the susceptible mice and defeated zQ175 showed reduced enkephalin levels in the ventral striatum. To determine if the reduced enkephalin levels cause depression-like behavior through disrupted DOR signaling, we infused DOR agonist SNC80 into the ventral striatum of the animals that experienced chronic defeat stress. Our results indicate that SNC80 can reverse the depression-like behavior in susceptible animals. To investigate the mechanisms that reduce enkephalins in depression-like conditions, we analyzed levels of enzymes that can degrade or produce enkephalins. We demonstrate that the decreased enkephalins may be attributed to increased levels of enkephalinases and reduced proprotein convertase 1 in susceptible animals. Overall, our data implicate that behavior induced by defeat stress is caused by reduced DOR signaling resulting from lowered levels of enkephalins, which may be mediated through elevated enkephalinases and decreased proprotein convertases.

31. Phagoptosis by microglia determines the size of the sexually dimorphic nucleus (SDN) of the POA

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The largest neuroanatomical sex difference in the mammalian brain was found in the preoptic area (POA) and named the sexually dimorphic nucleus (SDN) due to its larger size in males compared to females. Our lab has demonstrated that the immune cells, microglia, are essential for the masculinization of spine density on POA neurons, as well as male sexual behavior. We discovered that the female POA contains more phagocytic microglia than males during the first postnatal week. Inhibition of microglial phagocytosis with minocycline increased the volume of the SDN in both males and females indicating that microglia are engaging in phagoptosis (engulfment of viable cells) to shape the size of the SDN. This discovery, coupled with the known neuroprotective role of estradiol, predicts that estradiol suppresses microglial phagoptosis in the male SDN and microglia initiate phagoptotic events in females, in the absence of estradiol. Further studies are underway to determine the impact of estradiol on phagoptosis and the specific cellular targets being engulfed in the developing SDN. The experiments proposed challenge the dogma that estradiol prevents neuronal apoptosis in the male SDN; and could reveal novel hormone and neuroimmune mechanisms that regulate phagoptotic and neuroprotective cascades during normal brain development.

32. Dynamic control of synaptic substructure and function by adhesion molecules

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Recent work indicates that one critical factor likely to control synaptic strength is the nanoscale organization of proteins within the synapse. Our lab discovered that a protein called Rab3 Interacting Molecule (RIM), which is essential for neurotransmitter release, is clustered into ~100 nm subdomains within the active zone, and that vesicle exocytosis preferentially occurs where there is a higher subsynaptic density of RIM. Furthermore, these presynaptic sites of neurotransmitter exocytosis are aligned with postsynaptic nanoclusters of receptors, which has major implications for the regulation of receptor activation and synaptic efficacy through the subsynaptic positioning of receptors. Though many mechanisms may contribute to the trans-synaptic alignment of receptors to sites of release, a particularly attractive model is that synaptic cell adhesion molecules mediate alignment through high-affinity trans-synaptic protein binding. Leucine Rich Repeat Transmembrane neuronal (LRRTM2) participates in trans-synaptic protein binding with major pre- and postsynaptic scaffold molecules. Using an acute manipulation in a knockdown-rescue system and measured by dSTORM, we found that acute disruption of LRRTM2 results in rapid reduction in the nanoscale alignment of proteins at synapses. Furthermore, we performed whole-cell patch clamp of cultured hippocampal neurons and found that acute cleavage of LRRTM2 results in a substantial decrease in the evoked AMPAR-mediated EPSC amplitude. Together, these findings provide experimental support for the idea that trans-synaptic nanoscale organization plays an important role in maintaining synaptic strength. A structural role played by one or more specific cleft proteins provides further evidence for a molecularly guided “nanocolumn” architecture within the synapse.

33. Engineering Optogenetic Control of mTOR signaling

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As a critical hub of multiple growth signaling and metabolic pathways, mTOR responds with complex temporal and spatial properties that are critical to growth, proliferation, and oncogenic potential. The ability to perturb dynamic cellular and molecular processes like mTOR in vivo with spatio-temporal precision presents a significant methodological challenge and a valuable experimental asset for multiple fields of biomedical research. However, traditional pharmacological and genetic approaches lack the ability to target specific cellular subpopulations or subcellular areas. To address this technological gap, we are developing optogenetic tools for light-dependent inhibition and activation of mTOR. This novel toolset will allow us to probe the asymmetric growth dynamics in neurons and metastatic potential of tumors.

34. Hydroxynorketamine acts presynaptically in order to rapidly and selectively augment glutamatergic transmission in discrete hippocampal subfields

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There is preclinical evidence that the rapid antidepressant properties of ketamine arise from the pharmacological actions of its metabolite, (*2R,6R*)-hydroxynorketamine (HNK). However, in contrast to ketamine, (*2R,6R*)-HNK fails to inhibit the *N*-methyl-D-aspartate receptor (NMDAR) at concentrations relevant to its antidepressant effects, making its mechanism of action unclear. The aim of the current study was to examine the synaptic effects of (*2R,6R*)-HNK within discrete hippocampal subfields, with the hypothesis that rapid antidepressant compounds exert their effects by selectively enhancing glutamatergic transmission. Bath application of (*2R,6R*)-HNK enhanced Schaffer collateral (SC)-CA1 field excitatory postsynaptic potentials (fEPSPs) in the ventral hippocampus. This potentiation was insensitive to NMDAR inhibition, but completely eliminated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) blockade. Using an extracellular dual recording configuration, we show that the magnitude of this potentiation is comparable between proximal and distal dendrites in the stratum radiatum of ventral hippocampus, and between distal dendrites of ventral and dorsal hippocampus. However, in these same slices, (*2R,6R*)-HNK failed to induce a potentiation of fEPSPs when stimuli were delivered to temporoammonic (TA) afferents in the stratum lacunosum-moleculare of CA1. An increase in the probability of release, as evidenced by a decrease in the paired-pulse ratio, occurred under all conditions of (*2R,6R*)-HNK potentiation, suggesting that (*2R,6R*)-HNK acts presynaptically in order to selectively augment AMPAR-mediated synaptic transmission. Ongoing experiments are testing the involvement of distinct presynaptic calcium channels, whose contribution to vesicular release varies between SC-CA1 and TA-CA1 synapses. Additional processes that regulate presynaptic membrane depolarization and neurotransmitter release are also being actively investigated.

35. Identifying specific miswiring endophenotypes in neurodevelopmental disorders using CRISPR

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Aberrations in the CNS during development can cause cortical circuit miswiring. These abnormalities have been linked to several human neurodevelopmental and psychiatric disorders, including autism, epilepsy, and schizophrenia. Many gene mutations have already been identified as the underlying genetic etiopathologies and the list of new gene candidates is expanding at a rapid pace. In order to effectively test new mutations and identify specific endophenotypes, we have developed a genetic screen workflow that allows us to quickly test gene candidates in vivo. This workflow includes key technologies including: 1) Golden Gate Assembly for CRISPR construct creation, 2) in utero mosaic genome editing for internally controlled knockouts, and 3) tissue clearing for imaging native fluorescence in whole brains. With this fast turnaround method, we are able to identify miswiring endophenotypes along with novel molecular pathways that are involved in cortical development.

36. USP24 negatively regulates autophagy through the ULK1 and type III PI3-kinase pathway

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Autophagy is a lysosome-dependent intracellular degradation pathway essential for neuroprotection. Molecular defects in autophagy are linked to neurodegenerative diseases, including Parkinson's disease (PD) but the mechanisms causing its disruption are not fully understood. The deubiquitinating enzyme *USP24* is located on chromosome 1 in the *PARK10* locus associated with late-onset PD and was identified as a negative regulator of autophagy by our lab. We confirmed increased USP24 protein and mRNA levels in the substantia nigra of a subpopulation of idiopathic PD patients. In human cell lines and iPS cell derived dopaminergic neurons, *USP24* knock-down led to up-regulation of cellular autophagy flux, assessed by increased LC3-II levels and by lysosomal translocation of the mCherry-GFP-LC3 autophagy reporter. To determine where *USP24* functions in the autophagy pathway we studied its effect on the upstream regulators of autophagy. *USP24* knock-down caused accumulation of PtdIns3P (type III PI3-kinase product), demonstrated by quantification of the FYVE-dsRed reporter. Inducing autophagy by loss of *USP24* function was attenuated in the presence of type III PI3-kinase inhibitors. Furthermore, *USP24* knock-down lead to ULK1 protein stabilization and increased ULK1 activity. Our data suggests that *USP24* alters ULK1 protein stability, potentially by impacting ubiquitination. Together our data demonstrate that *USP24* regulates autophagy via ULK1 and the type III PI3-kinase pathway. Interestingly, *USP24* knock-down enhanced long-term survival and increased neurite length of iPS cell derived dopaminergic neurons, suggesting potential neuroprotective function. Our data highlight the mechanisms of *USP24* in regulation of autophagy and its potential role in PD.

37. Glutamatergic Ventral Pallidal Neurons Modulate Activity of the Habenula-Tegmental Circuitry and Constrain Reward Seeking

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The ability to appropriately integrate and respond to rewarding and aversive stimuli is essential for survival. The ventral pallidum (VP) plays a critical role in processing both rewarding and aversive stimuli. However, the VP is a heterogeneous structure, and how VP subpopulations integrate into reward networks to modulate these behaviors is unknown. We identified a non-canonical population of glutamatergic VP neurons that play a unique role in responding to aversive stimuli and constraining inappropriate reward seeking. Using neurochemical, genetic, and electrophysiology approaches, we characterized glutamatergic VP neurons. We performed patch clamp and in vivo electrophysiology recordings in the lateral habenula, rostromedial tegmental nucleus, and ventral tegmental area to determine the effect of glutamatergic VP neuron activation in these regions. Finally, we optogenetically stimulated glutamatergic VP neurons in a real-time place preference task and ablated these neurons to determine their necessity for reward seeking. Glutamatergic VP neurons exhibit little overlap with the canonical VP subtypes and show distinct membrane properties. Glutamatergic VP neurons innervate and increase firing activity of the lateral habenula, rostromedial tegmental nucleus, and gamma-aminobutyric acidergic ventral tegmental area neurons. While nonselective optogenetic stimulation of the VP induced a robust place preference, selective activation of glutamatergic VP neurons induced a place avoidance. Viral ablation of glutamatergic VP neurons increased reward responding and abolished taste aversion to sucrose. Glutamatergic VP neurons constitute a non-canonical subpopulation of VP neurons. These neurons increase activity of the lateral habenula, rostromedial tegmental nucleus, and gamma-aminobutyric acidergic ventral tegmental area neurons and constrain reward seeking.

38. Characterizing the dopaminergic activity in a mouse model of mania

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Bipolar disorder is characterized by episodes of depression, euthymia, and mania. A mouse model with a dominant negative mutation in the Clock gene (ClockΔ19) exhibits phases that mimics the manic and euthymic phase of bipolar disorder, locked to the time of the day. While previous studies have shown an increased firing frequency of VTA dopaminergic neurons in ClockΔ19 mice during the manic phase, little is known about the underlying mechanisms of these changes and how it affects the manic behavioral profile.
In this study, we aimed to characterize the excitatory and inhibitory input to the VTA dopaminergic neurons in vitro. We further aimed to investigate the VTA dopaminergic firing activity in freely behaving wild type or ClockΔ19 mice during a Pavlovian reward task. We found a decrease in the frequency but an increase in amplitude of spontaneous excitatory and inhibitory inputs to the VTA dopaminergic neurons in the ClockΔ19 mice. Additionally, we found an increased basal firing activity of VTA dopaminergic neurons in vivo of ClockΔ19 mice. Surprisingly, we found a seemingly decreased sensitivity to sucrose reward of ClockΔ19 mice in the Pavlovian reward task which seemed to be associated with a higher sensitivity to other distracting stimuli. Furthermore, we found that when applying high-frequency optical stimulation to mPFC inputs to the VTA we were able to induce long-term potentiation in ClockΔ19 mice but not in wildtype mice.

39. Optogenetic Dissection of Descending 5-HT-containing Neuron Function in nociception and persistent pain conditions

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The rostral ventromedial medulla (RVM) households major descending projections to modulate nociceptive input and transmission in spinal and trigeminal dorsal horn. The misbalance between descending inhibition and facilitation from the RVM has been involved in central mechanisms of chronic pain. Although functional subpopulation of RVM neurons in descending pain modulation have been characterized, 5-HT-containing neurons are best known as certain descending neurochemical projections. Accumulating data indicate that active 5-HT-dependent descending facilitation is implicated in central sensitization underlying the maintenance of persistent pain. However, it is still less known whether activation of 5-HT-containing neurons alone in the RVM sufficiently produce behavioral pain responses or active 5-HT-containing RVM neurons are required for persistent pain after nerve injury. By combination of 5-HT transporter transgenic (SERTcre) mouse line with optogenetic technique, we specifically manipulated endogenous serotonergic activity in the RVM to dissect their roles in descending pain modulation. First, we observed opsin expression in the RVM of SERTcre mouse after microinjection of Cre-dependent AAV9-EF1a-DIO-hChR2(H134R)-mCharry. Immunostaining showed that all ChR2-mCharry-expressing cells are serotoninergic and the infected soma are restrictedly distributed in the RVM. About 80 % of 5-HT-immunorwactive neurons per section of the RVM expressed ChR2. After implanted an optical fiber precisely targeting the RVM, we examined effects of blue light stimulation to ChR2-expressing 5-HT neurons on normal nociception in the freely moving animals. Single optical stimulation for 15 min produced significant mechanical allodynia in orofacial skin or hindpaw over 24 hr and conditioned place avoidance at the next day. Robust expression of c-Fos in RVM neurons including 5-HT-containing neurons after light stimulation indicates effective excitation of RVM 5-HT system. In addition, molecular depletion of 5-HT from 5-HT-containing RVM neurons with local Tph-2 shRNA could prevent blue light–induced behavioral hypersensitivity. These results confirm previous finding that selectively transient activation of 5-HT-containing RVM neurons in normal animals produces a prolonged pain behavior. Surprisingly, we did not find a wide and robust c-Fos expression in the spinal dorsal horn, along with restricted expression in the mid of the superficial layers of the dorsal horn. Next, we investigated the effects of inhibition of 5-HT-containing neurons in the RVM on trigeminal neuropathic pain induced by CCI-ION. Otogenetic inhibition of RVM 5-HT-containing neurons with intra-RVM injection of AAV9.hSyn.eNpHR3.0-eYFP attenuated behavioral hypersensitivity strongly at 14 d but less at 5d after CCI, further supporting our previous finding that active 5-HT-dependent descending facilitation is responsible to the transition of acute pain to chronicity.