The Program in Neuroscience presents:

22nd Annual Retreat

2019
Cover Image

Cell from a dissociated hippocampal neuronal culture expressing a fluorescent protein tagged probe (magenta) and stained for PSD-95, a postsynaptic marker (cyan/white). By Poorna Dharmasri, Doctoral candidate, Blanpied Lab

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:

https://lifesciences.umaryland.edu/Neuroscience/
PIN Gratefully Acknowledges

- Mary Kay Lobo for her outstanding service as the new Director of Graduate Education
- Donna Calu for chairing the Retreat Committee
- Marta Lipinsiki for 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 PIN Twitter feed.
  
  Follow us at @UMMedNeuro

- Quinton Banks, Ashley Marquardt, Amanda Labuza and Sam Bacharach 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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* student representatives
Acknowledgement for Educators

Faculty Course Directors:
- Tom Blanpied
- Mordecai Blaustein
- Joe Cheer
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- Bruce Krueger
- Mary Kay Lobo
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- Brian Polster
- Adam Puche
- Mark Rizzo
- Dennis Sparta
- Danny Weinreich

Volunteer Educators:
- Tara LeGates
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- Ramesh Chandra
- Cali Calarco
- Eldin Jasarevic
- Aaron Levy
- Katie Morrison
- Hyungwoo Nam
- Kate Peters
- Ryan Richardson
- Jon VanRyzin
- Natalie Zlebnik
- Sarah Metzbower
- Poorna Dharmasri
- Andrew Furman
- Kasey Girven
- Ashley Marquardt
- Austin Ramsey
## 22nd Annual Neuroscience Retreat

**Wednesday, June 5th, 2019**  
**Vollmer Center, Cylburn Arboretum**  
**Baltimore, MD 21209**

### Schedule

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| 8:30-9:00    | **Registration**  
(Coffee provided)                                        |
| 9:00-9:30    | **Introductory Remarks** (Mary Kay Lobo & Donna Calu)               |
| 9:30-10:30   | **Research Talks:**                                                  |
|              | **Hyungwoo Nam** (Research talk)                                     |
|              | “Reduced nucleus accumbens enkaphalins underlie vulnerability to social defeat stress” |
|              | **Kara Cover** (3-minute thesis)                                     |
|              | “Contribution of the rostral intralaminar thalamic nuclei to striatal physiology and behavior” |
|              | **Lace Riggs** (Research talk)                                       |
|              | “Synaptic mechanisms underlying the rapid hippocampal plasticity induced by the (2R,6R)-hydroxynorketamine metabolite” |
|              | **Ryan Richardson** (3-minute Tech Blitz)                            |
|              | “Combinatorial organization of CRISPR/Cas9-mediated knockin yields high-performance editing in vivo” |
|              | **Kelson Shilling-Scrivo** (3-minute thesis)                         |
|              | “Structural Changes in Primary Auditory Cortex Neuronal Networks Impact Encoding Fidelity in Noisy Environments” |
|              | **Amanda Labuza** (research talk)                                    |
|              | "Small Ankyrin 1 may regulate SERCA activity in astrocytes but not in neurons" |
| 10:30-11:30  | **“Neuroscience Advocacy: An Introduction and Practicum”**          |
|              | Adam Katz, M.S.                                                      |
|              | Advocacy Engagement Manager, Society for Neuroscience               |
11:30-12:15  Activities and Exploration

12:15-1:15  Lunch

1:15-1:30  1st Year Students’ Video

1:30-2:30  Keynote Address:
          “Bridging Motor Neuroscience and Neurorehabilitation”
          Amy Bastian, PhD
          Professor of Neuroscience
          Solomon H. Snyder Department of Neuroscience
          Johns Hopkins School of Medicine
          Kennedy Krieger Institute

2:30-3:45  Poster Session
          (Beverages & snacks provided)

3:45-4:00  Awards and Retreat Closing
KEYNOTE SPEAKER:

Amy Bastian, Ph.D., PT Chief Science Officer, Director Motion Analysis Laboratory Kennedy Krieger Institute and Professor of Neuroscience and Neurology, Johns Hopkins University School of Medicine

Dr. Amy Bastian is the chief science officer at Kennedy Krieger Institute, a role in which she identifies and promotes new directions for breakthrough research into the developing brain, spinal cord, and musculoskeletal system. She is also director of the Motion Analysis Laboratory that studies the neural control of human movement. Dr. Bastian is a professor of neuroscience and neurology at the Johns Hopkins University School of Medicine.

After completing her undergraduate degree in physical therapy at the University of Oklahoma, Dr. Bastian completed her doctoral degree in movement science at Washington University in 1995, and a post-doctoral fellowship in neuroscience at Washington University under Dr. W.T. Thach. Dr. Bastian came to Kennedy Krieger Institute in Summer 2001. Prior to that, she was an assistant professor in physical therapy in the Department of Anatomy and Neurobiology at the Washington University School of Medicine in St. Louis.

Dr. Bastian’s research uses computerized movement tracking techniques, non-invasive brain stimulation, novel devices and robotics to control walking and reaching movements. She studies how people with and without neurological damage control movement and learn new patterns. Some of her recent accomplishments include a series of papers on learning new walking patterns using a novel ‘split-belt’ treadmill published in the Journal of Neuroscience, Brain, and Nature Neuroscience. She has coauthored over 100 scientific papers and numerous book chapters and served as chair of the Musculoskeletal Rehabilitation Study Section at the National Institutes of Health (NIH). Dr. Bastian has given many named lectures, including a special lecture at the Society for Neuroscience meeting in 2014. She is currently the primary investigator on two R01 grants from the NIH, one of which recently received a prestigious Javits award from the National Institute of Neurological Disorders and Stroke (NINDS). Dr. Bastian has served on many national neuroscience and rehabilitation committees.
SPOTLIGHT ON ADVOCACY:

Adam M. Katz, M.S., Advocacy Engagement Manager at the Society for Neuroscience

Adam Katz is the Advocacy Engagement Manager at the Society for Neuroscience, where he equips and empowers researchers to engage their elected officials. He previously was a Policy and Advocacy Specialist at Research!America and an intramural postbaccalaureate researcher within the National Institute of Mental Health. Katz’s main research interests lie in neural plasticity. He received his undergraduate degree in Brain and Cognitive Science from the University of Rochester and his Master’s in Policy and Advocacy from Georgetown University.
The Cylburn Arboretum was originally the private estate of businessman Jesse Tyson, who started construction of Cylburn Mansion in 1863 as a summer home for himself and his widowed mother. Cylburn Mansion was designed by George A. Frederick, who was also the architect for Baltimore’s City Hall. Victorian Renaissance Revival in design, Cylburn was built of gneiss quarried and was noted for its inlaid floors, marble baths, leaded glass, plasterwork, tall windows and wide porches. Formal gardens and lawns with trees planted by Jesse Tyson himself were surrounded by natural woodland filled with wildflowers, wildlife and native and migrating birds.

Cylburn Arboretum, located in the northwest part of the City of Baltimore, is a beautiful 207-acre urban park with 3.5 miles of easy to moderately difficult woodland trails that wind through the grounds. Cylburn is one of the largest wooded areas in Baltimore City, making it an important habitat for local plants and animal life. Mature hardwood trees representative of the piedmont make up the bulk of the forested areas, though there are some conifers. The plantings alongside the trails contain an outstanding diversity of wildflowers. Cylburn has a wide variety of beautifully planted flower gardens displaying shrubs, perennials and annuals. From the “named” gardens that honor those who have played important roles in Cylburn’s history to the colorful plantings that enhance the mansion, carriage house, and points around the grounds, each garden offers a collection of colors and textures that delight visitors throughout the year.

The Vollmer Center at Cylburn features a lobby that opens directly out onto a terrace, which leads to the arboretum. A woodland amphitheatre connects the upper lobby to the arboretum below, and to terraces at both levels. The design incorporates many sustainable features, including the use of extensive green roofs over half of the building, composting toilets, natural ventilation, geo-thermal heating and cooling, and sustainable materials where appropriate.

Cylburn is managed by Baltimore City Recreation and Parks with support from the Horticultural Society and Cylburn Arboretum Association.
Illustration by Amanda Labuza
Ventral hippocampal neuron with projections to both nucleus accumbens and medial prefrontal cortex. By Natalie Hesselgrave and Andreas Wulff, Doctoral candidates, Thompson lab.
Reduced nucleus accumbens enkephalins underlie vulnerability to social defeat stress

Hyungwoo Nam

Enkephalins are endogenous ligands for delta opioid receptors (DORs) and are enriched in D2-MSNs in the striatum. Enkephalins are implicated in depression and enkephalinase inhibitors act as antidepressants. However, the specific role of enkephalins in depression is not fully investigated. To investigate enkephalin function we used mice that received chronic social defeat stress, after which the animals were either categorized as susceptible or resilient to stress based on their social behavior. First, we measured enkephalin levels using radioimmunoassay after defeat stress. Compared to the control animals, the susceptible animals showed reduced enkephalin levels. Then we studied the mechanisms that reduce levels of enkephalins by analyzing levels of enzymes that regulate enkephalin levels. Our results indicate that mRNA levels of enkephalinases were increased and proprotein convertase levels decreased. To determine if disrupted Enk-DOR signaling can cause depression-like behavior, we infused DOR agonist SNC80 into the animals that experienced social defeat stress. SNC80 were able to reverse the depression-like behavior. Overall, our studies show that depression-like behavior induced by social defeat stress is caused by reduced DOR signaling by lowered levels of enkephalins in the NAc, which may be mediated through elevated expression of enkephalinases and decreased proprotein convertases.
Contribution of the rostral intralaminar thalamic nuclei to striatal physiology and behavior

Kara Cover

Glutamatergic projections of the thalamic rostral intralaminar nuclei (rILN) innervate the dorsal striatum and are implicated in dopamine (DA)-dependent incubation of drug seeking. However, the mechanism by which rILN signaling modulates reward seeking and striatal DA release is unknown. Using patch clamp electrophysiology and fast-scan cyclic voltammetry in acute striatal slice, we find that rILN terminal activation drives cholinergic interneuron burst firing activity and evokes DA release in a cholinergic receptor-dependent manner. In vivo, optogenetic activation of this pathway drives behavioral reinforcement in a DA D1-receptor dependent manner and chemogenetic suppression of the rILN reduces dopaminergic nigrostriatal terminal activity as measured with fiber photometry. Together, these data provide evidence that the rILN activates striatal cholinergic interneurons to enhance the pursuit of reward through local striatal DA release and introduce an additional level of complexity in our understanding of striatal DA signaling.
Synaptic mechanisms underlying the rapid hippocampal plasticity induced by the (2R,6R)-hydroxynorketamine metabolite

Lace Riggs

Preclinical studies indicate that (2R,6R)-hydroxynorketamine (HNK) retains the rapid and sustained antidepressant-like actions of ketamine, but is spared its dissociative-like properties and abuse potential. While (2R,6R)-HNK is thought to exert its antidepressant-like effects by potentiating α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)-mediated synaptic transmission, its acute mechanism of action is unknown. Here, the acute synaptic effects of (2R,6R)-HNK were examined by recording field excitatory postsynaptic potentials (fEPSPs) and miniature excitatory postsynaptic currents (mEPSCs) in rat hippocampal slices. (2R,6R)-HNK bath application caused a rapid and persistent potentiation of AMPAR-mediated Schaffer collateral (SC)-CA1 fEPSPs in slices derived from male and female rats. The (2R,6R)-HNK-induced potentiation occurred independent of N-methyl-D-aspartate receptor (NMDAR) activity, was accompanied by a concentration-dependent decrease in paired pulse ratios, was occluded by raising glutamate release probability, and was blocked by presynaptic calcium channel inhibition. Additionally, in the presence of tetrodotoxin, (2R,6R)-HNK increased the frequency, but not amplitude, of mEPSC events, confirming a presynaptic site of action that is independent of glutamatergic network disinhibition. A dual extracellular recording configuration revealed that the presynaptic effects of (2R,6R)-HNK were synapse-selective, occurring in CA1-projecting SC terminals, but not in CA1-projecting temporoammonic terminals. Overall, we found that (2R,6R)-HNK enhances excitatory synaptic transmission in the hippocampus through a concentration-dependent, NMDAR-independent, and synapse-selective increase in glutamate release probability with no direct actions on AMPAR function. The current study provides novel insight regarding (2R,6R)-HNK’s acute mechanism of action, and shows that non-canonical presynaptic forms of synaptic plasticity can be engaged by compounds with rapid-acting antidepressant potential.
Combinatorial organization of CRISPR/Cas9-mediated knockin yields high-performance editing \textit{in vivo}

Ryan Richardson

Gene knockin enables tagging and experimental manipulation of endogenous proteins while retaining native regulation of spatial and temporal expression patterns. While genome editing using CRISPR/Cas9 has emerged as an effective strategy for producing such knockins, the high incidence of on-target genome damage by undesired nucleotide insertion or deletion (NHEJ-mediated indels) causes an intrinsic bias towards knockout rather than knockin. In culture, strategies can be employed to select for cells with precise knockin, thereby obviating the impact of undesirable editing outcomes. While CRISPR/Cas9-mediated knockin can be similarly performed directly in the brain, its utility is hampered by this inherently low efficiency and fidelity, coupled with the inability to apply selection \textit{in vivo}. In this study, to enable high-performance \textit{in vivo} knockin directly in the brain, we combine existing variants of Cas9 and donor DNA and quantify their cumulative effect on knockin \textit{in vitro} and \textit{in vivo}. Our findings show that fusing Cas9 to Ctip, a critical component of the endogenous homology-directed repair pathway, in combination with long, \textit{in-situ} linearized dsDNA donor templates, yields greater than 20-fold increases in knockin efficiency and editing specificity. Additionally, these optimizations result in a 40% increase in the occurrence of biallelic knockin, and, combined with in utero electroporation, provide an effective platform for \textit{in vivo} genome editing in the developing brain. Applying this approach with high-fidelity Cas9 variants increases on-target stringency with minor sacrifice to overall efficiency. This high-performance combinatorial \textit{in vivo} knockin strategy allows rapid and targeted experimental knockin into wild-type animals, and lays the groundwork for potential \textit{in situ} use of CRISPR/Cas9 in future gene therapy approaches in patients.
**Structural changes in primary auditory cortex neuronal networks impact encoding fidelity in noisy environments**

Kelson Shilling-Scrivo

Communicating in complex auditory environments requires the ability to separate behaviorally meaningful sounds from irrelevant ones. In mouse auditory cortex, sound features in a quiet environment are encoded by small populations of neurons (Francis et al. 2018; Liu et al. 2019). However, most sounds exist in the presence of other background sounds that overlap spectrally and temporally. To date it is unknown how populations of neurons encode sounds in the presence of a noisy background. We thus investigated how populations of neurons simultaneously encode both tonal foreground and noisy background sounds. We used *in vivo* two photon calcium imaging to record from 100s of neurons in primary auditory cortex (A1) in transgenic Thy1-GCaMP6s X CBA mice passively listening to tones in a broadband noise background. For each mouse, before imaging, A1 location was determined by widefield imaging. While imaging in A1, we presented 8 SAM tones (4-48 kHz ½ octave spacing, 5Hz carrier frequency, 10 repeats) at three levels (60, 50, 40dB) in the presence of 40dB broadband noise or in quiet. We imaged ~900 neurons and for each neuron, calculated the relative change in fluorescence for each trial (dF/F). Based on the average response to all stimuli, neurons clustered into three classes: (1) Neurons maximally responsive during the noise background (Noise-On); (2) Neurons maximally responsive during tone onset (Tone-On); and (3) Neurons maximally responsive during tone offset (Tone-Off). Initial results showed that as the signal to noise ratio (SNR) decreases, Noise-On neurons remain more responsive than Tone-On or Tone-Off neurons. Noise correlation analysis showed that as SNR decreases, there was a corresponding decrease in noise correlation in the two tone-responsive classes of neurons (Tone-On and Tone-Off). Since neurons can be embedded in functional subnetworks, we utilized Granger causality (GC) analysis to identify neurons’ functional connectivity among these three classes (Francis et al. 2018; Liu et al. 2019). GC analysis showed that for the two tone-responsive classes of neurons decreasing SNR increased the number of GC linked neurons. In contrast Noise-On neurons showed no change in the number of GC linked neurons across SNR. Finally, we trained a naïve Bayes classifier on our data and found that as SNR decreases, more neurons are required to correctly identify the tone presented. Together, our results support a model in which different features of a stimulus are encoded by different populations of neurons in A1 and that as SNR decreases, more neurons are needed to differentially encode those stimulus features. Thus, the size of the active population in A1 seems to relate to coding fidelity.
Small Ankyrin 1 may regulate SERCA activity in astrocytes but not in neurons

Amanda Labuza

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. Immunocytoschemistry (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.
Horizontal view of a cleared brain from a mouse injected with retrograde vira. Shows projections to the nucleus accumbens (red) and prefrontal cortex (green). By Andreas Wulff, Doctoral Candidate, Thompson Lab

Poster Presentations
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1. Perinatal exposure to opioids causes permanent deficits in somatosensory circuits

Jason B Alipio¹, Ying Li¹, Asaf Keller¹

University of Maryland School of Medicine, Program in Neuroscience, Department of Anatomy & Neurobiology

Fentanyl is a synthetic opioid that is commonly and readily prescribed for the treatment of chronic pain. The widespread clinical use of opioids like fentanyl has led to an opioid use epidemic, given that fentanyl shares many of the addictive properties of opioids used illicitly in recreational settings, like heroin. One underappreciated consequence of the opioid epidemic is a dramatic increase in the prevalence of opioid use during pregnancy. Perinatal exposure to opioids results in permanent complications to neonates later in life, including increased risk of autism spectrum disorder, attention deficit hyperactivity disorder, and disruptions in sensory function. Given that interactions between the thalamus and neocortex are involved in these behavioral outcomes, it is possible that these regions are particularly susceptible to the effects of perinatal fentanyl exposure. Sensory information is processed by different subregions of the cortex, and these structures interact primarily through reciprocal corticothalamic (CT) and thalamocortical (TC) projections to modulate behavioral responses to sensory input. We hypothesize that perinatal fentanyl exposure impairs somatosensory function by decreasing synaptic transmission in the CT/TC circuit. Here, we record spontaneous and evoked activity from neurons in the somatosensory (S1) cortex and the ventral posteromedial (VPM) thalamus of mice by using a combination of ex vivo optogenetic stimulation and whole-cell patch-clamp electrophysiology. By selectively activating genetically targeted corticothalamic synapses, we investigate the enduring consequences of perinatal fentanyl exposure on CT/TC function later in life. Our results suggest that excitatory synaptic transmission is persistently suppressed in thalamic and cortical neurons from adolescent mice exposed to perinatal fentanyl. To examine how perinatal fentanyl exposure influences S1 neural activity in vivo, we use electrocorticogram (ECoG) implants to record neural oscillations from S1 cortex in awake behaving mice. Ongoing studies are seeking to characterize aberrant behavioral outcomes throughout adolescence in mice exposed to perinatal fentanyl. These studies will contribute to our understanding of the enduring consequences associated with perinatal exposure to drugs of abuse, impact on infant neurodevelopment, and potentially inform improved treatment options.
2. Cellular and Neurodevelopmental Defects of a DCTN2 Missense Variant Causing Severe Intellectual Disability

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Wiring of cortical circuits is carried out by coordinated developmental mechanisms of neuronal migration, dendritic arborization, axon projection, and synaptogenesis. It is estimated that mutations in over 2500 genes can underlie cortical miswiring that leads to intellectual disability, a complex group of neurodevelopmental disorders characterized by significant impairment in cognition and adaptive behaviors. Up to 50% of intellectual disability cases have genetic etiologies. Identification of the specific gene variants underlying cortical wiring pathology has provided critical insights to the molecular processes behind physiological and pathological cortical circuit development.

In this study, using whole exome sequencing in a large cohort of consanguineous families, we identified a missense variant of DCTN2 that segregates with severe intellectual disability and epilepsy in an autosomal recessive fashion. DCTN2 is one of the 23 subunits of dynactin, a regulator of the dynein motor complex. It has been studied with respect to endocytosis in non-neural cells, and has been associated with motor neuron degeneration. We investigate DCTN2 in the developing brain and find it to be broadly expressed, including in neocortex and hippocampus. Using subcellular proteomics and immunolabeling, we find DCTN2 heavily enriched in growth cones of developing cortical axons. Using \textit{in} vivo CRISPR knockout in callosal projection neurons, we identify pervasive dysmorphic axon growth cones resulting from \textit{Dctn2} deletion both in the developing and adult cerebral cortex. These findings indicate that disruption of \textit{Dctn2} function results in specific abnormalities in the development of cortico-cortical circuitry, potentially underlying intellectual disability and epilepsy seen in patients.
3. Multiple Rare Inherited Human LRP1 Variants Associated with Aortic Disease Impair LRP1 Processing and/or Maturation in vitro

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Low-density lipoprotein receptor-related protein 1 (LRP1) is an endocytic receptor that regulates numerous ligands and participates in signaling pathways. Previous work from our lab demonstrates that a sm22 promoter driven knock-out of LRP1 results in aortic dilatation in mice. In collaboration with the Milewicz (UTHHealth) and Dietz (JHU) laboratories, we examined LRP1 molecules containing rare variants or *de novo* mutations that segregate in patients with aortic aneurysms. The mechanism by which these mutant LRP1 molecules are involved in aneurysm formation is unknown. We plan to biochemically characterize the functional defects in LRP1 imposed by these mutations. Our lab has developed a mini-receptor, a truncated form of LRP1 that recapitulates LRP1 function but isolates critical LRP1 domains for study. Site-directed mutagenesis was used to introduce mutations to the mini-receptor corresponding to the allelic variations described above. LRP1-deficient Chinese hamster ovary cells were transfected with mutant receptors to detect changes in LRP1 function. These studies have identified multiple mutant receptors that have clear deficits in LRP1 maturation and/or protein turnover compared to wild-type mini-receptors. One mutation results in a significant two-fold decrease in furin cleavage of LRP1, a process that is critical for LRP1 maturation. Two separate mutations exhibit significant changes in LRP1 turnover in a cycloheximide chase assay. These data demonstrate that LRP1 mutations associated with human aortic aneurysms confer biochemical deficits in LRP1 that could contribute to aortic disease. We predict that impairments in LRP1 function and maturation will cause dysregulation of LRP1 ligands and, thus, lead to aneurysm formation.
4. Identifying Imaging Biomarkers of Resilience to Drug Use: Interaction between Childhood Trauma History and Smoking Status on Gray Matter Structure in Adulthood

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The best public health tool to reduce smoking-related disease (#1 cause of preventable death in the US) is prevention. Childhood trauma (CT) is among the strongest predictors of substance use disorders (SUDs), including smoking. However, not all individuals with CT develop SUDs. These “resilient” individuals experience adaptive long-term outcomes despite a history of trauma. Delineating neurobiological mechanisms for resilience to SUDs may inform prevention strategies for at-risk youth. Here we considered variability in gray matter (GM) associated with resilience to smoking by comparing high-CT-nonsmokers to high-CT-smokers. Structural brain images and CT Questionnaire scores were collected at the NIDA-IRP from 147 smokers and 149 matched nonsmokers. Scans were processed in Freesurfer (ENIGMA pipeline) to measure GM structure. \textit{A-priori} region-of-interest analysis was conducted for 14 regions identified as sensitive to smoking and CT. Linear models considered factors of smoking status and CT scores; and cigarette exposure and dependence within smokers. In addition to main effects of smoking, smoking exposure, and CT, we found a CT x smoking status interaction in the Frontal Pole (FP). Structural development in the FP is normalized in the relatively resilient nonsmoking, high CT group, as compared to the smoking, high CT group. This interaction represents a presumptive neurobiological marker for resilience to cigarette smoking, despite high developmental risk as quantified by CT history. Primary prevention strategies that focus on improving FP function, including adaptation to changing environment (e.g. abusive to normative), may reduce later drug use in at-risk children.
5. Compositional changes in the gut microbiome contribute to stress-induced comorbid visceral pain

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Chronic overlapping pain conditions are highly prevalent in women and are commonly associated with psychological stress. In rats with existing orofacial neuropathic pain, 3-day repeated forced swimming stress leads to visceral hypersensitivity. Referred hyperalgesia in the low back area is more persistent in female rats than males. It is associated with a long-lasting upregulation of 5-HT3A receptors in the lumbosacral spinal cord and dorsal root ganglia. Intrathecally administered 5-HT3R antagonist transiently blocks referred hyperalgesia, suggesting 5-HT3R-mediated sensitization of spinal primary afferents from the gut. However, the underlying mechanisms are still unclear. This study expands on our previous observation that repeated forced swimming leads to gut dysbiosis in rats with orofacial pain. Using 16S rRNA-based analysis, we confirmed extensive compositional changes in fecal microbiota of rats with comorbid pain after stress. We found that some changes persisted for weeks, including an increase in the relative abundance of Firmicutes and a decrease in Bacteroidetes. We also found a reduction in microbiome biodiversity. Finally, fecal microbiota transfer (FMT) from rats with referred hyperalgesia induced comorbid pain in recipient rats with orofacial pain alone. However, naïve recipients did not develop this phenotype, suggesting that ongoing orofacial pain is a prerequisite for developing comorbid pain. Conversely, FMT from naïve rats attenuated referred hyperalgesia in recipient rats with comorbid pain. These findings indicate that compositional changes in the gut microbiome contribute to stress-induced persistent referred hyperalgesia in rats with orofacial pain. Further understanding of this causal relationship will help advance novel therapeutic strategies for comorbid pain conditions.
6. Exploring sex differences in the rewarding properties of juvenile rat social play

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Social play is widely expressed across mammalian juveniles, including human children. As the primary social behavior that occurs pre-puberty, social play is important for the development of social cognition that will guide an animal to flexibly interact with others in myriad contexts throughout life. Disrupted social play is a core symptom of male-biased neuropsychiatric disorders with developmental origins, such as autism spectrum disorders, which compels us to understand how the brain processes social interaction at younger ages when deficits generally appear. Social play is a highly rewarding activity that involves components of the mesolimbic dopamine (DA) system, particularly a population of DA neurons in the ventral tegmental area (VTA). Sex differences in the rewarding properties of other stimuli, such as drugs of abuse, are well characterized, and a recent study in Syrian hamsters demonstrated that adult females find same-sex social interaction more rewarding than males (Borland et al., Neuropsychopharmacology, 2018). We thus hypothesize that sex also impacts the reward value of juvenile social play. To test this, we will first quantify DA cells in the VTA of juvenile male and female rats by immunolabeling histological sections for tyrosine hydroxylase, a marker of DA cells. Though males play more than females, we hypothesize that juvenile social play is more rewarding to females and will test this using a conditioned place preference design. Future experiments will explore how sex may developmentally program VTA DA cell number and whether this underlies a sex difference in the rewarding properties of juvenile social play.
7. **CB1 receptor activation in the ventral tegmental area mediates learning about incentive stimuli in sign- and goal-tracking rats**


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Endocannabinoids are critical gatekeepers of dopaminergic signaling, and disrupting cannabinoid receptor-1 (CB1) signaling in the Ventral Tegmental Area (VTA) alters dopamine (DA) dynamics to attenuate cue-motivated behaviors. Prior studies suggest that DA release plays a critical role in driving sign-, but not goal-tracking. We first test the prediction that CB1 signaling mediates the attracting properties of a reward predictive lever cue. Here, we trained rats in Pavlovian lever autoshaping prior to systemic rimonabant injections (0, 1, 3 mg/kg) during early and late Pavlovian lever autoshaping test sessions. We show that systemic injections of rimonabant, a CB1 receptor inverse agonist, during Pavlovian lever autoshaping impairs the expression of both sign- and goal-tracking at the early test. With extended training, many previously goal-tracking and intermediate rats shifted to lever approach, which remained dose-dependently sensitive to rimonabant. Additionally, intra-VTA infusion of rimonabant selectively decreases sign, but not goal-tracking at the early test suggesting that endocannabinoids in the VTA are responsible for specifically mediating sign-tracking. We next examine whether rimonabant blocks the reinforcing properties of the Pavlovian lever cue in a conditioned reinforcement test. Here, we trained rats in Pavlovian lever autoshaping prior to systemic rimonabant injections (0, 1 mg/kg) during a conditioned reinforcement test. We find that 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.
8. The Cerebellar Dentate Nucleus in Autism: Are Neurons and their Perineuronal Nets Preserved Despite Missing Purkinje Cells?

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Purkinje cell (PC) dysfunction is the most consistent neuropathological finding in autism, with as many as 75% of cases showing reduced numbers. The lateral hemisphere displays the greatest PC decrease reported and projects to the dentate nucleus (DN). Therefore, we hypothesized that neurons targeted by PCs in the dentate would be lost as well. The DN harbors the greatest percentage of neurons in the brain surrounded by a perineuronal net (PNN). PNNs around the DN neurons were quantified and western blots for expression are underway.

Immunohistochemistry was performed on human postmortem brain tissue from 19 control and 18 autism cases for nickel DAB anti-HPLN1, a link protein in the PNN. Neutral red was used as a counterstain to identify all neurons. The density of neurons with PNNs, based on HPLN1 expression, was not different between control and autism. The density of neurons without a PNN also showed no differences and therefore total neuron numbers were similar.

Despite reports of significant reductions in the number of PCs in the lateral hemisphere of the cerebellum, similar reductions are not evident in the DN. Thus, the targets of PC output within the DN appear to be preserved by the remaining PCs and inferior olive input. Furthermore, the proportion of neurons surrounded by PNNs compared to those without appear to be unaltered. Further work is underway to determine whether activity-dependent components of the PNN, such as aggrecan, are affected while total PNN numbers remain unchanged.
9. CG, a lncRNA that regulates cortico-striatal projections in the developing mouse embryo

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As the brain develops, neurons use non-activity dependent mechanisms to guide their projections to make functional circuits. Understanding the genetic and molecular components of what drives these circuit formations has been an open question in developmental biology. In the following work we highlight the use of a novel screening process that integrates datasets of genes upregulated in the axonal growth cone with the novel technique, \textit{in utero} Mosaic Analysis Phenotyping (\textit{iuMAP}). The \textit{iuMAP} technique utilizes \textit{in utero} electroporation and the CRSIPR/Cas9 system to focally manipulate developing neurons while being able to track the functional consequences of the genetic manipulation. This technique allows us to identify circuit miswiring without \textit{a priori} knowledge of gene function, making it particularly suitable for investigating non-cannonical factors that may be novel drivers in the development of brain connectivity, such as non-coding RNAs(ncRNAs). Recently, extranuclear function of ncRNAs has begun to gain a lot of attention but there is no to little evidence the ncRNAs have critical roles outside regulating transcription or translation. Utilizing the \textit{iuMAP} technique we knocked out a novel long non-coding RNA (lncRNA) at e14.5. We observed that by knocking out this lncRNA the ipsilateral cortico-striatal projections were missing at p7. This is the first finding that suggests non-coding segments of the genome are critical for making specific projections in the developing brain.
10. Spatial and temporal characterization of LC3+ axonal bulbs formed in injured spinal cord

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Autophagy involves autophagosome formation which sequesters cell contents including protein aggregates and damaged organelles and delivers the cargoes to lysosomes for degradation. After traumatic axonal injury, autophagy markers accumulate in the axons. However, the exact role of autophagy in axonal degeneration in vivo after traumatic axonal injury remains elusive although studies on neurodegenerative diseases suggest dysfunctional autophagy is associated with axonal degeneration. Therefore, here as a first step, we tried to characterize the changes of autophagosome marker LC3+ in the axons in a mouse spinal cord injury model by using transgenic and/or immunohistochemical labelling. Acutely after contusive spinal cord injury, we found abundant LC3+ axonal bulbs, which are believed to be degenerative as opposed to regenerative growth cones, form at both rostral and caudal to the injury. The LC3+ axonal bulbs become abundant 6 hours after the spinal cord injury and its size and number increase at 1 day time point. We further demonstrated that LC3+ axonal bulbs are more abundant in the dorsal column, where the injury impact is from, than the ventral white matter. More specifically, LC3+ axonal bulbs are more abundant rostral to the injury site in both fasciculus gracilis and corticospinal tract of the dorsal column, while the bulbs in the fasciculus gracilis are larger in size than those in the corticospinal tract. Ongoing study includes dissecting the molecular interplay between autophagy and axonal bulb formation as well as axonal degeneration.
11. Role of membrane-bound glucocorticoid receptors in modulating hippocampal activity and neuroendocrine regulation

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Depression is a widespread psychiatric disease and a leading contributor to health burden in the United States. Understanding the biological underpinnings of psychiatric conditions such as depression is paramount to developing novel treatments and improving patient outcomes. One of the most consistent biological findings in patients with major depression is dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, the major controller of the neuroendocrine stress response. Furthermore, chronic stress has an extensive association with depression, and causes noted changes in hippocampal structure, function, and glucocorticoid receptor (GR) expression that are independently associated with depression phenotypes. The hippocampus also has axons that project to the hypothalamus and has been suggested as important in HPA axis regulation. The hippocampus has high levels of expression of GR, and binding of the principle stress hormones, glucocorticoids (GCs), to these receptors is known to cause changes similar to those seen in chronic stress and result in depression phenotypes in rodent models. Recent evidence suggests the existence of a novel form of glucocorticoid receptor existing at the cellular membrane (mGR) that signals via non-canonical pathways. This receptor is postulated to enable GCs to exert rapid actions in the nervous system. Preliminary data I have gathered demonstrates rapid changes to \textit{in vivo} hippocampal local field potentials following administration of membrane-impermeant GCs, suggesting these changes are caused at the cell surface and mediated by non-canonical mechanisms. My project proposes to investigate the functional significance of these mGRs in the hippocampus and subsequent regulation of the HPA axis. To do this, I first propose to fully characterize the hippocampal response to glucocorticoid administration and isolate the membrane-specific effects on hippocampal physiology using patch-clamp electrophysiology. I will investigate the hippocampal regulation of the HPA axis by probing specific hippocampal-hypothalamic (vHipp-PVN) synapses as well as a likely intervening inhibitory synapse. Finally, I will use a validated multimodal chronic stress model of depression to study the effects of a clinically-relevant dysregulation of HPA axis activity on these particular aspects of neuroendocrine physiology. In this way, I will test the hypothesis that chronic stress dysregulates mGR function and vHipp-PVN synapse communication. The proposed experiments will reveal whether glucocorticoids act at membrane-associated glucocorticoid receptors in the hippocampus to alter neuronal discharge rapidly and thereby suppress HPA axis function. Furthermore, I will test the hypothesis that these processes are dysregulated by chronic stress. The proposed aims will explore long-studied facets of stress and depression through a new and exciting avenue; the membrane-associated glucocorticoid receptor, provide critical insight into the significance of these receptors in neurophysiology, neuroendocrine regulation, and behavioral outcomes that could lead to novel treatments for psychiatric illness.
12. Receptor contributions to ApoE signaling in neurons and endothelial cells

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Expression of the apolipoprotein E (ApoE) E4 allele is a prominent genetic risk factor for Alzheimer’s and cerebrovascular disease, but the mechanisms underlying ApoE’s contribution to both remain unclear. Isoform-specific differences in ApoE lipid transport and/or signaling functions may directly impact both neuronal and vascular cell health. Members of the LDL receptor family, including LRP1, VLDLr, and ApoER2, are major cell surface receptors for ApoE and play critical roles in maintaining vascular integrity. Recent studies indicate that ApoE induces signaling events in neurons in an isoform-dependent manner that are inhibited by receptor associated protein (RAP, nonspecific LDL receptor inhibitor), indicating the involvement of an unidentified LDL receptor. Here, we aim to identify the receptor contributing to ApoE-induced signaling events in neurons, and investigate coinciding signaling in vasculature. Using ClariomS microarray analysis on primary neuronal cell culture from VLDLr knockout mice treated for 48 hours with 10 µg/mL ApoE4 +/- 100 nM RAP we identified 43 genes changed by ApoE4, 4 of which were prevented by RAP. Comparison to ongoing experiments in neurons from wildtype mice will identify VLDLr-dependent ApoE-induced changes in gene transcription. To assay for ApoE signaling effects in endothelial cells, HUVECs were incubated with 10 µg/mL ApoE2, ApoE3, or ApoE4 +/- 100 nM RAP and subsequent immunoblotting indicated that ApoE induces increases in ERK phosphorylation in endothelial cells that are both isoform-specific (induced by E2 and E3, not E4) and LDL receptor-dependent (inhibited by RAP).
13. The role of Ca\(^{2+}\) signaling in anti-NMDAR encephalitis

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N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors required for normal synaptic transmission at excitatory synapses in the central nervous system. Calcium conductance through NMDARs is especially critical to learning and memory. Anti-NMDAR encephalitis is an autoimmune disease in which antibodies are generated against the obligate GluN1 subunit of NMDARs. These antibodies crosslink NMDARs and induce internalization, resulting in neuropsychiatric symptoms, coma, and death in severe cases. The mechanism by which this internalization occurs is poorly understood, and limited evidence exists for the acute effects of NMDAR antibodies on intracellular signaling pathways. This internalization of NMDARs is dependent on activation of the receptor and an influx of Ca\(^{2+}\). Therefore, we hypothesize that Ca\(^{2+}\)-dependent signaling is disrupted by anti-GluN1 antibodies upon binding to NMDARs. We measured NMDAR-dependent miniature, spontaneous Ca\(^{2+}\) transients (mSCaTs) in cultured neurons treated with anti-GluN1 human monoclonal antibodies (hMAbs) derived from an anti-NMDAR encephalitis patient, and found that Ca\(^{2+}\) influx through NMDARs is not altered at early time points prior to receptor internalization. We also tested a longer incubation of the anti-GluN1 hMAb on CaMKII\(\alpha\) at the post-synaptic density, and found a decreased response in total CaMKII\(\alpha\) after NMDAR activation. These findings suggest that synaptic Ca\(^{2+}\)-dependent signaling is likely dysregulated in response to anti-GluN1 hMAbs at longer timepoints. We also present data from unbiased phosphoproteomic analysis to identify additional signaling pathways that may be dysregulated following anti-GluN1 hMAb treatment. Together, these studies may aid in identifying candidate pathways for novel therapeutics to reduce morbidity and mortality in anti-NMDAR encephalitis.
14. Glutamate Receptors as Directors of Synaptic 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. Both genetic and more acute manipulations will be employed to test the necessity and sufficiency of the receptors for organizing nanostructure. Presented here are preliminary results indicating the feasibility of proposed approaches and the role of receptor structure in governing synaptic organization. First, we demonstrate the use of optical dimerization to acutely recruit receptors to the synapse. We predict alignment of RIM1/2 nanoclusters will reflect the positioning of recruited receptors. Furthermore, overexpression of a chimeric transmembrane protein bearing the N-terminal domain of the GluA2 subunit of AMPA receptors seems to disrupt PSD-95 nanostructure. 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.
15. Transcriptional adaptations in the ventral pallidum following cocaine self-administration

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Growing evidence suggests the ventral pallidum (VP) is critical for drug intake and seeking behavior. Receiving dense projections from the nucleus accumbens as well as dopamine inputs from the midbrain, the VP plays a central role in the control of motivated behaviors. Repeated exposure to cocaine is known to alter VP neuronal firing and neurotransmission. Surprisingly, there is limited information on the molecular adaptations occurring in VP neurons following cocaine intake. To provide insight into cocaine-induced transcriptional alterations we performed RNA-sequencing on VP of mice that underwent 10 days of cocaine self-administration (0.5mg/kg/infusion) followed by twenty-four hours of abstinence. We observed differential gene expression in 363 genes between animals that self-administered cocaine and saline controls. Subsequent Gene Ontology analysis pointed toward alterations in dendrite- and spine-related genes. Searching for a common regulator for these sets of genes, we found that the expression of the transcription factor Nr4a1 showed a robust increase following cocaine self-administration. Further, we observed an increase in the Nr4a1 transcriptional target Plk2, a molecule important for synaptic and structural plasticity. Analysis of Plk2 molecular targets showed alterations in Actin and Rap2 dynamics after cocaine exposure, confirming alterations in dendritic and spine functions. Overexpression of Nr4a1 in the VP reduced cocaine seeking supporting its role in drug-related behavior. Using fluorescent in situ hybridization, we are now determining which VP projection neuron population displays increased Nr4a1 and Plk2 levels after cocaine self-administration. This includes VP-ventral tegmental area, VP-lateral habenula, VP-mediodorsal thalamus, or VP-nucleus accumbens projection neuron populations. Additionally, we are using adenoassociated virus (AAV) overexpression and CRISPR knockdown manipulation of Nr4a1 and Plk2 to interrogate the role of these molecules in VP circuits during cocaine self-administration and relapse-like behavior. Altogether, our work can provide crucial information into the molecular adaptations occurring in VP neuron supporting cocaine self-administration and relapse-like behavior.
16. The role of extended amygdala in incubation of Fentanyl seeking

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Drug addiction is a chronically relapsing disorder that consists of cycles of compulsive drug seeking and periods of abstinence and withdrawal. Drug associated cues are potent triggers of relapse, which heightens with time away from the drug. The time-dependent increase in cue-triggered drug seeking, which is termed ‘incubation of drug craving,’ is modeled in rodents by examining responding for drug-associated cues after a period of abstinence. With opiate drugs, withdrawal symptoms may further heighten cue reactivity by recruiting brain systems involved in both reward seeking and stress responses. The central nucleus of amygdala (CeA) and its corticotrophin releasing factor (CRF) projections to the bed nucleus of the stria terminalis (BNST) are critical drivers of stress-induced relapse of drug seeking. We hypothesize that opioid withdrawal recruits CeA→BNST CRF to drive incubation of opiate craving. To begin testing this, we examine the effect of CRF1 receptor antagonist R121919 (1 µg/0.3μL) in BNST on incubation of fentanyl seeking. We train rats to self-administer fentanyl (2.5µg/kg/infusion) and examine extinction responding after 1 or 30 days of fentanyl withdrawal to assess incubation of opiate seeking. On day 30, we inject either vehicle or R121919 into BNST. Rats tend to respond more for fentanyl-associated cues on day 30 relative to day 1, and R121919 attenuates protracted responding for drug-associated cues on incubation day 30. In ongoing experiments, we incorporate opioid-dependence as a factor to investigate whether CeA→BNST CRF pathway is recruited more strongly in dependent rats than non-dependent rats to drive heightened opiate cue-reactivity.
17. Isolation and Characterization of Extracellular Vesicles from Mouse Spinal Cord Tissue

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All cells release extracellular vesicles (EVs) carrying bioactive molecules whose composition can change during pathology. To date, the majority of EV studies have been conducted in vitro after their isolation from cell culture media or biological fluids. Additional support for the physiological role of EVs requires the ability to identify these structures within solid tissues. Here we provide the first evidence for EVs isolated from ex vivo mouse spinal cord tissue. We performed an enzymatic digestion of spinal cord followed by differential centrifugation to remove cells and cellular debris. The remaining supernatant was overlaid onto a three-step sucrose gradient to isolate EVs by density gradient ultracentrifugation. We found at least two populations of EVs differentiated by density that pelleted at 100,000g. High density EVs that equilibrated in 1.3M sucrose showed strong expression for classical EV markers including CD81, Flotillin-1, and LAMP-1 by Western Blot (WB); low density EVs equilibrating in the 0.6M sucrose layer were only positive for CD81 and to a lesser degree. We further analyzed these fractions at an individual particle level using the ExoView R100™, a technology that captures and images EVs on surface-coated antibody spots for tetraspanin proteins (e.g., CD81, CD9, CD63) that are highly enriched in EVs. We detected about twice as many CD81+ EVs in the high density fraction (1747 particles) compared to the low density fraction (874 particles). Future work in our lab will apply this methodology to analyze how EVs contribute to local inflammatory and neurodegenerative processes after spinal cord injury.
18. Stress-Induced Impairments in Fear Learning are Causally Related to Increased Kynurenic Acid Formation in the Prefrontal Cortex

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Severe and/or prolonged stress has many detrimental effects and often causes cognitive impairments. Experimental manipulation of kynurenic acid (KYNA) affects learning and memory in animals. However, the link between a stress-induced change in KYNA and a change in cognitive function has yet to be explored. Here we examined whether acute stress increases KYNA levels in the brain and, specifically, if a stress-induced increase in KYNA is causally related to impairments in fear learning. Adult male Sprague-Dawley rats (250-300 g) were tested using three different acute stress models (restraint, exposure to fox urine, or inescapable shocks). Extracellular KYNA levels were determined in the prefrontal cortex by in vivo microdialysis (n=6/group). In rats that received inescapable shocks, extracellular KYNA levels were increased by ~85%. The other two stressors were much less effective (<25% change in KYNA levels compared to baseline). Another cohort of rats (n=8/group) underwent a fear discrimination procedure immediately after the termination of stress. During training, rats were exposed to two different auditory tones - one paired with a foot shock (CS+) and the other unpaired (CS-). One week later, rats were tested for freezing behavior during re-exposure to both auditory stimuli in a novel context without any shocks delivered. Only rats receiving inescapable shocks were unable to discriminate between CS+ and CS-. All other groups exhibited greater freezing to CS+ than to CS-. To further examine the impact of KYNA manipulation on behavior, a separate group of animals received a s.c. injection of the irreversible KYNA synthesis (kynurenine aminotransferase II) inhibitor PF-04859989 1 h before initiation of the stress challenge. Application of the KYNA synthesis inhibitor blocked the stress-induced KYNA increase (n=6-7/group) and normalized the impairment in fear discrimination (n=12-17/group) in rats that received inescapable shocks. Taken together, these findings indicate a causal relationship between a stress-induced KYNA increase and fear learning impairments. Pharmacological inhibition of KYNA synthesis may therefore be a therapeutic option for treating cognitive dysfunctions in stress-related disorders.
19. Caudate Volumes and Cognition in “Pure” Ketamine and Poly-drug Ketamine users

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Aims: Enlarged striatum in stimulant use disorder might be related to chronic dopaminergic stimulation. Ketamine is an abused drug that leads to strong craving, but only mildly increased striatal dopamine release. This study aims to evaluate striatal volumes in primarily ketamine (K) verses Poly-drug+ketamine (Poly+K) users.

Methods: T1-weight MRI image was obtain on 116 subjects (43 Non-drug users, 35 K, 38 Poly+K users). Volumes of caudate, putamen and globus palidus were segmented and compared using one-way ANCOVA, covared for age and sex. Cognition and mood were also assessed.

Results: Right caudate was larger in Poly+K than non-drug users (p_{post hoc}=0.015) with a linear trend across groups (Poly+K > K > non-users, p=0.015). Both user groups performed poorer on verbal and visual learning tasks (p=0.001) and had more depressive symptoms (p<0.001) than Non-drug users. Larger right caudate correlated with better verbal-learning in all participants (r=0.18; p=0.019) and lower depression score in users (r_{users-only}=-0.34; p_{group-by-volume}=0.032). Greater lifetime ketamine used correlated with higher depression scores (r=0.27; p=0.008), while more cocaine use days tended to correlate with larger right caudate (r=0.30, p=0.12).

Conclusions: The even larger caudate volumes in Poly+K, compared to K and Non-drug users, might be due to their greater cocaine use, which supports the hypothesis that chronic dopaminergic stimulation leads to glia activation and neuroinflammation. Larger caudate in those with better cognition and lesser depressive symptoms suggest a compensatory response. Additional evaluations of tobacco and alcohol co-use and multi-parametric imaging (including DTI and MRS) will allow further delineation of these volume changes.
20. Elucidating the role of ALS-linked UBQLN2 on Mitochondria Function

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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 the Pxx domain of UBQLN2 have been found to cause ALS with frontotemporal dementia. UBQLN2 is a vital protein for maintaining cellular proteostasis through binding ubiquitinated misfolded proteins and transferring them to autophagic and proteasomal degradation systems. These functions help to deter the accumulation of protein aggregates, which is a hallmark of ALS pathology. ALS is a multifactorial disorder and has been linked to alternative disease mechanisms, such as mitochondrial dysfunction. However, the role of mitochondria in ALS disease pathology remains poorly understood. 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. Through transmission electron microscopy, UBQLN2 P497S mice show significant mitochondrial structural defects in the spinal cord, such as loss of cristae. Seahorse respiration assays on isolated mitochondria from the spinal cord and hippocampus indicated that mitochondrial state 3 respiration is perturbed in P497S mice, suggesting a possible defect in ATP production. Using CRISPR-Cas9, we generated stable knock-out cell lines of UBQLN2. In HeLa cells and in motor-neuron like NSC-34 cells, UBQLN2 KOs show an attenuation of mitochondrial respiration and ATP production. In addition, transfection of COX7a2L, a mitochondrial complex IV protein into HeLa UBQLN2 KOs show that COX7a2L aggregates when UBQLN2 is knocked-out, suggesting that UBQLN2 may chaperone mitochondrial proteins. Future studies in evaluating mitochondrial dynamics, function, and structure within the knock-out model will help to elucidate the role of UBQLN2 in maintaining mitochondrial health.
21. Sex-specific transcriptional networks in the medial amygdala underlie differences in expression of juvenile social play

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Social play behavior, or rough-and-tumble play, is a characteristic pattern of social behavior exhibited by mammals during the juvenile period which likely contributes to the development of social and emotional skills needed throughout life. Importantly, there is a sex difference in expression of social play, with males exhibiting greater intensity and frequency of play than females, though as with any behavior, there is individual variability. To identify the transcriptional signatures associated with play in both a sex-dependent and sex-independent manner, we performed RNA sequencing (RNA-seq) of the medial amygdala (MeA), the site of masculinization of play, in high- and low-playing males and females at the juvenile age. As social play is a dynamic behavior likely produced by complex interactions among many genes, we then utilized a network approach, Weighted Gene Co-expression Network Analysis (WGCNA), focusing on 4,261 genes that showed nominal differences in expression related to sex or play. We identified 22 gene co-expression modules, many (11 modules with p < 0.05) of which are sex-specific in expression, as they are correlated with expression of play behavior in one sex but not the other. Future analysis will identify and validate “hub” genes driving differences in play in particular modules of interest. Additionally, we will integrate this analysis with published single cell RNA sequencing experiments (scRNA-seq) to explore potential cell-type-specific expression of these modules. Together, these novel analyses will greatly improve our understanding of how differential transcriptomic regulation in the medial amygdala drives sex differences in MeA circuitry and social play.
22. Ethanol modulates inhibitory synaptic transmission in the nucleus accumbens

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The nucleus accumbens (NAc) is an essential integration center for circuits governing reward learning and motivated behavior. Plastic changes at synapses onto NAc medium spiny neurons (MSNs) enable reward learning. While NAc MSNs receive both excitatory and inhibitory input to regulate their output, very little is known about the mechanisms underlying plastic changes at inhibitory synapses and how this may be modulated by alcohol to hijack the reward system. Here we employ whole cell patch clamp electrophysiology to record inhibitory synaptic currents from MSNs in mouse nucleus accumbens core (NAcc). We discover a form of inhibitory synaptic plasticity: a postsynaptically expressed tropomyosin kinase B (TrkB) receptor-mediated long term depression of GABAergic transmission onto MSNs (NAcc-iLTD). We find that signaling through mitogen-activated protein kinase kinase (MEK) and dynamin downstream of TrkB may be necessary for NAcc-iLTD to occur, as their inhibition results in abolishment of NAcc-iLTD. Notably, we also find that acute ethanol exposure significantly augments NAcc-iLTD. Moreover, we reveal that inducing NAcc-iLTD in vivo is rewarding in a TrkB-dependent manner. Taken together, our data demonstrate that BDNF/TrkB signaling may play a role in regulating inhibitory synaptic plasticity in the NAcc to encode reward and suggest that ethanol disinhibits the NAcc through this mechanism to modulate alcohol reward.
23. Chemogenetic activation of the Ventral Pallidum Npas1-neurons induces susceptibility to social defeat stress

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Depression is a serious disorder intrinsically related to stressful events, with an enormous impact on individual health. However, we are still seeking to understand how stress experience impacts the brain and develop more effective treatments for depression. Studies using the social defeat stress model (SDS) demonstrate an important role of the nucleus accumbens (NAcc) and ventral pallidum (VP), key brain areas in motivated behaviors, in the etiology of depression. Considering the extensive and reciprocal connections that exist between these regions, VP projections to NAcc could be interesting targets related to the susceptibility to stress. To address this question, we used a chemogenetic approach to manipulate VP Npas1 neurons, which heavily projects to NAcc, during a subthreshold protocol of SDS (SSDS). Npas1-Cre-2A-tdTomato mice stereotaxically received AAV2-hSyn-DIO-hM3Dq (excitatory Designer Receptor Exclusively Activated by Designer Drugs - DREADD) or AAV2-hSyn-DIO-hM4Di (inhibitory DREADD) into the VP 2 weeks before a SSDS protocol. Thirty minutes before the SSDS, animals received Clozapine N-Oxide (CNO) i.p. at a dose of 1 mg/kg. Twenty-four hours later the animals underwent a social interaction test (SI) and a forced swim test (FST). Chemogenetic activation of Npas1 neurons during SSDS decreased time spent in the interaction zone and increased time spent in the corners in the SI when a social target was presented. It also increased immobility in the FST. In contrast, inactivation of Npas1 neurons had no effect on such behaviors. Our results demonstrate that increased activity of VP Npas1 neurons during stressful events induces susceptibility to social defeat stress.
24. Development of a custom multi-site fiber photometry system for monitoring claustrum activity in freely behaving mice

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The claustrum is proposed to mediate a variety of functions ranging from sensory binding to top-down cognitive control of action, but direct functional assessments of this telencephalic nucleus are lacking. Here we employ the guanine nucleotide-binding subunit beta-4 cre driver line in mice to selectively monitor and manipulate claustrum projection neurons. Using fiber photometry, we find elevated claustrum activity prior to an expected cue during correct performance on a cognitively demanding five-choice response assay relative to a less-demanding one-choice version of the task. Claustrum activity during reward acquisition is also enhanced when cognitive demand is higher. Furthermore, we use optogenetic inhibition of claustrum prior to the expected cue to demonstrate that claustrum is critical for accurate performance on the five-choice, but not the one-choice, task. These results suggest the claustrum supports a cognitive control function necessary for reward acquisition under cognitively demanding conditions.
25. Effects of sex on expectancy and placebo analgesia in chronic orofacial pain patients

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Temporomandibular pain disorder (TMD) is approximately three times as common in women as in men. Symptoms of TMD are also more severe in women and vary in intensity across the menstrual cycle. In pain studies reporting sex difference, women trend more sensitive to pain than men. Placebo studies are emerging to investigate influence of sex on expectancy-induced analgesia, though as in pain, sex- and gonadal hormone level-dependency is undetermined.

Between- and within-sex differences in placebo analgesia were assessed through a behavioral pain modulation task in 260 patients with at least a three-month history of TMD to evaluate sex differences, controlling for menstruation pattern and birth control regimen in women. Mediation analysis was conducted to explore if expectancy favors sex difference in placebo effects.

Among TMD patients, significant differences were observed in expectancy between men (mean=61.12) and women (mean=70.26, t173=-2.60, p=0.010), as were differences in placebo effect between men (mean=14.22) and women (mean=18.97, t258=-1.91, p=0.050). Among women with self-reported regular menses, no significant effects on expectancy or placebo were found based on menstrual pattern (expectancy: p=0.47; placebo: p=0.99) or birth control method (expectancy: p=0.072; placebo: p=0.46). Mediation analysis results underlined sex effects, indicating expectancy does not mediate dimorphic placebo effects (indirect=-1.00, SE=0.83, 95% CI [-3.01,0.18]).

We conclude strong sex differences in expectancy and placebo analgesia in TMD patients, controlling for menstrual pattern and birth control method. Our results emphasize the need to consider sex as a biological variable when expectancy and placebo effects are explored while taking into account hormonal factors.
26. The BDNF-TrkB pathway acts through nucleus accumbens D2 expressing neurons to mediate susceptible behavior to social defeat stress

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Depressive disorders affect a significant portion of the world's population. In the United States, for example, 16.2% of inhabitants will experience at least one depressive episode during their lifetime. In addition, according to WHO, depressive disorders are the leading cause of disability in the world, which also highlights the economic relevance associated with depression. Social defeat stress (SDS), which involves a resident intruder paradigm, has been widely used to induce negative affective states that model symptomatology of depression. Previous studies demonstrate that increased levels of BDNF from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) mediates susceptible behavior to SDS. While it is known that BDNF acts on NAc medium spiny neurons (MSNs) it is unclear if it is acting through dopamine receptor 1 or 2 (D1 or D2) expressing MSNs. To provide insight into this, we generated a Cre-inducible adenoassociated virus (AAV) expressing truncated TrkB (Trkb.T1), which lacks the kinase domain thus preventing downstream TrkB signaling. We infused this virus into the NAc of D1-Cre or A2A-Cre mice. Following viral expression we performed SDS followed by social interaction, the splash test, sucrose preference, and the forced swim test. Our data demonstrate that blocking BDNF-TrkB signaling, through TrkB.T1 overexpression in D2-MSNs prevents stress susceptibility and causes resilient behavior to SDS. In contrast TrkB.T1 expression in D1-MSNs causes a susceptible outcome to a subthreshold (S)SDS. Our studies implicate D2-MSNs as mediating the BDNF-TrkB effects of stress susceptibility, while BDNF acting on D1-MSNs has a protective role in stress.
27. The necessity of striatal fast-spiking interneurons in habitual alcohol consumption

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Habit formation is an adaptive learning process that allows for efficient reward acquisition. If the reward is a drug of abuse, such as alcohol, habit learning is accelerated and habitual use is promoted. The dorsolateral striatum is necessary for habit formation and the function of its resident parvalbumin expressing fast-spiking interneuron (FSI) population is significantly modulated by alcohol. To determine whether FSIs are necessary for the development of habitual alcohol drinking, we selectively ablated dorsolateral FSIs in mice undergoing a voluntary chronic intermittent ethanol consumption paradigm. Adult male and female C67B6J mice voluntarily consumed alcohol for 4-weeks before being challenged on a habitual ethanol drinking assay. Selectively ablating FSIs in the DLS prevented binge-like drinking in mice and significantly blocked the expression of habitual ethanol consumption. Collectively these results demonstrate causal involvement of FSIs in habitual ethanol consumption thereby highlighting this neuron population as a key target for therapeutic intervention in alcoholism.
28. Claustrum circuit mechanisms for large-scale cognitive brain networks

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The claustrum is a subcortical telencephalic nucleus with widespread cortical connections. We recently showed that the human claustrum is activated upon initiation of a cognitively demanding task that is met with the emergence of task-positive, cortical cognitive network activity. Thus, we hypothesize that the claustrum mediates cognitive network dynamics. To test whether there is a structural connectivity basis for this, we employed neuronal tract tracing and channelrhodopsin-assisted circuit mapping approaches to test the connectivity of the claustrum with key cognitive network nodes. Results of this study stand to inform circuit mechanisms of cognitive networks, which are disrupted, along with cognition, in several neuropsychiatric disorders including schizophrenia and addiction.
29. Dynamic control of synaptic substructure and function by cell 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 PSD-95 and several neurexin isoforms. In order to test the ongoing role of LRRTM2 in established synapses, we adapted an approach to acutely disrupt LRRTM2 binding interactions in the cleft. Using 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 to test how acute disruption of the LRRTM2 extracellular binding interaction impacts synaptic transmission. We find 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. Broadly, these results also indicate that synaptic cell adhesion molecules can play specific and unexpected roles in regulating function at established synapses well after synaptogenesis.
30. Prenatal Tobacco Exposure on Brain Morphometry and Cognitive Measures in the Adolescent Brain Cognitive Development (ABCD) Cohort

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INTRODUCTION: Prenatal tobacco exposure (PTE) may be associated with sex-specific effects on brain structural and cognitive development. However, the specific brain regions involved and the extent of sex-specific effects remain unclear. Using data from the ABCD Study, we cross-sectionally evaluated brain and neurocognitive measures in 9-10 year old children with (n=194) and without (n=4,202) PTE.

METHODS: Structural magnetic resonance imaging (sMRI), neurocognitive measures, PTE status and exposure intensity, and socio-demographic information, were obtained from the first data release for these children. sMRI were processed by the Data-and-Informatics Center using FreeSurfer. Neurocognitive measures were acquired using the NIH Toolbox. Two-way analyses of covariance were performed to compare the groups on brain and cognitive measures, and assess for possible sex-specific effects associated with PTE, while covarying for the children’s age, parents’ education and ABCD sites.

RESULTS: Compared to unexposed children, PTE children tended to show thinner superior-frontal gyrus (p=0.09), poorer working-memory (p=0.030) and episodic-memory (p=0.029). Regardless of PTE, compared to boys, girls showed smaller total brain area (p<0.0001) and volume (p<0.0001), thinner lingual gyrus (p=0.014), thicker anterior cingulate cortex (ACC, rostral-p=0.002, caudal-p=0.031), and had better episodic-memory (p=0.042). Relative to unexposed children, PTE-boys showed larger, while PTE-girls showed smaller cortical areas (interaction-p=0.045). Furthermore, only boys showed an association between more daily-cigarettes-exposed prenatally and thinner superior parietal (interaction-p=0.036) and middle temporal cortices (interaction-p=0.001).

CONCLUSION: These results support previous findings of PTE and sex effects on neuroimaging measures among school-age children. Additionally, PTE may have sex-specific effects on brain development, and greater PTE-intensity may preferentially affect the boys.
31. Circuit endophenotypes: A novel approach to investigate rare variants in schizophrenia

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Schizophrenia is a highly heritable psychiatric disorder that arises from neural circuit miswiring in the developing brain. A majority of mutations associated with schizophrenia are rare genetic variants, for which classical genetic linkage analyses and animal models have not been successful in identifying etiopathologic mechanisms. A new approach is needed to study the link between the complex genetics of schizophrenia, its multifactorial clinical manifestations, and the circuit pathology that underlies them. Using circuit endophenotypes to simplify aspects of the schizophrenia phenotype provides a bridge between the upstream genetics and the downstream behavior, allowing us to identify and investigate wiring in new rare variants. An example of such a variant is a mutation in the regulatory region of the cell-adhesion molecule, IgLON4, which was recently found upregulated in the dorsolateral prefrontal cortex of a small subset of schizophrenic patients. IgLON4 has been implicated in neurite extension in vitro, but has not previously been studied in the context of specific circuits in vivo. Here we show that IgLON4 overexpression results in aberrant axon collaterals in the cerebral cortex in vivo from pyramidal neurons in cortical layer II/III, thus suggesting a role in miswiring of the canonical neural circuit. CRISPR knockout also exhibits a circuit endophenotype resulting in a loss of projections connecting sensory to prefrontal integration areas. Changes to the canonical cortical circuit can have dramatic effects on information processing and may lead to the development of psychiatric disorders like schizophrenia. Investigating IgLON4 overexpression by identifying circuit endophenotypes will provide an insight on how the cortex normally develops and how cortical miswiring can become pathological.
32. USP24 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 and neuronal survival. 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.
33. Sex differences in descending noxious inhibitory control (DNIC) and the related brain circuitry

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DNIC, the phenomenon by which pain in one body part inhibits pain in distant body parts, is compromised in several chronic pain disorders. Sex differences in chronic pain prevalence and DNIC are observed in humans, but animal models that would allow the study of mechanisms underlying these differences and their clinical implications are not available. We have developed several rodent models to examine sex differences in DNIC. Initially, we compared latency to withdraw a hindpaw from a noxious thermal stimulus after capsaicin injection, a conditioning stimulus, in the forepaw in rats and mice of both sexes, observing that males exhibited more prolonged and stronger DNIC. Continuing with rats, we showed that this difference is consistent when a noxious mechanical stimulus is used as a test stimulus. We then paired capsaicin with mechanical stimulation of a CFA-sensitized masseter muscle as a test stimulus, maintaining the same sex differences. Valuably, these observations support that sex differences in DNIC are preserved across different species and experimental conditions. We then conducted fMRI experiments to determine which regions are involved in this process and how their functional connectivity differs between male and female rats. Using the PAG and ACC as seeds, we found stronger functional connectivity between PAG and ACC, as well as PAG and insula and prelimbic cortex in males. In females, we found stronger connectivity between ACC and hippocampus and thalamus. These findings will guide our future research on investigating central mechanisms that underlie sex differences in chronic pain conditions.
34. Role of the Parabrachial Nucleus in Migraine Pain

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Migraine headaches affect 13\% of the USA population, causing significant disability and contributing heavily to healthcare costs. Despite this severity, there is a relative lack of effective drugs to prevent and treat migraines. It is thought that migraine pathology includes irritation of the dura (the outermost layer of the meninges), and transmission through the trigeminal pathway, which relays sensory information from the head, face, and dura to central centers. However, we have not elucidated the central changes that underlie migraines. Clarifying these pathways could provide new targets for migraine management and prevention. One central nucleus receiving input from the trigeminal pathway is the Parabrachial Nucleus (PB). PB is involved in pain transmission, and we have shown previously that its activity is amplified in a rat model of trigeminal neuropathic pain. Using \textit{in vivo} extracellular recordings, we have identified for the first time a subset of PB neurons that respond directly to electrical stimulation of the dura (36 cells from 13 rats). We hypothesize that in a rat model of migraine, hyperactivity of dural-stimulus-responsive PB neurons underlies migraine headache pain. We predict that if we apply low pH solution to the dura (a commonly used rodent migraine model), firing rates for PB neurons of interest will increase. In preliminary experiments measuring PB activity several hours after applying low pH to the dura, we have identified dural-stimulus-responsive PB neurons in which either receptive field or neural activity is increased compared to baseline.
35. 5-HT1B receptor activation increases synaptic strength at stress-sensitive synapses

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Depression is a devastating mental illness that severely impairs quality of life. Selective Serotonin Reuptake Inhibitors (SSRIs) like fluoxetine are the most commonly prescribed antidepressants. However, we have a very limited understanding of how SSRIs act on neuronal circuits to treat depression. Antidepressant actions of SSRIs may be mediated by potentiating synapses which transmission is impaired by chronic stress. Determining the mechanism by which SSRIs potentiates these stress-sensitive synapses may be key to developing new fast-acting antidepressants. Here, we determine if activation of the serotonin receptor 5-HT1B is sufficient to potentiate two stress-sensitive synapses, the temporoammonic (TA-CA1) and the hippocampus-nucleus accumbens (Hipp-NAc) synapses.

We have previously shown that fluoxetine potentiates the TA-CA1 synapse by activating the 5-HT1B receptor using slice electrophysiology. We follow these studies up by determining whether this potentiation is also observed in vivo in anesthetized mice. We recorded optically evoked local field potentials in the SLM layer of CA1 of anesthetized mice to determine if an injection of the 5-HT1B receptor agonist Anpirtoline was sufficient to potentiate the TA-CA1 synapse.

Fluoxetine also restores synaptic strength at the Hipp-NAc synapse. However, it is not yet determined whether 5-HT1B receptor activation mediates the strengthening at this synapse. Here, we test whether 5-HT1B receptor activation is sufficient to potentiate the Hipp-NAc synapse. We performed whole-cell recordings of accumbal D1 Medium Spiny Neurons and measured evoked Excitatory Postsynaptic Currents (eEPSC). We found that washing on Anpirtoline, potentiated eEPSCs and that this was blocked when slices had been pretreated with the 5-HT1B receptor antagonist Isamoltane. Anpirtoline-induced potentiation was further prevented by pretreating the slice with the CaMKII inhibitor KN-62.

Together, our data suggests that 5-HT1B receptor activation potentiates Hipp-NAc synapses mediated by the activation of CaMKII. This provides a possible mechanism by which SSRIs can restore synaptic strength at stress-sensitive synapses.