Texas A&M Chapter of the Society for Neuroscience

Winter Symposium 2019

Thursday, December 5th, 2019

Interdisciplinary Life Sciences Building Lobby

Acknowledgements

The Texas A&M chapter for the Society of Neuroscience would like to thank all participants for sharing their work and all judges who volunteered their time. In addition, this would not have been possible without the hard work of TAMIN students who assisted in setting up the event. We hope to see you next year.

Thank you,

The Texas A&M Chapter for the Society of Neuroscience Committee

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Program

Texas A&M Chapter of the Society for Neuroscience

Poster Session& Vendor Show

Vendor Show: 10:00 AM - 2:30 PM
Poster Session A: 10:00 AM - 11:30 AM
Lunch (Food Provided): 11:30 AM – 12:30 PM
Poster Session B: 1:00 PM - 2:30 PM
Guest Speaker Seminar: 3:00 PM - 4:00 PM
Poster winners will be announced after the talk

Guest Speaker: Dr. Weichun Lin

Associate Professor, Effie Marie Cain Scholar in Medical Research
Department of Neuroscience, UT Southwestern Medical Center

Understanding Synaptic Biology Through the Neuromuscular Junction

Gift/Essentials Drive:
Voices for Children: Casa of Brazos Valley.
Please bring ***unwrapped*** *toys or essentials (socks, underwear, diapers) and gifts for teens (purses, wallets, jewelry) in the Brazos Valley.*
Please e-mail sfn.tamu@gmail.com for questions and inquiries

Table of Contents

Abstracts

The following abstracts are classified as follows:

Poster # – Category (U/J/S/P) – Session (A/B)

Undergraduate Students

1.U.A Memories at the Neuromuscular Junction are Resistant to Extinction

Tarbet, M; Hudson, K; Lout, E; Grau, J

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Prior studies have shown that neurons within the spinal cord can support instrumental learning (Grau et al., 1998, Behav. Neurosci., 112, 1366). In a typical experiment, rats undergo a thoracic (T2) transection and are subsequently tested by applying electrical stimulation (shock) to one hind leg whenever the leg is extended. Over time, transected animals learn to maintain the stimulated leg in a flexed position that minimizes net shock exposure. Treatments that impair spinal cord function (e.g., i.t. lidocaine), or that disrupt communication between the periphery and the spinal cord (e.g., cutting the sciatic nerve), block learning. Interestingly, if communication with the spinal cord is cut after the response is acquired, animals given response-contingent shock continue to maintain the leg in a flexed position. This implies that a peripheral modification contributes to the maintenance of the learned response. The present study explores the circumstances under which the learned response is weakened (extinguished). In Experiment 1, rats underwent a spinal transection and were trained for 30 min with response contingent shock the next day. After 25 min of training, half the animals had the sciatic nerve cut. Performance was tested for an additional 30 min, with or without response-contingent shock. During testing, all animals continued to maintain a flexion response. Next (Experiment 2), we examined whether exposure to non-contingent stimulation would weaken the learned response. Spinally transected rats received 30 min of training followed by 6 min of variable intermittent shock given independent of leg position. Performance was then assessed for 30 min. Exposure to non-contingent shock had no effect on the maintenance of the learned response. Further work is being conducted to examine whether a longer period of non-contingent stimulation, applied to other dermatomes, will lead to the extinction of the learned response.

1.U.B Pain Input Increases Lesion Site Hemorrhage After Traumatic Brain Injury

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Traumatic brain injury (TBI) is a major cause of death and disability in the United States. Many of these injuries are the result of events such as car accidents, combat violence, and falls, and often result in additional injuries (polytrauma) that serve as a source of pain (nociceptive input). Previous work in our laboratory has shown that nociception is detrimental to recovery after spinal cord injury (SCI) and leads to increased lesion site hemorrhage. It is not known whether these findings generalize to other

neurologic insults. The present study sought to determine whether nociceptive input expands the area of hemorrhage after TBI. Male Sprague-Dawley rats (N=12) weighing between 275-300 grams were given either a moderate traumatic brain injury in the right frontal region, using the Leica One controlled cortical impact device (2mm impactor tip, 4 m/s, deformation depth 3mm), or underwent a sham surgery. A day later, the irritant capsaicin (3%, 50 ml) or vehicle was applied to the contralateral hindpaw via a subdermal injection. Behavioral tests, including cylinder test and catwalk gait analysis, were performed at zero and three hours post nociceptive input. Animals were then euthanized, perfused with 4% paraformaldehyde, and brain tissue was collected. Collected tissue was sectioned and stained with hematoxylin and eosin to quantify hemorrhage extent at the lesion site, and sections were imaged. Images were analyzed for hemorrhage, quantified by percent area of hemorrhage, by a blinded experimenter using Image J software. The results showed that nociceptive input increased the area of hemorrhage at the injury site ($p < .05$) and produced an acute disruption in behavior. Pain-induced hemorrhage was limited to the injured hemisphere. On-going work is examining the circumstances under which pain input fuels hemorrhage after TBI, how it affects behavioral function, and the neurobiological processes involved.

2.U.A Effects of Host/Graft Sex Mismatch on Survival and Integration of Neural Progenitor Cell Transplants for Spinal Cord Injury

Pitonak, Michael; Jonika, Michelle; Bain, Gregory; Tucker, Ashley; Letchuman, Sunjay; Loesch, Kimberly; Blackmon, Heath; Dulin, Jennifer N.

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Spinal cord injury (SCI) is a devastating event that frequently results in permanent loss of neurological function. Transplantation of neural progenitor cells (NPCs) into sites of SCI has high potential for improving functional outcomes through the provision of new neurons that can reconstruct lost neural circuitry. However, little is still understood about the basic biological guidelines determining the success of NPC graft survival, integration with the host nervous system, and functional efficacy. Mismatch between the sex of donor and recipient tissue has been shown to contribute to organ transplant rejection in humans. Although there have been a large number of preclinical studies and clinical trials examining NPC transplantation for SCI, the potential effects of host/graft sex mismatch have not previously been investigated. Indeed, in the majority of experimental studies, the sex of donor tissue has not been reported. We therefore sought to determine whether sex mismatch between graft and host tissue influences the survival and integration of transplanted NPCs in a mouse model of spinal cord injury. Donor sex was determined for individual GFP+ mouse embryos by rapid genotyping of the X chromosome gene Rbm31x, which possesses the divergent Y chromosome gametolog Rbm31y. Either male or female spinal cord NPCs were isolated and transplanted into spinal cord lesion sites of either male or female adult mice immediately following injury. Four weeks after transplantation, we performed immunohistochemical analysis to determine graft cell survival, proliferation, neuronal differentiation, integration with host tissue, and extension of graft-derived axons into the host spinal cord. Results from this study will illuminate the effects of sex as a biological variable in the success of experimental NPC transplantation studies, and moreover will highlight an important consideration for

cell transplantation approaches in human clinical trials. Future work will examine the effects of donor cell sex on the host immune response following NPC transplantation.

2.U.B Characterizing the Role of Transcription Factor Hb9 in Glial Cell Development

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Hb9 (Mnx1) is a transcription factor described as a motor neuron specific marker in embryonic development and a critical factor for the development of spinal cord motor neurons. We have found that within the postnatal and adult spinal cord, Hb9-expressing cells are distributed in an increasing gradient along the rostro-caudal axis. This distinctive localization of molecularly-defined cells has yet to be described in the literature, and in fact this phenomenon has only been described for a handful of genes. Furthermore, immunohistochemical analysis of both neonatal and adult mouse spinal cord tissue has shown Hb9 expression in astrocytes, a population of glial cells in the spinal cord. In the peripheral nervous system, we observed a similar phenomenon with Hb9-expressing Schwann cells present in an increasing rostro-caudal gradient throughout the body. These preliminary observations have several exciting implications. Hb9 may play an important role not only in astrocyte development, but also in development of Schwann cells, which are the glial cells associated with the peripheral nerves. Additionally, characterization of the differences between Hb9+ and Hb9- glial cells may reveal new functional roles for glia throughout the developing spinal cord. Through characterizing the role of Hb9 in glial cell development, describing the molecular pathways involved, and determining the differences in gene expression between Hb9+ and Hb9- spinal cord glial cells, the developmental function of Hb9 can be better understood.

3.U.A The Role of Fragile X Mental Retardation Protein in Regulating Striatal Synapses

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The fragile X mental retardation protein (FMRP) is an RNA-binding protein that regulates the translation of hundreds of brain RNAs and has an important role in synaptic plasticity. This protein is encoded by the FMR1 gene located on the X chromosome, and includes RNA-binding regions such as the RGG and KH2 domains, the latter of which is critical to FMRP's regulation of synapses in hippocampal cells. A CGG trinucleotide expansion mutation in the FMR1 gene results in the absence of FMRP, leading to fragile X syndrome (FXS). FXS is the leading monogenic cause of autism and is characterized by impaired social and cognitive development, hyperactivity, restricted motor behaviors, and changes in synapse morphology. Despite the fact that multiple FXS symptoms relate directly to function of the striatum, very little is known about FMRP's role in this brain region. We have shown that cultured striatal cells lacking FMRP have a significant deficit in dendritic spines and synaptic puncta at 14 days in vitro (DIV), a sharp contrast to Fmr1 knockout (KO) phenotypes that are well characterized in cortex and hippocampus, indicating a unique role for FMRP in striatal synaptic development and stability at this time point. To investigate whether acute expression of FMRP is sufficient to rescue this deficit and

whether specific RNA-binding domains (e.g. KH2, RGG) are involved in FMRP's regulation of striatal synaptic puncta, we transfected Fmr1 KO cortical-striatal co-cultures with either wildtype- or mutant-Fmr1 expressing plasmids. The cells were then fixed at 14 DIV and stained for the presynaptic marker synapsin and the postsynaptic marker PSD-95 via immunocytochemistry, and their colocalization was used to quantify synaptic puncta. Our preliminary data suggest that the RGG, and not the KH2, domain is necessary for FMRP's role in regulating striatal synapses. Overall our results indicate that FMRP has a unique striatal function, which must be further elucidated in order to fully understand and provide effective treatment for FXS.

3.U.B Effect of Fetal Alcohol Exposure and Striatal Cholinergic Interneurons on Alcohol Intake in Adult Mouse Offspring

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Fetal alcohol spectrum disorder, resulting from prenatal alcohol exposure (PAE), is known to cause significant cognitive defects. One feature of these deficits is decreased cognitive flexibility. Cognitive behavioral flexibility is mediated, in part, by cholinergic interneurons (CINs) of the dorsomedial striatum (DMS). In this experiment we sought to elucidate the effects of PAE on CIN-dependent behavioral flexibility in the context of alcohol consumption. Female Ai14 mice were trained to consume high levels of alcohol using an intermittent access 2-bottle choice (IA2BC) drinking procedure. These mice were then crossed with male, non-PAE ChATCre mice, generating ChATCre;Ai14 offspring. The ChATCre;Ai14 mice were matched with non-PAE controls and all were exposed to alcohol via the IA2BC procedure. Bottles were weighed before and 24 hours after each drinking session to calculate alcohol intake. Surprisingly, we discovered that PAE decreased alcohol intake in adult female, but not male, offspring. In addition, female control mice consumed more alcohol in the presence of quinine, as compared to male control mice. However, this difference was disappeared in the PAE group. To explore the role CINs play in alcohol addiction, an AAV-hM3Dq-mCherry stereotaxic virus was infused into the DMS to trigger the selective expression of excitatory DREADDs in DMS CINs. CNO was then administered to half of the animals while the other half received a saline injection. We found that chemogenetic excitation of DMS CINs caused an increase in alcohol intake in both control and PAE offspring. It is known that striatal dopamine terminals contain nicotinic acetylcholine receptors (nAChRs) and that CIN excitation activates nAChRs leading to dopamine release. We discovered that a blockade of nAChRs extinguishes the effects of the CNO ligand, implying that these receptors play a key role in modulating drinking behavior. Together, the data suggests that PAE differentially affects alcohol intake in male and female and that CIN positively regulates alcohol intake. These changes contribute to PAE induced behavioral inflexibility.

4.U.A The Role of Fragile X Mental Retardation Protein in Cocaine Intravenous Self- administration and Synaptic Plasticity

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Cocaine, and other drugs of abuse, are known to induce synaptic plasticity in the dorsal striatum (DS) and nucleus accumbens (NAc), brain regions that play a key role in the early reinforcing effects of cocaine and the goal-oriented and repetitive behaviors involved in the development of addiction. Previous studies have identified a role for the fragile X mental retardation protein (FMRP) in mediating drug-induced changes in dendritic spine density in these regions, with Fmr1 knockout (KO) mice showing a significant increase in total spine density following repeated cocaine exposure. FMRP is an RNA-binding protein that regulates dendritic changes, transports messenger RNA, and regulates the translation of RNA into protein. In this study, we further elucidate the role of FMRP in regulating drug-related behaviors and cocaine-induced synaptic plasticity following repeated drug exposure in an operant cocaine intravenous self-administration assay. Twenty-four hours after the final IVSA session, tissue was collected and synaptic fractions from the NAc and DS were isolated. We found that, despite normal learning, Fmr1 KO mice fail to make a normal upward shift in self-administration behavior during dose-response testing and earn significantly fewer reinforcers than WT mice with increasing schedules of reinforcement. In addition, compared to wildtype mice, Fmr1 KO mice show significantly decreased levels of Arc, an mRNA target of FMRP and immediate early gene involved in synaptic plasticity, suggesting that FMRP may be important in regulating the expression of Arc in the synaptosome. Here we show that FMRP plays a key role in regulating cocaine self-administration behavior and consequent protein expression and localization. Ongoing work in our lab is examining specific RNA-binding regions and targets of FMRP that may be important in mediating drug-related synaptic plasticity. Our findings provide a better understanding of the biological basis of addiction and may lead to the improvement of therapeutic interventions.

4.U.B Aberrant Sprouting and Migration of Immature Granule Cells in the Adult Hippocampal Dentate Gyrus in Female Mice Following Spinal Cord Injury

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Numerous brain insults, as well as exercise and environmental enrichment, have been shown to alter adult hippocampal neurogenesis. In most brain injury models, including epilepsy, this altered neurogenesis includes the aberrant growth of basal dendrites into the dentate gyrus hilus and the ectopic migration of immature granule cells into the hilar region. However, such aberrant changes, that are known to alter hippocampal circuitry, have not been examined following spinal cord injury(SCI). Spinal cord injury is known to result in numerous changes to the brain and is known to exacerbate the development of post-traumatic depression. Numerous studies have linked depression that is not caused by SCI, to an observed decrease in adult hippocampal neurogenesis. There are scant reports that spinal cord injury can influence adult hippocampal neurogenesis. However, the data are contradictory, in that

some models, injury severity and post-SCI timepoints caused an increase in the number of newborn hippocampal granule cells, whereas others reported no change. Moreover, the aberrant sprouting of hilar basal dendrites from immature neurons, as well as the ectopic migration of immature granule cells into the hilus have not been previously examined in the context of SCI. These data are also obtained primarily from male rodents, and data are lacking regarding the influence of SCI on adult hippocampal neurogenesis in female rodents. These missing data are especially important because women are almost twice as likely to be diagnosed with depression than men. Therefore, the following study was performed to test the hypothesis that, SCI in female mice, will result in altered growth and migration of immature neurons in the hippocampal dentate gyrus.

5.U.A AAV5-mediated Alpha-synuclein Overexpression in the Mouse Midbrain as a Model for Early Parkinson's Disease: A Pilot Study

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Parkinson's Disease (PD) is a central nervous system disease that affects 60,000 Americans each year and is the most common neurodegenerative motor disorder. PD is characterized by muscle rigidity, loss of coordination, and cognitive deficits. PD patients can receive L-Dopa or deep brain stimulation; however, these only treat symptoms of PD because there is currently no cure. The lack of a cure can be partly attributed to a gap in knowledge of early pathology and symptoms of PD. One issue may be that current animal models of PD, which use 6-OHDA and MPTP degenerate dopaminergic neurons in the substantia nigra pars compacta (SNc) at a rapid pace. The immediate degeneration of these neurons does not allow for observations of early-stage PD. Therefore, there is an urgent need for an early-stage PD animal model in order to better characterize initial PD symptoms, which could lead to earlier diagnoses and preventative treatments. This study aims to develop an early PD model through overexpression of alpha-synuclein in the midbrain. Alpha-synuclein is a protein that has emerged as a major player in the progression of PD. Alpha-synuclein aggregates have been found in early stage multiple system atrophy (MSA) and in late stage PD brains; however, it is unknown how synucleinopathy begins in early PD. In order to monitor alpha-synuclein in early stages of PD, we injected C57BL/6 wild-type mice with an AAV5 overexpressing alpha-synuclein in dopaminergic neurons in the SNc. Mice were assessed every two weeks for behavioral deficits using established tests such as open-field, pole test, foot slips, and beam-walk. Unlike current PD models, mice that received alpha-synuclein did not display major behavioral deficits until almost four months post-injection. Compared to GFP injected controls, alpha-synuclein mice displayed longer beam walk cross times and a higher incidence of foot slips. In parallel experiments, mice were sacrificed at 1, 2 and, 4 months after injection with AAV5-alpha-synuclein or AAV5-GFP for histological analysis. A comparison between alpha-synuclein and GFP mice showed differences in the spread of alpha-synuclein and GFP to other brain regions. We also observed no robust difference in tyrosine hydroxylase (TH) staining for dopaminergic neurons in the SNc. Surprisingly, we see the progressive encroaching of alpha-synuclein containing dopaminergic nerve terminals on cell bodies of dorsal lateral striatal (DLS) astrocytes. Future work will determine if alpha-syn localizes within striatal astrocytes in our model of PD. Based on our data we suggest that alpha-synuclein overexpression in the midbrain can be used to model early PD in mice. "

5.U.B Toxin-Based Chronic Demyelination and Remyelination Mouse Model of Multiple Sclerosis

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Cuprizone is a copper chelator known to target mature oligodendrocytes and induces well-characterized demyelination in cerebral white matter with multiple pathological characteristics of type III Multiple Sclerosis (MS) lesions. The Cuprizone–mediated demyelination and remyelination occur independently of T cell activation, and the blood-brain barrier remains intact. In this study, we examined demyelination, glial responses and remyelination following chronic cuprizone exposure for 12 weeks. The mice were put on a 0.2% cuprizone diet for 12 weeks and then returned to a normal diet for 4, 8, and 12 weeks. Novel placement behavioral analysis as well as immunohistological analysis were used to characterize how the cuprizone diet impacted the mice. Tissue analyses were focused on the hippocampal and corpus callosum area. Behavioral analysis indicated that mice recovered for 12 weeks after chronic cuprizone exposure exhibited nearly identical preference for a novel object as those intoxicated with cuprizone for 12 weeks without any recovery. This suggests that spatial memory is not dependent on remyelination mechanisms. Immunohistochemistry was used to examine activated astrocytes, microglia, and myelin. Tissue analysis showed marked demyelination in corpus callosum and hippocampus at 12 weeks that was associated with reactive astrocytes and microglia. Time-dependent remyelination was evident during the recovery period, while astrogliosis and microgliosis decreased over time. By 12 weeks of recovery, myelin was largely returned in both regions. Future directions include understanding the molecular mechanisms of remyelination following chronic loss of myelin and identification of oligodendrocyte lineage cell populations that are capable of differentiating into myelin-producing cells. Knowledge gained from such studies could provide insights for future development of neuroprotective and regenerative strategy for patients with progressive multiple sclerosis.

6.U.A Dopaminergic Neuron and Synaptic Bouton Quantification in the Hippocampus Following Traumatic Brain Injury

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Traumatic brain injury is a major contributor to health problems within the US and can lead to various debilitating conditions such as neurological deficits, disabilities, and other morbidities. Notably, after a TBI, there is an increased risk for alcohol and drug consumption and abuse. As many as one third of patients that suffer a TBI, consume alcohol in what is considered the moderate to heavy range, following their injury.1 Increased Alcohol and drug use after TBI is further exacerbated by the fact that, from 1994 to 2004, approximately 37-51% of patients presenting at the emergency room with a TBI, had alcohol in their blood.2 Therefore, alcohol is a significant risk factor for, and co-morbidity of, a TBI. Because alcohol seeking behaviors and alcohol abuse have been linked to plasticity of the dopaminergic system, illuminating how a TBI can alter this system could provide insight into understanding how addiction-related systems are influenced by brain injury. The dopaminergic system is considered to be a major component of the reward system in the brain.3 In the hippocampus, a structure that is notably altered

following a TBI, a major source of dopaminergic innervation is by the ventral tegmental area (VTA). 4 The VTA is activated when the hippocampus detects novel information. Upon detection, dopaminergic signaling is transmitted through the nucleus accumbens and ventral pallidum,4 to the VTA, resulting in hippocampal dopamine release. The release of dopamine in the dorsal hippocampus is integral to promoting attention and synaptic plasticity, as well as facilitating long-term potentiation of memory pathways.4 Considering the role of the hippocampus in spatial learning and memory, it is possible that this pathway represents an anatomical substrate for a spatial component to the rewarding effects of dopamine. Consistent with this notion, D1 and D2 receptors within the pyramidal cell layer, can enhance consolidation of novel information into long-term memories, possibly via actions on the limbic reward system pathway.4 Studies in epilepsy suggest that brain insults can alter dopaminergic signaling in the hippocampus. Therefore, it is possible that other types of brain insults, including TBI, might also modulate dopaminergic signaling in the hippocampus. For this study, we hypothesize that, TBI can cause alterations to D1 and D2 receptor expression in the hippocampus. To test this hypothesis, we utilized transgenic D1 and D2 expressing mice to quantify D1 and D2 expressing cells and synaptic boutons in the hippocampus. A lateral fluid percussion injury was utilized as the TBI model.5 To quantify the neuronal and bouton counts a manual counting regimen was used. The hippocampus, within a range of -1.46 to -2.14 from bregma, was subdivided into regions based on Paxinos atlas. The D1- and D2-expressing neurons and boutons were counted, assigning a specific color to the cells in each subregion of the hippocampus. The anatomical regions counted include the three layers of the dente gyrus (hilus, granular cell layer, and molecular cell layer), CA1, CA2, and CA3 regions of the hippocampus, as well as the fasciola cinereum region. The two groups of bouton axons that extend within the CA2 and CA3 pyramidal layer, presumably mossy fibers, were also quantified.

6.U.B Theiler Murine Encephalomyelitis Virus and Effect on Collaborative Cross Mice's Gait and Grip Strength

Alec Pinon; Candice Brinkmeyer-Langford; Colin Young; Katia Amstalden; David Threadgill; Jane Welsh; Aracely Perez; Koedi Lawley

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Theiler's Murine Encephalomyelitis Virus (TMEV) is used as a model in mice to study the neurological pathology of diseases and conditions similar to that of Multiple Sclerosis and many other neurological conditions such as amyotrophic lateral sclerosis and Parkinsons. Genetic background of the mice can play a significant role in determining the reaction to infection with TMEV and overall progression of neurological conditions. The reaction and disease course can be monitored through phenotypic means, by utilizing tools such as DigiGait and BioSeb Grip Strength tests. These tools allow us to monitor and quantify symptoms such as paralysis, paresis, weakness, gait, and other movement and strength related variables, in order to track progression of the infection and related effects.

Junior Graduate Students

7.J.A Differential Effects of Various Opioids and Sociability Levels on Affect and Anxiety-like States

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Social environment influences the trajectory of developing opioid use disorder (OUD). Thus, this study tested the hypothesis that sociability levels will affect the responses to different opioids. Mice were tested for their baseline sociability, anxiety levels, pain sensitivities, and their acute locomotor response to opioids. Then, they were administered daily saline, hydrocodone, or morphine, and re-tested for locomotor sensitization and the effects of opioids on their sociability, anxiety levels and pain sensitivity. Finally, mice acquired and extinguished conditioned place preference (CPP) before reinstatement was assessed. Based on their baseline sociability level, mice were classified as Socially Avoiding (SA) or Socially Exploring (SE). SA and SE mice did not differ in their baseline weight and anxiety sensitivities. SA mice had higher baseline heat sensitivity. Anxiety sensitivity increased in SA mice and deceased in SE mice. Acute locomotor response was stronger to hydrocodone in both social groups. However, stronger locomotor sensitization was developed to morphine, especially in SE mice. Morphine-injected SA, but not SE, mice spent more time in the center zone of the OFT and in the light zone of L/D boxes, and developed heat hyperalgesia. Mice previously exposed to morphine, but not hydrocodone, reinstated conditioned place preference to a 1mg/kg priming dose whereas all other groups reinstated at higher doses. In both social groups, repeated morphine administration had overall stronger effects compared to hydrocodone. Sociality exploring animals are more sensitive to the sensitizing effects of opioids. Opioids have greater effects on the stress and pain systems of socially avoiding animals. The underlying mechanisms for developing OUD might differ in individuals with various sociability levels. Pre-exposure to morphine but not hydrocodone during adolescence may increase the risk of relapse in adulthood.

7.J.B The Necessity of Microglial Activation in Morphine-Induced Attenuation of Recovery Following Spinal Cord Injury

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One of the most significant consequences of spinal cord injury (SCI) is acute and chronic pain. In the emergency setting, opioids are used to manage this pain. Approximately 80% of patients are treated with morphine within the first 24 hours following injury (Stampas et al., unpublished data). Unfortunately, we have also found that the amount of opioids administered in the first 24 hours post injury are positively correlated with the severity of pain that patients report at one year post SCI. In rats, we also found that morphine treatment in the first 7 days post SCI not only increases symptoms of pain at 42 days post injury (Hook et al. 2009), but also undermines the recovery of locomotor function. Prior research implicated microglia in opioid-induced hyperalgesia and the development of neuropathic pain, we hypothesized that morphine may be increasing the inflammatory response post-SCI to produce these

adverse effects. To test this, we used flow cytometry to quantify the expression of microglia/macrophages after a moderate contusion injury and morphine administration. We found that, irrespective of the route of administration, morphine increases the number of activated microglia present at the injury site, relative to saline-treated SCI controls. Significantly, administration of minocycline prior to intrathecal morphine not only blocked the morphine-induced increase in microglial expression, it also protected locomotor recovery. Extending this study to a clinically-relevant paradigm, the current study will determine the necessity of microglia in the morphine-induced development of chronic pain and attenuation of recovery using an intravenous morphine administration model.

8.J.A Striatal Cholinergic Interneurons and Fetal Alcohol Exposure-induced Behavior Inflexibility

Xie, Xueyi; Binette, Annalise; Purvine, William; Ramos, Joseph; Wang, Xuehua; Walters, Kallan; Chainaranont, Anna; Hoang, Duc; Wang, Zhangyang; Smith, Laura; Miranda, Rajesh; Wang, Jun

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Prenatal alcohol exposure (PAE) is a leading cause of fetal alcohol spectrum disorder (FASD) which is often characterized by cognitive impairment. Several studies have suggested that PAE could reduce cognitive flexibility, which is mediated, in part, by cholinergic interneurons (CINs) in the dorsomedial striatum (DMS). In this study, we are trying to understand whether PAE-induced behavioral inflexibility is due to changes in DMS CINs. We first trained female Ai14 mice to consume high levels of alcohol using an intermittent two-bottle choice drinking procedure for 6 weeks, and then mated them with male alcohol-naïve ChATCre mice. We found PAE preferentially increased locomotor activity in male offspring. The number of ChAT+ neurons in the striatum of adult offspring as well as cholinergic activity were significantly reduced by PAE. To examine whether CIN activity regulates alcohol intake, we infused AAV-flex-hM3Dq into the DMS of adult offspring and found that chemogenetic CIN excitation by CNO increased alcohol intake. Since it has been established that CIN excitation increases dopamine release through its action on nicotinic receptors, we then tested whether nicotinic receptors contribute to CIN-mediated alcohol intake. We applied nicotinic AChR antagonist DhβE and observed that CIN-mediated increase in alcohol intake was abolished. Finally, we tested whether PAE induces behavioral inflexibility by operant reversal learning paradigm and found that male mice showed a deficit in the learning tasks. Our data suggested that PAE might induce changes in DMS CIN which essentially lead to behavior inflexibility.

8.J.B Novel Mediators of Prenatal Alcohol Effects in Neural Stem Cells: Gag-like Proteins as RNA Chaperones for Intercellular Communication

Pinson, Marisa R.; Tseng, Alex M.; Chung, Dae; Nair, Vijay; Miranda, Rajesh C.

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Extracellular vesicles (EVs) are nanometer-sized, membrane-bound vesicles released by cells that may serve as a means of intercellular communication by transporting biological information between cells. Recently the Gag-Like Protein (GLP) Arc was identified in neuron-derived EVs, and found to transport its own mRNA between neurons. This raises the possibility that other members of the brain-enriched GLP

family may function like Arc, and the ancestral retrovirus Gag protein, in their ability to interact with mRNA and self-target for membrane bound export in EVs. GLPs may chaperone RNA in intercellular transport and therefore, serve as a means for programming the multipotency and differentiation of neural stem cell (NSC) ensembles. GLPs may also mediate effects of alcohol in developing tissues. In line with this reasoning, we evaluated the expression of GLP mRNAs in neural development under basal and ethanol exposure conditions by using qRT-PCR and of proteins using immunoblot. The GLPs PEG10 and PNMA2 were knocked-down (KD) and the impact on NSC differentiation was determined by qRT-PCR and immunoblot. Furthermore, effect of KD of GLPs on cellular metabolic activity was determined by MTT assay, on cell cycle progression Click-iT EdU flow cytometry, and apoptotic activity by Caspase-Glo 3/7 assay. Results show that transcript expression of PNMA2 increased during differentiation, suggesting a role in NSC maturation. Furthermore, ethanol exposure resulted in a dose related-increase in mRNA and protein for GLPs, PEG10 and PNMA2. Ongoing studies are evaluating the effect of GLP KD on NSC differentiation, cell cycle progression, and apoptotic activity. These data support a hypothesis that GLPs mediate compensatory mechanisms for cell survival or maturation following ethanol exposure. Further understanding how GLPs mediate and coordinate neural development may lead to interventions that moderate the harmful effects of prenatal alcohol exposure.

9.J.A Sex Specific Differences in Patients with Alcohol Spectrum Disorders

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Fetal Alcohol Spectrum Disorder (FASD) is a complex neurodevelopmental disorder compromising of a constellation of variable cognitive and physical abnormalities as a result of in utero alcohol exposure. While in-utero alcohol exposure effects both male and female fetuses, sex specific differences have been shown and outlined in animal models. In this paper, a literature review was done to outline sex specific differences in FASD in humans. Studies have shown that alcohol exposure during pregnancy can affect the sex ratio prevalence. Other studies have shown sex specific differences in brain development including white matter myelination and change in brain cortical thickness and total brain volume in children with FASD. May et al. conducted a epidemiological retrospective study in South Africa comparing sex ratios between children with FASD and healthy controls. Urban et al. used Diffusion Tensor Imaging (DTI) to study white matter myelination changes in participants with FASD. Treit et al. similarly used DTI to study brain volume and cortical thickness differences between the sexes in participants with FASD. Tesche et al utilized Magnetoencephalography (MEG) to investigate brain dynamics of adolescents with FASD during the performance of an auditory oddball task. Studies have shown that alcohol exposure during pregnancy can effect the sex ratio prevalence with higher demise of males prenatally in comparison to control population without alcohol exposure. Aged matched females with FASD were shown to have delayed myelination in comparison to males. Both sexes with FASD were shown to have decreased brain volume and cortical thickness compared to participants without FASD. However, the difference in brain volume and cortical thickness were more prominent in males with FASD than females with FASD when compared to sex matched controls.6 Another study showed presence of differential activation of the auditory pathway between the sexes in participants with FASD.5 Conclusion: These findings outline epidemiological and physiological differences between the sexes in FASD. This highlights the potential for further studies into the sex specific physiological changes in FASD and potential development of individualized management plans based on the differences.

9.J.B Ventral Hippocampus Inactivation Reduces Context-Dependence of Signaled Active Avoidance

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There are many factors that can affect learning and memory including mood, drugs, or even the context in which learning occurs. Context has become a focus in the emotional memory literature because it strongly affects where pathological fear responses are expressed. This is particularly relevant to the relapse of fear that occurs in some individuals after therapy, which is due in part to the context-dependence of the memories formed during therapy. Although there is considerable work on the role for context in the expression of reactive fear responses, such as freezing behavior or autonomic arousal, there is little work on how context regulates active fear responses, including avoidance. Given the criticality of avoidance behavior in both normal defensive responses to threat, as well as pathological avoidance behavior that accompanies a range of psychiatric disorders, it is important to examine the context dependence of avoidance. In both rats and humans, avoidance behavior can be modeled in the laboratory using instrumental conditioning procedures in which subjects can avoid an aversive outcome by making a particular behavioral response. We specifically use a two-way signaled active-avoidance (SAA) task in rats, in which animals learn to shuttle from one compartment to another in response to an auditory warning signal that predicts an aversive footshock. Preliminary data we have collected show for the first time that SAA is context-dependent after 4 days of conditioning but becomes context-independent after 8 days of conditioning. Specifically, after 4 days of conditioning, rats exhibit higher levels of avoidance responding (assessed during an extinction test) in the conditioning context, relative to a novel context. The decrease in avoidance in the novel context is accompanied by an increase in freezing behavior. Previous research has shown that context-dependence of avoidance depends on the hippocampal-associated cortices. Moreover, the ventral hippocampus (VH) is necessary for increases in freezing in a novel context after extinction, a mechanism that might contribute to the loss of avoidance responding after a context shift. We have recently found that inactivating the VH with a GABAA agonist, muscimol prevents the context-shift deficit. Our next steps will be to look how the importance of the VH changes over time and to examine the projections from the VH to the infralimbic cortex and basolateral amygdala as potential pathways involved in the context shift deficit.

10.J.A The Role of Astroglial Sphingosine-1-Phosphate Receptor 1 in the Developing Brain

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Sphingosine 1-phosphate (S1P), a bioactive lipid molecule, regulates diverse biological processes including cell proliferation, migration, and activity through a family of G-protein coupled S1P receptors (S1PRs). Among S1PRs, S1PR1 is highly expressed in the central nervous system (CNS); however, its physiological function in the CNS remains poorly understood. In situ hybridization analysis of mouse brains at various postnatal developmental stages reveal abundant and increased expression of S1PR1 in Bergmann glia of the cerebellum and protoplasmic astrocytes of the grey matter overtime. We

hypothesize that S1PR1 mediated signaling contributes to normal brain development and that disruption of astrocytic S1PR1 may result in functional alterations of Bergmann glia and consequently in differential cell migration, cell proliferation, and cerebellar functionality. To investigate the

functions of astroglial S1PR1 in vivo, we generated conditional Aldh1l1CreERT2:S1pr1fl/fl mice where S1pr1 is selectively ablated in astrocytes, including Bergmann glia, in a tamoxifen- inducible manner. Immunohistochemistry analysis reveal strong immunoreactivity and membranous localization of S1PR1 in Bergmann glial processes from wild-type mice but not in those from the conditional knockout mice. We are currently employing double immunohistochemistry and EdU tracing methods to determine the effects of S1pr1 ablation on proliferation and maturation of Bergmann glia and on migration of granule cells over the first two weeks of postnatal cerebellar development. We will further examine the role of S1PR1 in development of astrocytes using Ai14 reporter mice to visualize all astrocytes that underwent S1pr1 deletion. This study shall provide new insights into the in vivo functions of S1PR1 in astroglial development and their interactions with neurons.

10.J.B MiR-20a-3p Mediates Mitochondrial Function in Normoxic and Ischemic Conditions

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Astrocytes from middle-aged reproductively senescent rats, who typically have larger infarct volumes after stroke, have been shown to have a reduced functional capacity for glutamate clearance and production of trophic factors compared to astrocytes from adult rats, who typically have smaller infarct volumes. Epigenetic analysis revealed greater H3K4 trimethylation of the promoter region of the mir17-92 cluster in adult astrocytes, and qRT-PCR analysis confirmed increased expression of all members of this cluster including a 240-fold elevation of mir20a-3p. Intravenous injection of mir20a-3p mimics 4h after induction of ischemia improved stroke outcomes in middle-aged female rats. Bioinformatics indicate that mir20a-3p modulates a number of mitochondrial genes, and this study tested the effect of mir20-3p on mitochondrial function in astrocytes in normoxic and ischemic conditions. Cultures of male and female human astrocytes were assigned to normoxic (21% O2, 25 mM glucose) or ischemic (1% O2, 0 mM glucose) conditions for 6h. Cells were treated with 50 nM mir20a-3p, 50 nM scrambled control miR or vehicle. Mitochondrial function was assessed by Fluorescent Recovery After Photobleaching (FRAP) and Seahorse XFe96 Analyzer. Imaging of astrocyte cultures indicates FAM-labeled mir20a-3p uptake, and qRT-PCR analysis indicates a significant sex difference in mir20a-3p expression after OGD that recapitulates the in vivo phenotype. Treatment with mir20a-3p significantly accelerated fluorescent recovery compared to cells treated with scrambled miR or vehicle in normoxia and OGD for both sexes. A significant sex difference was observed in fluorescent recovery, with the male cells experiencing a dampened effect in response to mir20a-3p or scrambled treatment. Additionally, preliminary data using the Seahorse XFe96b analyzer suggest that miR-20a-3p may be suppressing mitochondrial respiration in ischemic conditions. The results from the Seahorse XFe96 analyzer validate bioinformatics predictions stating that miR-20a-3p represses mitochondrial genes. However, the results from the FRAP data suggest that suggest that this microRNA may promote other aspects of mitochondrial function. Since the rate of fluorescent recovery measures the continuity of the mitochondrial membranes, these data

suggest that mir20a-3p promotes mitochondrial fusion. If greater continuity of the mitochondrial membrane compensates for the greater energy demand required after ischemic injury, these data may explain why stroke recovery is better in young females compared to males.

11.J.A The Effects of Spinal Cord Injury on Hippocampal Neurogenesis

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External stimuli, such as exercise and environmental enrichment, have been shown to increase adult hippocampal neurogenesis. Stress, as well as brain insults, including seizures, stroke and traumatic brain injury (TBI), have also been shown to reduce neurogenesis, which is detrimental to hippocampal circuitry. Decreased hippocampal neurogenesis has also been associated with depression. Interestingly, the incidence of depression is significantly increased in patients who've suffered spinal cord and traumatic brain injuries (Hill et al., 2015). Whereas there are numerous studies examining neurogenesis after brain injuries, very few studies have examined changes to adult hippocampal neurogenesis following a spinal cord injury (SCI). In one study, neurogenesis was decreased at 60 days following three different intensities of thoracic spinal cord compression in 8-week old male rats. The decrease was most robust in the most severe SCI group (Jure et al., 2017). Conversely, at 6 weeks following a thoracic contusion SCI, no change in hippocampal neurogenesis was observed (Franz et al., 2014). Therefore, it is possible that different spinal injuries, or injury severities, might differentially influence neurogenesis, and these differences might be related to why some patients will develop post-traumatic depression, whereas others will not. This study will assess the number of immature neurons in the hippocampus of 12-week-old male and female mice, at 4 weeks after a mild thoracic SCI. Mild T8 spinal cord contusion was induced (with 40 kdyne of force) as previously described (Hook et al., 2017), with a protocol adapted for mice. The mice were sacrificed 4 weeks after the SCI, via transcardial perfusion with sterile saline followed by 4% paraformaldehyde. After sacrifice, brains were extracted, cryoprotected and serial sections were obtained using a freezing microtome. Immunohistochemistry was performed for anti-doublecortin (DCX), to visualize immature neurons in the hippocampal dentate gyrus and subgranular zone. To quantify the DCX-labeled cells, 5000 μm2 numbered grids were successively placed in the sub-granular zone and upper layers of the granule cell layer. A random number generator was used to select >60% of the boxes to stereologically estimate the number of DCX-labeled cells in the hippocampal dentate gyrus. Data collection is ongoing, by a rater blind to the condition of the mice. Once completed, the results will be analyzed using a t-test.

11.J.B Gut Dysbiosis Modulates the Impact of Ischemic Stroke in Male Rats Contributing to Their Worse Outcome Compared to Age Matched Females

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Sex differences in stroke have been well documented with young adult males having a higher risk for and a worse outcome after a stroke event than young adult females. This has been attributed to the

neuroprotective properties of the gonadal steroid estrogen, however, recent evidence indicates that the gut microbiome may play an important role in stroke outcomes. The gut response to stroke has been shown to be immediate and significant. Here we tested the hypothesis that males have a greater permeability of the gut blood barrier and experience gut dysbiosis both constitutively and in response to stroke. Male and female Sprague Dawley rats (5-7 months of age) were subject to endothelin-1 induced middle cerebral artery occlusion (MCAo) simulating an ischemic stroke event. Sensory motor tests were conducted pre and 2d after MCAo. Blood and fecal samples were also obtained at the same time points. We report that male rats have greater mortality and sensory motor deficit post MCAo as compared to age matched female rats. Additionally, males have significantly higher serum levels of Mucin-2 and LPS post MCAo indicating leakier gut. Males also have higher levels of proinflammatory cytokines IL-17A, MCP-1 and IP-10. Fecal mucin-1 was significantly decreased post MCAo only in the males likely caused by a loss of mucolytic bacteria indicating gut dysbiosis. In order to analyze gut morphology, the villus length to crypt length ratio was quantified and showed a significant decrease in the male MCAo group and a significant sex different indicating a stronger damage response in the males. We also stained for tight junction proteins Claudin-2 and ZO-1 and found continuous expression along the villi in the female sham, female MCAo and male sham groups, however, the male MCAo group had gaps in expression along the villi. Taken together, these results indicate that the worse stroke outcome in the male rats is associated with deterioration of normal gut architecture and a more permeable gut.

12.J.A Retrograde Mapping of Inputs to Direct- and Indirect-Pathway Neurons in the Posterior Dorsomedial Striatum

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The dorsomedial striatum (DMS) is necessary for goal-directed learning and strongly implicated in alcohol use disorder. The posterior DMS receives multiple extrastriatal inputs and primarily consists of dopamine D1 receptor-expressing (D1Rs) medium spiny neurons (D1-MSNs) and D2R-expressing MSNs (D2-MSNs). These neurons show aberrant synaptic plasticity due to excessive alcohol consumption, however, the afferent inputs onto pDMS D1- vs. D2-MSNs are uncertain. To classify the presynaptic neurons projecting onto either D1- or D2-MSNs in the pDMS, we used cutting-edge monosynaptic retrograde tracing technology to label presynaptic neurons in the whole mouse brain. Next, we established the degree to which presynaptic neurons projecting onto pDMS D1-MSNs (or D2-MSNs) also express D1Rs (or D2Rs). We found numerous projections from the distinct cortical regions, thalamus, amygdala, and midbrain. Remarkably, we discovered that extrastriatal D1-MSN-projecting neurons did not contain D1Rs; similarly, most D2-MSN-projecting neurons did not contain D2Rs. We found limited expression of D1-MSN-projecting neurons in cortical and thalamic regions that contained D1Rs; some D2-MSN-projecting neurons in the cortical, thalamic, and midbrain also expressed D2Rs. Our results indicate that linked corticostriatal and thalamostriatal neurons do not express the same type of dopamine receptors; this has never been addressed in the addiction field. Further characterization of these pathways will also advance understanding of the pDMS circuit in drug and alcohol addiction.

12.J.B The Parkinson's Disease-related Gene LRRK2 Plays a Crucial Role in Controlling Innate Immune Responses During Mycobacterium Tuberculosis Infection

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Mutations in the Parkinson's disease (PD)-associated gene leucine rich repeat kinase 2 (LRRK2) have been implicated in susceptibility to mycobacterial infection, but little is known about its function outside of the central nervous system. To investigate possible roles for LRRK2 in the immune response to bacterial pathogens, we infected two strains of LRRK2-mutant mice—LRRK2 KO and LRRK2 G2019S, a knock-in strain that expresses the most common gain-of-function PD allele, with the important human pathogen Mycobacterium tuberculosis (Mtb). We found that LRRK2 KO and WT mice harbored similar bacterial burdens; however, loss of LRRK2 led to increased inflammation and neutrophil infiltration in the lungs of Mtb-infected mice at an early infection time point (21 days), suggesting a role for LRRK2 in regulating innate immune responses in the lung. Consistent with a role for LRRK2 in controlling inflammation, Mtb-infected LRRK2 KO mice demonstrated hyperactivation of astrocytes in PD-relevant regions of the brain relative to Mtb-infected control mice. These results suggest that peripheral infection can alter immune cells in the brain—it is tempting to speculate that such changes may precipitate neurodegeneration, especially in individuals harboring genetic susceptibilities. Extensive experiments in ex vivo macrophages suggest that LRRK2's role in innate immune outcomes stems at least in part from its crucial role in maintaining mitochondrial homeostasis and influencing basal type I interferon levels. In contrast to LRRK2 KO mice, Mtb-infected G2019S mice had increased bacterial burden, high levels of pro-inflammatory cytokines, and displayed a dramatic increase in the extent and severity of pulmonary inflammation compared to WT mice. These results illuminate complex, yet underappreciated, links between LRRK2 and the innate immune response and indicate that there are intimate connections between the peripheral immune response, neuroinflammation, and activation of brain-resident glial cells that may play a critical role in triggering or exacerbating PD.

13.J.A Curcumin Nanoparticle Therapy Modulates Neuroinflammation, Neurogenesis and Mitochondrial Function and Improves Brain Function in a Model of Gulf War Illness

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Gulf War Illness (GWI) is a multi-symptom illness, which affects approximately 30% of veterans who served in the first Gulf War. Epidemiological studies have suggested that exposure to a combination of chemicals that inhibit acetylcholinesterase activity, and stress, during the war caused GWI. Indeed, concurrent exposure to low doses of chemicals widely used in GW and mild stress for 28 days in rats

leads to symptoms seen in veterans with GWI. The central nervous system related symptoms in GWI include cognitive and mood dysfunction in association with chronic neuroinflammation and decreased neurogenesis in the hippocampus. We investigated the efficacy of curcumin encapsulated biodegradable nanosystems (nCUR, a compound having robust antioxidant and anti-inflammatory properties) for improving brain function and modulating neuropathological changes in animals exposed to GWI-related chemicals and stress (GWI rats). We first exposed young rats to low doses of GWI-related chemicals such as the nerve gas prophylactic drug pyridostigmine bromide (PB), mosquito repellant DEET, insecticide permethrin (PM) for 28 days. Two months later, GWI rats received nCUR at 10, 20 or 40 mg/Kg (3 days/week) or empty nanoparticles (vehicle) for eight weeks. A group of age-matched naïve control rats was included as controls. A battery of behavioral tests performed in the last four weeks of treatment revealed cognitive and memory dysfunction, pattern separation deficit, and anhedonia in GWI rats that received empty nanoparticles. Moreover, the hippocampus of these animals displayed activated microglia, hypertrophied astrocytes, decreased neurogenesis and increased expression of genes that encode proteins relevant to mitochondrial electron transport with elevated levels of mitochondrial complex proteins I, II and IV. In contrast, GWI rats that received different doses of nCUR showed better cognitive, memory and pattern separation function and no anhedonia. Besides, the hippocampus of these animals displayed reduced density of activated microglia and hypertrophied astrocytes, increased neurogenesis, and normalized expression of mitochondrial genes and complex proteins II and IV. Interestingly, a maximal increase in neurogenesis was observed in GWI rats that received the lowest dose of nCUR (10mg/Kg). Thus, eight weeks of low-dose nCUR treatment is sufficient for improving brain function in a model of GWI. Reduced neuroinflammation, enhanced neurogenesis, and normalized mitochondrial activity likely underlie the improved brain function mediated by nCUR treatment.

13.J.B Inactivation of Prelimbic Projections to Rostromedial Tegmental Nucleus Enhances Cue- induced Reinstatement of Cocaine Seeking

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The prelimbic cortex (PL) has been implicated in the regulation of drug-seeking behavior but has been shown to have a degree of functional flexibility that supports roles in both promoting and suppressing of drug seeking. Within PL we found distinct neuronal subpopulations that project to either the nucleus accumbens core (NAc core) or the rostromedial tegmental nucleus (RMTg). Previous studies have established PL projections to NAc core are necessary for reinstatement of cocaine seeking in rats. Given that RMTg has been implicated in behavioral inhibition, we hypothesized that PL projections to RMTg may suppress drug seeking, indicating that PL-NAc core and PL-RMTg pathways play opposing roles in regulating drug-seeking. To test this hypothesis, we used a functional disconnection to temporarily disrupt the PL-RMTg pathway. Male Sprague Dawley rats self-administered cocaine during daily 2-hour sessions for 12-15 days, in which lever presses were reinforced by intravenous cocaine infusions (0.2 mg/infusion; fixed ratio 1) paired with light/tone cues. Rats then underwent extinction training for 7-14 days, during which cocaine and cues were no longer available. Reinstatement of drug-seeking behavior was elicited by tone/light cues or cocaine prime (10 mg/kg, i.p.). To temporarily disconnect the PL-RMTg pathway prior to reinstatement, rats received a unilateral microinjection of GABA agonists baclofen/muscimol in PL (1 mM/ 0.1 mM) and a contralateral microinjection of AMPA receptor

antagonist NBQX in RMTg (1 mM). We found that functional disconnection of PL-RMTg increased cue-induced reinstatement, as compared to within-subject vehicle control, indicating that this circuit normally plays a suppressive role in cocaine seeking. In contrast, we found that functional disconnection of PL-RMTg had no effect on cocaine-induced reinstatement. Taken together with previous evidence supporting a role for PL projections to NAc core in driving drug seeking, these data indicate that PL projections to RMTg play an opposing role by suppressing drug seeking.

Senior Graduate Students

14.S.A Dorsal Hippocampus Mediates Covert Retrieval of a Contextual Fear Memory

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Memories enter a liable state after retrieval, and administration of protein synthesis inhibitors interferes with the reconsolidation of reactivated memories. Although inhibition of protein synthesis within the amygdala interferes with the reconsolidation of fear to a first-order auditory conditioned stimulus (CS), indirectly reactivated fear memories are not sensitive to protein synthesis within the amygdala. Given that higher order S-S associations are thought to depend on the hippocampus, we conducted a series of experiments to examine the role of the dorsal hippocampus (DH) in the reconsolidation of indirectly retrieved fear memory. Previous reports suggest backward (BW) conditioning, a procedure in which the unconditioned stimulus directly precedes the CS is mediated by contextual fear. To confirm this, we tested whether extinction of the conditioning context selectively reduced fear to a BW CS. Accordingly, animals were fear conditioned using either forward (FW) or BW trials (context A). Next, animals underwent context extinction (A) or novel context exposure (B). Finally, animals were tested for fear to the CS in a third novel context (C). Results revealed that CS-elicited fear was attenuated in BW-conditioned but not FW-conditioned animals. In a separate cohort, we also examined whether the opposite was true. That is, does extinction of an auditory CS in a novel context (B) reduce fear to the conditioning context (A) selectively in BW-conditioned animals. Similar Exp. 1, impairments in contextual fear retrieval following CS extinction were selective to animals that had received BW conditioning. In the final experiment, animals were implanted with bilateral cannulae aimed at DH and after recovery were conditioned to a FW or BW CS as in Exp.1 and 2. 24 hours later rats received a single CS presentation in a familiar context (B), which we hypothesized would reactivate the memory of the conditioning context (A) in BW but not FW-conditioned animals. To target the reconsolidation of this reactivated context memory we infused a protein synthesis inhibitor (rapamycin) or vehicle into the DH immediately following the retrieval session. Lastly, we assessed fear in the conditioning context (A). Consistent with our hypothesis, intra-DH infusion reduced fear selectively in animals that were conditioned to a BW CS. These results provide evidence that the BW, but not FW, CS reactivated a hippocampal representation of the original conditioning context, and that reconsolidation of this memory required hippocampal protein synthesis. This has important implications for novel therapeutic approaches to target and selectively erase traumatic memories.

14.S.B Pre-existing Inflammation Increases the Likelihood of Depression after Spinal Cord Injury

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Previously, we have shown that approximately one-third of spinally injured rats exhibit behavioral, physiological, and immunological correlates of depression. This incidence corresponds with the clinical spinal cord injury (SCI) population, where 18-26% of patients experience significant depression symptoms. Concomitant with humans, rats exhibiting depression behaviors post-SCI also have elevated serum cytokine levels, compared to not-depressed conspecifics. Additionally, these rats have higher serum IL-6 levels before injury, indicating that inflammation may be a predictor for depression. We hypothesized that IL-6 may not only indicate depression susceptibility, but also cause it. To test this, we daily administered IL-6 (0, 1.6, or 3.2 g, i.p.), to male Sprague-Dawley rats, for 7 days prior to SCI. We assessed an array of depression-like behaviors prior to injury as well as on days 2, 9, and 19 post-injury. Serum cytokine levels were analyzed prior to injury, after IL-6 administration, and 10 days post-injury. Using hierarchical cluster analysis, we divided subjects into 2 groups based on their depression behaviors from days 9 and 19 post-injury. One group displayed depression symptoms: decreased social interaction, sucrose preference, and burrowing. Replicating our previous findings, depressed subjects expressed higher serum IL-6 prior to SCI, supporting IL-6 as a predictor for depression susceptibility.
Further, IL-6 seemed to increase the likelihood of developing depression, as 67% of the subjects treated with the low dose of IL-6 clustered in the depressed group, compared to 35% of the vehicle-treated group. These data suggest that inflammation prior to SCI may increase susceptibility to depression post injury.

15.S.A Effects of Morphine on the Function of Activated Microglia and Macrophages after SCI

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Opioids are the most effective and commonly prescribed analgesics for the treatment of acute pain after spinal cord injury (SCI). However, our laboratory has previously shown that morphine administration in the early phase of SCI undermines locomotor recovery in our rodent contusion model. We hypothesize that the adverse effects of morphine might be mediated by the activation of Opioid Receptors (ORs) on immune cells. Previous data suggests that morphine increases the expression of immune cells after an SCI but the extent to which morphine changes the function of these immune cells is not known. To test this, we used flow cytometry to determine whether morphine administration changes the phagocytic activity of microglia and macrophages. Additionally, using western blots we examined whether morphine activation of immune cells engages signaling pathways associated with inflammation and proliferation of immune cells. Briefly, subjects were giving a moderate contusion injury. On the day following surgery half of the subjects received morphine (i.v.) or saline for 1, 3, or 7 days. A section of

the injured cord was collected and cells were incubated with fluorescent beads and antibodies to identify microglia and macrophages. Our results indicate that after 3 days of morphine administration there is reduced phagocytic activity in macrophages but not microglia. Additionally, our western blot data shows that morphine up-regulates the production of dynorphin and pro-dynorphin in microglia and macrophages. These results suggest that morphine might be changing the innate immune response after SCI and thus affecting recovery of function for patients. Given the clinical utility of opioid analgesics, it is imperative that we fully understand the effects of morphine in mediating the immune response after SCI. We must develop safe and effective therapeutic strategies for the use opioids in pain management after SCI.

15.S.B Axonal Survival and Schwann Cell Regeneration after Schwann Cell Death

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The neuromuscular junction (NMJ) has long been studied as a model synapse because of its large size, accessibility, and ease of manipulation, and the role that Schwann cells play at the NMJ is of increasing interest. Experiments that selectively ablate terminal Schwann cells (tSCs) have shown that they are dispensable for the acute function of the NMJ, but within a week, NMJs stripped of their tSCs begin to display functional deficits. Whether and to what extent Schwann cells can recover after such an insult, however, has remained undetermined. Here, we have selectively ablated Scwhann cells at the NMJ and observed their recovery after the injury. When Schwann cells die off, their associated motor axons largely do not degenerate, but remain at the NMJ. Scwhann cells regenerate and re-cover the synapse within three weeks, but through this regenerative process hyper-proliferate and remodel the axon terminal. The ability of Schwann cells to replicate and regenerate after the selective death of Schwann cells and the lack of degeneration of the bare motor axons have wider implications for demyelinating diseases and gliopathies in general.

16.S.A Fetal Sex is a Determinant of Maternal Plasma MicroRNA Responses to Prenatal Alcohol Exposure: Evidence From an Analysis of a Ukraine Cohort

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We previously reported (Balaraman et al.,2016) that miRNAs secreted into plasma of alcohol-exposed pregnant mothers from a Ukraine cohort could predict growth and other deficits in the new-born infant. Pregnant women were classified as unexposed to alcohol (UE), heavily exposed, with affected infants (HEa) or heavily exposed with apparently unaffected infants (HEua), and plasma samples were obtained at mid-pregnancy and at the end of the third trimester. Here, we assessed the coordinated expression of secreted maternal miRNAs, and whether patterns of coordinate miRNA expression differed between mothers in the HEa and HEua groups compared to the UE group. In aggregate, our analysis shows that ethanol exposure increased significant cross-wise miRNA correlations in maternal plasma samples collected at the second and the third trimester. The number of significant correlations was higher in the

HEa group compared to HEua group, and both groups exhibited a higher number of significant cross-correlations compared to UE mothers. Maternal data were next segregated by infant sex. Re-analysis showed that in HEa and HEua groups, mothers that gave birth to female infants showed increased cross-correlations in second and third trimester, compared to mothers who subsequently gave birth to male infants. To overcome the loss of statistical power due to segregation by infant sex, we used iterative bootstrap resampling with replacement to estimate the stability of cross-wise correlation patterns in simulated populations. Group differences in the resulting data representing the number of significant correlations in each iteration were assessed by computing means and 95% confidence intervals. These analyses confirmed the stability of increased cross-correlations in miRNA patterns specifically in HEa and HEua mothers of female infants. Additionally, we combined ANOVA and bootstrap resampling analyses and identified likely ethanol sensitive-infant sex specific maternal miRNAs. Those miRNAs will help identify sex-differences in the consequences and mechanisms of prenatal alcohol exposure. These data indicate firstly that fetal sex and outcome (affected vs. unaffected) determines correlated miRNA responses to ethanol exposure in pregnant mothers. Secondly, coordinated expression of miRNAs in the HEa group, suggests that alcohol exposure may result in coordinated secretory activity of tissues that contribute to circulating miRNAs in the pregnant mother.

16.S.B Nucleus Reuniens Influences Medial Prefrontal Cortex And Hippocampal Neuronal Activity During Retrieval Of Extinguished Fear Memories

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Coordinated activity between the medial prefrontal cortex (mPFC) and hippocampus (HPC) is essential for encoding and retrieving spatial, working and contextual memories. The nucleus reuniens (RE) is a ventral midline thalamic nucleus that has a role in synchronizing activity in the HPC and mPFC. In Pavlovian fear conditioning, we have recently showed that RE inactivation impairs both the acquisition of hippocampal-dependent contextual fear memories as well as the extinction of fear to an auditory conditioned stimulus (CS). We hypothesized that the extinction deficit may be due to RE inactivation impairing behaviorally relevant neural activity in the mPFC and HPC. To test this idea, we examined the influence of RE inactivation on the induction of c-fos in mPFC and HPC by an extinguished conditional stimulus (CS). Consistent with our hypotheses, we found that inactivation of RE impaired the expression of extinction and this was associated with decreased c-fos expression in both the mPFC and HPC. We are currently exploring the functional role for RE projections to mPFC or HPC (or both) in extinction retrieval using an intersectional optogenetic strategy. Taken together, these data show that RE has a crucial role regulating neuronal activity in the mPFC and HPC that promotes successful retrieval of extinguished fear memories.

17.S.A Social Stress During Adolescence Followed by Western-Style Diet Leads to Physiological Dysregulation, Depressive Phenotype, and Decreases in Reward Sensitivity in Adulthood.

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The prevalence of major depressive disorders (MDD) and obesity in adolescence has steadily increased over the last decade. This comorbidity has been reported by clinicians and a relationship between depression- and anxiety-like states with cardiac and metabolic dysfunction has also been demonstrated in rodents. While much has been elucidated about the neural basis of depression and obesity, how they converge is unknown. It is unclear whether chronic stress induces physiological and neurobiological changes associated with metabolic dysfunction or vice versa, thus understanding potential mechanism(s) and/or directionality is paramount. To this end adolescent (postnatal day [PD]30) male C57bl mice were exposed to HFD either before or after chronic social defeat stress (CSDS), and then tested for behavioral and physiological dysregulation. Mice were given free access to HFD (Research Diet: D12451) for 14 days (PD30-44) prior to CSDS exposure (10 days; 10 minutes/day), and subsequently tested for sucrose/saccharin preference. A group was given access to HFD alone for 4 weeks and tested in the conditioned place preference (CPP) paradigm to assess for changes in drug reward. A separate group was tested in the social interaction test (SIT) to measure changes in social avoidance. Mice did not show changes in caloric intake or total body weight regardless of diet. However, those in the HFD condition showed a significant decreased in preference for sucrose when compared to the normal chow (NC) exposed mice. Mice pre-treated with HFD did not develop preference to the side compartment paired with morphine (1.0 mg/kg), which promoted CPP in the NC-exposed mice.
Combined, these results demonstrate that pre-exposure to HFD blunts responses to both natural and synthetic drug reward. Mice exposed to CSDS before the introduction of the HFD showed no change in calories consumed, but a significant increase in body weight after only 10 days of HFD was observed. The NC-exposed mice showed significant recovery in social interaction whereas the HFD-exposed mice showed profound social avoidance. Together, these findings indicate that exposure to HFD during adolescence blunts reward sensitivity, and that stress exposure followed by consumption of HFD induces neurobiological changes that lead to physiological and mood related deficits (i.e. anhedonia) which could lead to the development of maladaptive behaviors and negative health outcomes in adulthood.

17.S.B The Brain Regulates Pain-induced Hemorrhage in the Spinal Cord after Injury

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Spinal cord injury (SC) is often accompanied by additional injuries (polytrauma) that provide a source of pain input in addition to the initial trauma to the spinal cord. We have shown that pain input after SCI leads to an acute increase in hemorrhage and impaired long-term recovery. Recent work has suggested that the effect of pain on hemorrhage is dependent on rostral brain systems. When the communication

between the lesion site and the brain is disrupted, the adverse acute effects of pain input on secondary injury mechanisms are blocked. Given this, we sought to elucidate the role of the brain in the development of acute hemorrhage and impaired long-term recovery. To do this, we gave nociceptive stimulation above the injury or below the injury to determine if the brain is playing a mediator (sufficient) or regulator (necessary). Adult male Sprague-Dawley rats received a contusion injury at T12. The next day, subjects were either given noxious input in the form of six minutes of uncontrollable shock or capsaicin injection above the injury, below the injury, or no shock/vehicle (Exp. 1 & 2). To control for pain from 6 minutes of shock above the SCI, the rats were given a dose of morphine (20 mg/kg) before noxious treatment (Exp. 1 & 2). Previous studies in our lab have shown that morphine treatment does not block the effects of nociceptive stimulation on hemorrhage and recovery. Three hours after noxious input, the rats were sacrificed and 1 cm of spinal cord tissue encompassing the injury site was collected for hemorrhage analysis. It was found that only noxious input below the injury increases acute hemorrhage in the spinal cord. For Experiment 3, subjects' received a T12 contusion injury. The next day, a baseline locomotor score was recorded. Then, subjects received shock treatment as described above and locomotor recovery was monitored and recorded for 21 days. It was found that subjects only showed a deficit in recovery when shock was received below the injury. Together, this data suggests that the brain is playing a regulatory role (necessary) in the development of acute hemorrhage and long-term locomotor impairment.

18.S.A Axonal Damage and Behavioral Correlates in CNS Myelin Repair

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The proper function of the vertebrate central nervous system (CNS) is critically dependent on the myelination of axon fibers. Loss of myelin or myelin-producing oligodendrocytes (OLs) in the central nervous system (CNS) contributes to neurological disorders such as multiple sclerosis and Alzheimer's disease, as well as to age-related neurological decline. It is generally believed that the remyelination of denuded axon fibers confers protection. However, our understanding of the myelin repair process, specifically the interaction between axons, myelin, and glial cells, remains limited. In this study, we utilized the Cuprizone (CPZ) model to investigate cellular interactions during myelin repair in an autoimmune-independent fashion. To facilitate direct visualization of myelin, we utilized "green-myelin" mice that express membrane-anchored green fluorescence protein in mature OLs under the CNP1 promoter. Adult mice were fed a 12-week "chronic" CPZ diet, which induced substantial demyelination and glial activation in the corpus callosum (CC) and cortex (Ctx). After 4-8 weeks of recovery, significant remyelination and decreased microgliosis were achieved, but astrogliosis was maintained. Further, staining of amyloid precursor protein and non-phosphorylated Neurofilament H revealed significant axonal damage in both the CC and Ctx, which persisted even after 8 weeks of recovery. DigiGait and Open Field behavioral analyses demonstrated a worsening of bilateral sensorimotor function after chronic demyelination, indicated by a shorter, shuffling stride, decreased distance traveled, and prolonged postural stability adjustments, which was not rescued after myelin recovery. Transcriptome profiling of the CC at the early stages of demyelination and myelin repair revealed several immune and signaling pathways for early myelin repair, which are currently being compared against remyelination at the chronic stage. Together, our data suggest that persistent axonal damage due to a chronic demyelinating insult may underlie aberrant remyelination mechanisms and prolonged motor deficits in

demyelinating disorders.

18.S.B Drug-induced Synaptic Plasticity from Striatal Medium Spiny neurons to Cholinergic Interneurons and Behavioral Inflexibility

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Increasing evidence indicate that acetylcholine levels in the dorsal striatum significantly increase in a reversal learning task. Striatal acetylcholine mainly arises from cholinergic interneurons (CINs), which are tonically active and constitute about 2% of the total neural population in this area. The striatal principal neurons are GABAergic medium spiny neurons (MSNs), which express either dopamine D1 receptors (D1Rs) or D2Rs. It has been reported that MSNs make synaptic connections with CINs. We thus hypothesize that MSNs inhibit CIN activity. Using slice electrophysiology, we found that selective optogenetic excitation of D1R-MSNs or D2R-MSNs induced inhibitory postsynaptic currents (IPSCs) in striatal CINs, with a greater magnitude for D1R-MSNs than D2R-MSNs. A rabies-mediated retrograde monosynaptic neural tracing study found that D1->CIN connections were significantly stronger than D2-
>CIN connections. The same rabies study also revealed that CIN-innervating D1R-MSNs projected to the substantia nigra pars reticulata (SNr), which forms the direct pathway and controls reinforcement of addictive drugs. Next we examined how exposure to addictive substances, alcohol and cocaine, altered GABAergic inputs on CINs and CIN firing activity. We found that the spontaneous IPSC frequency and the D1->CIN IPSC amplitude were increased, whereas the spontaneous firing rate of CINs was significantly reduced in drug-administered animals, as compared to their water or saline controls. Importantly, we found that excessive alcohol consumption or repeated cocaine self-administration impaired reversal learning of operant sucrose and food self-administration. Our data suggest that drug-induced inflexibility may be mediated by the aberrant plasticity at D1->CIN connections.

19.S.A Pain-induced Hemorrhage after Spinal Cord Injury: Pentobarbital Anesthesia and Local Application of Lidocaine Prevent Hemorrhage When Given Before, But Not After, Noxious Stimulation

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Nociceptive stimulation delivered to spinally contused rats has been shown to exacerbate tissue loss and leads to deficits in long-term recovery. Recent work suggests that application of anesthesia shortly before pain stimulation blocks the adverse effects of nociceptive stimulation We have explored this effect across both general (pentobarbital) and local (lidocaine) anesthetic interventions. Disrupting communication with the brain by means of a rostral transection causes the same effect. These results suggest that the adverse effect of pain input after injury depends, in part, on brain processes. Here, we examine the efficacy of both anesthetics administered before or after noxious electrical stimulation in spinal cord injured rats. Rats received a contusion injury at T12 and, 24 hours later, were given an

anesthetic dose of pentobarbital (50mg/kg) or an infusion of lidocaine at T2. Drug infusion took place either before or immediately after 6 min of electrical stimulation (shock) or nothing. Three hours after shock, rats were sacrificed and one cm of tissue was collected enveloping the injury site. The extent of hemorrhage was assessed by measuring the absorbance of light at 420nm and western blotting targeting hemoglobin. As expected, nociceptive stimulation increased the extent of hemorrhage in vehicle treated (awake) rats. This effect was attenuated by anesthesia only when given before stimulation. On-going studies are examining whether anesthesia attenuates capsaicin-induced hemorrhage.

19.S.B Response Properties of the Bat Primary Auditory Cortex to Frequency-Modulated Stimuli

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Neural selectivity to the direction and rate of sensory stimuli is a common feature across sensory modalities. In the auditory system, frequency-modulated (FM) sweeps are essential elements of vocal communication in animals and for human speech. Echolocating bats provide an ideal model for studying the neural circuits underpinning FM sweep selectivity due to their reliance on FM sweeps for biosonar. We used multichannel microelectrode arrays in anesthetized Mexican free-tailed bats (Tadarida brasiliensis) to evaluate how FM sweeps were encoded in the primary auditory cortex (A1). Pure tone stimuli were presented to determine the response properties of principal cells and their surrounding local field potentials (LFP), including characteristic frequency (CF), best frequency (BF), minimum threshold (MT), best level (BL) at CF, and frequency response area bandwidth (BW). We used these parameters to map the topographical organization of response properties throughout A1, and then investigated how FM sweep selectivity was represented within this map. Spiking neurons responding to pure tone stimuli were tonotopically organized rostrocaudally with lower CFs represented caudally. Frequency response areas were significantly lower and broader for LFPs than for spikes, indicating that local interneurons receive convergent inputs from an asymmetrical frequency range to produce feedforward inhibition that may bias spiking neuron response properties in favor of downward sweeps. The majority of A1 neurons responded preferentially to downward FM sweeps, with more neurons tuned to slower sweep rates. These results are consistent with hypotheses that intracortical inhibitory processes, such as sideband inhibition, shape FM tuning and suggest that in the free-tailed bat, the A1 is preferentially sensitized to sounds occurring within the context of downward FM sweeps.

20.S.A Alcohol Effects On the Proteome Of Fetal Neural Stem Cell-derived Extracellular Vesicles

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Prenatal alcohol exposure (PAE) can result in craniofacial abnormalities, growth deficits, and is the leading cause of neurodevelopment disability worldwide. Neural stem cells (NSCs) are particularly vulnerable to alcohol (ethanol) exposure during the late first through the second trimester, when they are most extensively involved in neurogenesis. NSCs reside in a complex microenvironment rich in sub-

200 nanometer-sized extracellular vesicles (EVs), which are shown to traffic protein, lipid, and RNA cargo between cells, that can serve as a mode of intercellular communication. Using fetal mouse derived cortical neuroepithelium, cultured ex-vivo as non-adherent neurosphere cultures, we previously found that ethanol exposure resulted in significant elevation of miRNA cargo like miR-140-3p in EVs, which direct NSCs towards an aberrant astroglial lineage. Subsequently, EVs may be amplifying PAE's temporal and spatial effects in the stem cell niche to result in a neurogenic capacity decline. For this study, we further investigated the impact of ethanol on the proteome of NSC-EVs. Analyses of our EVs identified ~86% of proteins needed for eukaryotic translation initiation, implying that EVs carry with them the ability to translate EV-chaperoned mRNAs in recipient cells. Statistical and pathway overrepresentation analyses showed that moderate ethanol, ~26 mM, resulted in a significant increase in proteins of the Nonsense-Mediated Decay (NMD) pathway in EVs, whereas a higher dose, ~70 mM, resulted in EV overexpression of mitochondrial proteins that constitute a Danger-Associated Molecular Pattern (mito-DAMP) pathway. NMD is an important surveillance pathway that reduces errors in gene expression by eliminating premature stop codon-containing mRNA transcripts. Multivariate analysis of our complementary RNAseq data indicates that ethanol globally suppresses NMD pathway transcripts in NSCs. Consequently, NMD pathway proteins sequestered in EVs are hypothesized to transfer neuroprotection to cells where the capacity to correct errors in protein translation may be depleted. Eukaryotic cells under high stress, expel mitochondrial proteins as a 'danger' signal that activates 'pattern recognition' receptors and pro-inflammatory responses in target cells. Moreover, our RNAseq analyses indicate that ethanol exposure selectively reduces the expression of the S100 family of DAMPs, but importantly, not DAMP receptors (TLRs and RAGE). Consequently, mito-DAMPs in EVs from heavy- ethanol-exposed NSCs are predicted to compensate for the loss of S100 DAMPs, and to spread inflammation through the NSC microenvironment, compromising NSC growth and differentiation.
Collectively, these studies identify EVs as a novel source for communicating stress responses, in an ethanol dose-related manner, to cells within the fetal neural stem cell niche.

20.S.B CRISPR/Cas9 Editing of Myosin-Va Gene in a Rat Model of Griscelli Syndrome Type1

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Griscelli Syndrome (GS) type 1 is an ultra-rare, autosomal recessive, severe neurological disease that affects vesicle and membrane trafficking due to a mutation in the Myosin-Va gene, leading to a loss of respective protein expression(Çağdaş et al., 2012). Myosin-Va (MYOVA) is an unconventional motor protein that is essential for transporting mRNA(McCaffrey & Lindsay, 2012; Salerno et al., 2008) and mRNPs(Yoshimura et al., 2006), smooth endoplasmic reticulum(Wagner, Brenowitz, & Hammer Iii, 2010), secretory granules(Eichler, Kögel, Bukoreshtliev, & Gerdes, 2006), and other proteins in the neuron to dendritic spines. Due to the loss of intracellular neuronal transportation, patients with type 1 GS exhibit severe neurological deficits such as developmental delays, intellectual disabilities, seizures, and motor impairment, as well as hypopigmentation of the hair and skin. We have a line of rats with a point mutation in a donor splice site early in the MyoVa gene, which leads to a loss of functional protein(Landrock et al., 2018). This line of rats is an ideal model for GS Type 1 that shows the characteristic dilute hair color, loss of dopaminergic neurons, muscle weakness, seizures, and has a

shortened life-span that is seen in Griscelli patients (Landrock et al., 2018; Stoica et al., 2012). We hypothesize that by using clustered regularly interspaced short palindromic repeats (CRISPR) with caspase 9 (CRISPR/Cas9), we will be able to edit the genome in primary cells of this model for GS type 1 and restore the Myosin-Va gene. Primary rat fibroblasts or neurons will undergo cationic lipid mediated transfection with CRISPR/Cas9 plasmids 5' and 3' of the mutation. After 24-72 hours of treatment, cells will be harvested and DNA or RNA will be extracted. PCR and cloning will be performed and the nucleic acids will be sequenced using sanger sequencing for evidence of editing. Through sanger sequencing, we have demonstrated qualitative evidence of targeted editing of the MyoVa gene with our CRISPR/Cas9 constructs in primary cell cultures. We plan to transfect organotypic striatal cultures with a 5' gRNA, 3' gRNA, and a DNA minicircle to correct the mutation and restore functional MYOVA protein/.

21.S.A A Lifetime Analysis of Myofiber Pathology in Mdx Mice

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Duchenne muscular dystrophy (DMD) is a fatal x-linked degenerative disease affecting 1:5000 males. Resulting from mutations that prevent expression of dystrophin protein, affected individuals are susceptible to contraction-induced injury to their skeletal muscle fibers. Muscle fibers repeatedly degenerate and regenerate, but eventually fail to repair leading to loss of muscle fibers. The muscular dystrophy x-linked (mdx) mouse is the most commonly used animal model for DMD, however, it is far from a perfect model. Mdx mice show no overt phenotype, have only marginal deficits in neurotransmission and show little fibrosis compared to the human disease. On the other hand, mdx mice have a shortened lifespan, undergo repeated bouts of muscle damage and repair, and replicate many of the morphological changes seen in human patients. Despite decades of research on the mdx mouse, the field lacks a thorough characterization of myofiber pathology, especially in aged mdx mice. Therefore, we set out to characterize features of myofiber pathology from 2 weeks to 2 years of age. The goal of this project was to assess the degree to which the pathology mimics the human disease in order to determine which pathological features might serve as reliable markers for testing therapeutics.

21.S.B Goal-directed and Habitual Cocaine Seeking: Further Assessment of Noncontingent Cocaine as a Method to Cause Satiety and Outcome Devaluation

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Addiction has been hypothesized to stem from a failure to exert goal-directed control over habits. To evaluate the role of habits in addiction, our lab recently developed a novel outcome devaluation procedure to assess goal-directed and habitual responding for intravenous (IV) cocaine in rats. We hypothesized that noncontingent administration of IV cocaine would mimic preferred blood levels, resulting in temporary satiety and outcome devaluation. Previously, our lab found that pre-training NMDA lesions of dorsomedial striatum (DMS) caused rats to be insensitive to outcome devaluation, whereas lesions of the dorsolateral striatum (DLS) caused rats to be sensitive to outcome devaluation.

Supporting the validity of this procedure, these findings are consistent with established roles of DMS and DLS in goal-directed and habitual responding, respectively. We found that non-lesioned rats show a great degree of variability in their sensitivity to outcome devaluation, regardless of schedule of reinforcement. This may reflect individual differences in response strategies for cocaine self-administration, or instead may be explained by differences in preferred cocaine levels, which might affect sensitivity to non-contingent cocaine. Here, we sought to evaluate individual differences in preferred levels of cocaine using pharmacokinetic modeling to estimate cocaine levels during free accessself-administration sessions. Male Sprague Dawley rats were trained on a seeking-taking chained schedule of cocaine self-administration (0.5 mg/kg/infusion). Outcome devaluation was carried out via noncontingent administration of IV cocaine (1mg/kg) followed by evaluation of responding on the seeking lever for 10 minutes under extinction conditions. Similar to our previous results, we found variability among animals in their sensitivity to outcome devaluation. However, there was no correlation between sensitivity to outcome devaluation and preferred blood levels of cocaine. In addition, there was no difference in preferred blood levels of cocaine when comparing animals with DMS or DLS lesions, despite clear differences in sensitivity to outcome devaluation. Finally, pharmacokinetic modeling revealed that noncontingent IV cocaine (1 mg/kg) resulted in blood levels that are greater than the preferred levels for all animals, indicating that this dose might result in temporary satiety across animals. These findings support the premise that noncontingent IV cocaine induces temporary satiety, and that individual differences in subsequent responding are related to differences in response strategy (goal-directed vs. habitual), rather than differences in satiety.

22.S.B **The Bed Nucleus of the Stria Terminalis Regulates Context-dependent Flight Behavior in Rats**

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Fadok and colleagues (2017) have developed a modified Pavlovian fear conditioning procedure in which a serial conditioned stimulus (SCS) consisting of serial presentations of pure tone (7 kHz) and white noise (1-20 kHz), followed by a footshock unconditioned stimulus (US). After conditioning, mice exhibit freezing to the tone, but transition to flight (e.g. escape jumps and increased movement speed) during the noise. The transition from freezing to flight behavior is gated by the central nucleus of the amygdala (CeA), and flight responses are only elicited within the conditioning context (Fadok et al., 2017). Here, we replicate these behavioral findings in male and female Long-Evans rats and further investigate how flight responses are contextually regulated. After SCS conditioning, rats either received unsignaled footshocks in a novel context (Shock) or were exposed to the same novel context for an equal amount of time (No-Shock). The next day, Shock animals displayed flight responses to SCS-alone presentations within the unsignaled footshock context, whereas No-Shock animals did not. We therefore conclude that flight responses are dependent upon contextual fear, irrespective of where SCS conditioning occurs. The bed nucleus of the stria terminalis (BNST), central amygdala (CeA), and the ventral hippocampus (VH) have been implicated in contextual fear. In the second experiment, we show that muscimol inactivation of either the BNST or the CeA, but not the VH, diminishes flight responses in the conditioning context. These findings advance our understanding of the neural circuitry underlying the contextual regulation of active defensive behavior by demonstrating that flight responses are dependent upon contextual fear and that this effect is mediated by the BNST.

Postdoctoral Fellows and Research Scientists

22.P. A Molecular Alterations in the Aging Cortex Associated with an Age-Dependent Decline in Axon Growth

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Spinal cord injury (SCI) is a life-long condition that places great burden on the individual and society, however there has been a shift in the demographic population effected. Currently in America ~80% of all people with SCI are 40 years or older, and the average age at injury has increased to ~43. Therefore, understanding SCI in middle aged and aging populations is of great importance. Despite intensive studies into enhancing axon growth after trauma, how age can impact axon growth is still not known. The dynamics of axonal growth are altered with age and impact recovery from injury. Experimentally, SCI is commonly modeled in young adult animals. This contrasts with the aging human SCI population and hampers the translation into clinical application. Understanding the underlying mechanisms of this decline in axon growth is critical for the development of novel therapies that can stimulate repair in an aging population. One promising target is Signal Transducer and Activator of Transcription 3 (STAT3). STAT3 is involved in a range of cellular functions, many of which have been associated with a decline in axon growth. We have found significant differences in both RNA and protein expression of STAT3, and associated molecules, between young and aging mice without injury. In the aging cortex STAT3 expression is reduced. Kinases involved in STAT3 functionalization, by both phosphorylation and acetylation, and in the translocation of STAT3 are altered. We will present here a characterization of the molecular players altered in the middle aged cortical neuron compared to their younger counterparts.

23.P.A The Long-term Epileptogenic Impact of Stroke in Mature Adult and Reproductive Senescent Female Rats

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Epileptogensis is triggered by diverse factors such as stroke, brain injury or prolonged seizures. Stroke is a major risk factor for development of epilepsy, especially in older populations, and exhibits a strong sex difference. However, the mechanism underlying the post-stroke epilepsy (PSE) remains unclear. There are few validated animal models of PSE in the disease prone females. In this study, we investigated PSE in female rats by using monitoring epileptogenic seizures after stroke induced by middle cerebral artery occlusion (MCAO). MCAO was induced by intracerebral injection of endothelin-1 in mature adult (MA, 5 months, cyclic) and reproductively senescent (RS, 11 months, acyclic) female rats. Animals were recorded for the occurrence of behavioral and electrographic seizures by a continuous 24/7 video-EEG system for one week at 2, 4, 6, 8, 10 and 12 months. The extent of brain damage was assessed 12 months after MCAO or pilocarpine injections by neuropathology analysis of neurodegeneration and neuronal injury. Epileptiform seizure discharges were detected in 25% of RS and 40% of MA female rats

at 4 months; and in 50% of RS and 33% of MA female rats at 6 month after stroke ($p<0.05$ vs control; . n=4-13). However, spontaneous seizures were not evident at 8, 10 or 12 months after stoke. Stroke induced epileptogenesis was significantly accelerated after pilocarpine challenge (second hit) wherein all animals showed spontaneous seizure (1-2 seizures/day; $p<0.05$ vs MCAO control without pilocarpine). After 12 months of MCAO, neuropathology findings showed significant neurodegeneration (20-40% vs control) in NeuN staining in ipsilateral hippocampus of the MCAO group alone and bilaterally in the hippocampus in the group that received pilocarpine 8 weeks post MCAO group ($p<0.05$ vs. control). Overall, these results indicate that MCAO-induced stroke is a potential epileptogenic trigger with low incidence but highly sensitive to a second hit injury with severe spontaneous seizures. These outcomes are consistent with clinical reports of greater incidence and/or worst outcomes of stroke in elderly women. Therefore, the inclusion of sex, age, and disease models in the study design of epilepsy research is of utmost importance.

23.P.B Chronic Spinal Cord Injury in the Mice Induces cardio-metabolic risk factors

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SCI can disrupt the neural circuitry signals to vital organs in the body and induce an inflammatory response. About 50% of SCI patients develop metabolic disease which is considered as risk factor for cardiovascular disease. The liver is a key organ in regulating metabolic pathways, and about 80% of people with chronic SCI display liver abnormality. The mechanisms of liver dysfunction and other associated diseases remain poorly understood. Here, we examined the effects of chronic SCI on the cardiometabolic disease in adult mice after 18months post-SCI. Mice were randomly assigned to either a sham or SCI groups. SCI was induced at T8 by the crush forceps model. Serum levels of alanine transaminase (ALT) were measured by ELISA, high-density lipoproteins (HDL) were measured by CardioChek analyzer, IL-17A, IL-1β, IL-6 cytokines, c-peptide, insulin, and glucagon were measured by Meso Scale Discovery biomarker assays (MSD). mRNA expression of collagen Type 1 Alpha 1 (Col1α1), Peroxisome proliferator-activated receptor gamma (PPARγ), α-smooth muscle actin (α-SMA), and tumor necrosis factor (TNFα) were determined by qPCR in liver tissue. Serum levels of ALT, IL-17A, IL-1β, IL-6, C-peptide, and Insulin, were increased in SCI versus sham group. However, Serum levels of HDL and glucagon were decreased in SCI group. Gene expression of Col1α1, α-SMA, were higher in hepatic tissues of SCI versus sham. PPARγ gene expression significantly reduced in SCI versus sham. These data provide evidence that chronic SCI elevates inflammatory cytokines and fibrotic markers, which could predispose individuals to cardio-metabolic disease. Supported by Mission Connect (TIRR Foundation).

24.P.A Combined Neural Stem Cell Grafting and Ganaxolone Therapy Greatly Eases Spontaneous Seizures and Co-morbidities in a Model of Chronic TLE

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Epilepsy, typified by spontaneous recurrent seizures (SRS), affects ~60 million people worldwide. Temporal lobe epilepsy (TLE), seen in ~30% of epilepsy patients, is a condition where seizures originate from the hippocampus. Memory and mood impairments are co-morbidities of chronic temporal lobe epilepsy (TLE). Since >35% of TLE patients acquire intractable epilepsy, alternative approaches such as cell therapy, either alone or in combination with drugs, have received much interest. We tested the hypothesis that combined intrahippocampal grafting of neural stem cells (NSCs) with short-term oral administration of ganaxolone (GAN, a synthetic analog of the neurosteroid allopregnanolone), facilitates better seizure control and improved cognitive and mood function than NSC grafting alone in rats afflicted with chronic TLE. We induced status epilepticus (SE) in young adult F344 rats via graded intraperitoneal injections of Kainic acid. After 2 hours of SE, the seizures were terminated through a dose of diazepam, and the frequency of behavioral SRS was quantified at 2-4 months after SE. Chronically epileptic rats (CERs) exhibiting comparable frequency of SRS were next randomly assigned to one of the five groups: CERs receiving sham surgery, CERs receiving oral GAN treatment for 14 days, CERs receiving intrahippocampal NSC grafting, and CERs receiving NSC grafting plus two-weeks of GAN. The donor NSCs were expanded from the subventricular zone of postnatal F344 rats expressing the green fluorescent protein in all cells. The frequency of SRS or Stage V-SRS, measured through continuous video-EEG recordings for 14-21 days at 4-5 months after treatment, was reduced in all treated groups. Furthermore, grafting of NSCs with or without GAN treatment reduced neuroinflammation, enhanced neurogenesis, and improved cognitive function. However, the overall efficacy for suppressing SRS was much higher in CERs receiving combined NSC and GAN treatment (63-65% reduction), than CERs receiving GAN (20-29% reduction) or NSC grafts alone (37-38% reduction). Besides, unlike with NSC or GAN treatment alone, combined NSC and GAN therapy improved pattern separation function and reversed anhedonia. Combined therapy also mediated superior enhancement of hippocampal neurogenesis and repression of activated microglia without altering the survival and differentiation of graft-derived cells. The results underscore that combined NSC and GAN therapy is superior to NSC or GAN treatment alone for alleviating seizures and easing the co-morbidities of TLE.

25.P.A Efficacy of Melatonin for Improving Cognitive and Mood Function in a Model of Gulf War Illness

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Gulf War Illness (GWI) affects 30-40% of 700,000 military personnel who served in the first Gulf War (GW). GWI is typified by a lingering cognitive, memory and mood impairments. Animal model studies employing exposures to GWI-related chemicals (GWIR-Cs) and moderate stress have linked these symptoms to persistent neuroinflammation, increased oxidative stress, mitochondrial dysfunction, and decreased hippocampal neurogenesis. These observations have prompted testing of various antioxidant

and anti-inflammatory compounds for their effectiveness to improve brain function. We investigated the efficacy of different doses of melatonin for improving cognitive and mood function in a rat model of chronic GWI. We also examined whether brain impairments and the associated pathological changes, continue into the middle age in GWI rats. Male SD rats were exposed daily to GW-related chemicals, pyridostigmine bromide, (PB, 2 mg/kg), DEET (60 mg/kg), and permethrin (PM, 0.2 mg/kg), and 15- minutes of restraint stress for 28 days. Six months later, the animals were treated with different doses (5, 10, 20, 40 and 80mg/Kg) of melatonin for eight weeks (5 days/week). Following the treatment regimen, animals were tested for cognitive and mood function through a battery of behavioral tests. The tasks comprised an object location test (OLT), a novel object recognition test (NORT), pattern separation test (PST), an object in place test (OIPT), and a sucrose preference tests (SPT). The animals belonging to the GWI receiving vehicle displayed impairments in all tests, implying that cognitive and mood impairments seen at earlier time-points persist for prolonged period. While all doses of melatonin were effective for improving a simple recognition memory function in GWI rats, improvements in more complex behavioral tests seemed to require a higher dose of melatonin. Indeed, GWI animals receiving 80mg/Kg melatonin displayed improved ability for discerning minor changes in the environment in an OLT and OIPT, better pattern separation in PST, and no anhedonia in SPT. Lower doses of melatonin did not facilitate improvements in these tasks. Immunostaining analyses of brain tissues revealed the persistence of activated microglia, reactive astrocytes and reduced hippocampal neurogenesis in middle-aged GWI rats. Higher dose of melatonin treatment could able to reduce the percentage of

activated microglia in the GWI. Measurements of the effects of melatonin for modulating the population of reactive astrocytes, and increasing hippocampal neurogenesis, are currently in progress. Thus, melatonin therapy has promise for improving cognitive and mood function in GWI at relatively higher doses.

25.P.B Single Intranasal Administration of MSC-Derived A1-Exosomes Thwarts Moderate TBI- Induced Long-term Cognitive Mood Impairments

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A significant percentage of people with moderate or severe traumatic brain injuries (TBIs) develop chronic neuroinflammation and persisting cognitive and mood dysfunction. The chronic effects are driven primarily by excessive inflammation in the early phase, which can prompt tissue damage and chronic neuroinflammation. In patients, the chronic effects produce severe behavioral deficits months or years after recovering from the acute symptoms. Many drugs have been shown to reduce neuroinflammation following TBI, but none have been incorporated into the standard medical care because of limited functional efficacy or undesirable side effects. Therefore, there is a need for alternative therapeutic strategies. One strategy is to administer exosomes secreted by mesenchymal stem cells (MSCs), which have shown similar or better efficacy than MSCs for modulating inflammation in several disease models. Exosomes are particularly attractive for application in neurological disorders as they can readily cross the blood-brain barrier, quickly get incorporated into neurons and microglia following intranasal (IN) administration, and are unlikely to cause thrombosis. Our previous study showed that intravenous administration of MSC-derived A-1 exosomes is efficacious for reducing

inflammation in the hippocampus, and improving learning and pattern separation function in the early post-injury period (Kim et a., PNAS, 2016). In this study, we investigated the efficacy of a single intranasal (IN) administration of A1-exosomes early after moderate TBI for thwarting long-lasting impairments in cognitive, memory and mood function. Nine-week-old mice were first subjected to unilateral controlled cortical impact injury using a velocity of 5m/s, dwell time of 300ms and injury depth of 0.8 mm. Two hours after the induction of TBI, mice received intranasal administration of A-1 exosomes (~5 billion/nostril, total, 10 billion) or vehicle. The mice were examined for cognitive and mood function using a series of behavioral tests at 7 months post-TBI. Animals that received vehicle after TBI displayed cognitive dysfunction, recognition memory problems, pattern separation deficits as well as anhedonia. Remarkably, animals that received A-1 exosome treatment after TBI showed similar cognitive, memory and pattern separation function as naïve control animals. These animals also exhibited a higher level of neurogenesis in the hippocampus contralateral to injury. Analyses of microglia and astrocytes are currently in progress. Thus, IN administration of A-1 exosomes shortly after moderate or severe TBI has promise for preventing long-term cognitive and mood impairments.

26.P.B **Sex Differences in Ethanol's Effects on Fetal Neural Stem and Progenitor Cells**

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Prenatal alcohol exposure can result in growth and neurodevelopmental deficits, collectively termed 'Fetal Alcohol Spectrum Disorders'. We have found that ethanol reprograms neural stem and progenitor cells to favor premature maturation and decrease self-renewal. Recent evidence has shown that genetic sex is an important determinant gene expression and lineage progression in the adult ventricular-subventricular zone. Here, we investigated sex differences in the developing neural stem cell niche ex vivo, using neurosphere cultures derived from murine dorsal neuroepithelium at GD 12.5. Separate suspension cultures were prepared from male and female tissue samples based on the qPCR assessment of the presence of the Y Chromosome (YMT region). Female neurospheres were more stem-like than male neurospheres, with higher rates of secondary neurosphere formation. In contrast, male neurospheres showed fewer secondary neurospheres but with a higher number of viable cells. This increased cell number in males is associated with a larger proportion of cells in mitotic S-phase and higher rates of DNA synthesis, indicating that male neurospheres were enriched for more lineage committed, transit amplifying-like populations. Sex-segregated neurospheres were exposed to ethanol for 5 days and were assessed for the expression of markers of neural stem cells and differentiation lineages using qPCR. Control male and female neurospheres had similar levels of stem cell and differentiation markers, with the exception of progenitor/gliogenic GFAP mRNA which was higher in the male neurospheres. Ethanol exposure at low doses (60 mg/dL) affected mRNA transcript levels only in female neurospheres, which had increased mRNA expression of stem/progenitor marker nestin mRNA and decreased expression of neurogenic and gliogenic transcripts. Ethanol exposure at chronic alcohol use levels (320 mg/dL) decreased neural, astrocytic, and oligodendrocytic markers in male neurospheres while the female neurospheres exhibited a moderate reduction in oligodendrocytic markers. Preliminary analysis of RNAseq data indicates a monotonic relationship between the number of transcripts altered at a large effect size and ethanol dose. These data provide evidence of sex differences in neural stem

and progenitor cell dynamics in the developing cortex. Ethanol exposure alters the neurosphere transcriptome and further work will elucidate the contribution of sex to these ethanol-induced alterations. More research is needed to understand how these interactions between sex and alcohol shape cortical development and neurobehavioral deficits following prenatal alcohol exposure.

27.P.B **Cytisine and Estrogen Exert Synergistic Neuroprotection in a 6-OHDA Mouse Model of Parkinson's Disease**

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Parkinson's disease (PD) incidence rates predict a worldwide pandemic that will affect over 12 million people by 2040, underscoring an urgent need for neuroprotective drugs. Unfortunately, there are no neuroprotective drugs on the market and most proposed neuroprotective drugs fail clinical trials because PD is caused by a variety of insults not recapitulated in any single animal model. We approach this barrier by focusing on hyperactivated endoplasmic reticulum (ER) stress, which is a convergent apoptotic mechanism for multiple PD-related toxicities. In addition to hyperactive ER stress, PD shows strong sex differences, affecting twice as many males than females with a milder disease phenotype in females and an earlier age of onset in males. Interestingly, we discovered that a smoking cessation drug and partial neuronal nicotinic acetylcholine receptor (nAChR) agonist, cytisine is neuroprotective only in female mice. Based on our data, we hypothesize that cytisine and estrogen exert neuroprotection in PD by synergistically inhibiting apoptotic ER stress in dopaminergic (DA) neurons. We found that primary mouse midbrain cultures, 200 nM cytisine, which is incapable of activating surface receptors, binds to and chaperones nAChRs out of the ER resulting in the upregulation of Endoplasmic Reticulum Exit Sites (ERES) in DA neurons. We predicted that cytisine-mediated ERES upregulation increases ER protein export, thereby reducing ER stress. As predicted, cytisine inhibited 6-hyroxydopamine (6-OHDA) induced ER stress by attenuating proteins in two of the three main arms of the ER stress pathway – ATF6 and XBP1. Based on this result, we tested if cytisine is neuroprotective in a mouse model of striatal 6-OHDA lesions. Surprisingly, alternate day low dose i.p. injections of cytisine (0.2 mg/kg) in 6-OHDA lesioned mice reduced apomorphine rotations only in females, suggesting that cytisine requires estrogen to exert neuroprotection. We further confirmed these effects in cultured DA neurons exposed to 6-OHDA, 10 nM β-estradiol inhibits CHOP, a key apoptosis inducing protein in the third arm of the ER stress pathway. Our data suggest that estrogen and cytisine work synergistically to convey a neuroprotective effect by increasing ERES and reducing apoptotic ER stress.