SYNAPSE 2018
Abstract Booklet
Alphabetical, by First Author

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Molecular mechanisms of mitochondrial dysfunction in regulated necrotic cell death: Implications for stroke and traumatic brain injury
Traumatic brain injuries and stroke disrupt several signaling cascades that cause regulated cell death and lead to damage in neurons and glia long after an initial injury or impact. These injuries are referred to as secondary injuries. One type of regulated cell death involved in secondary injuries is ferroptosis, a form of regulated necrotic cell death. Ferroptosis is morphologically and biochemically distinct from apoptosis and autophagy and depends largely on the glutamate/cysteine antiporter (system xc). This antiporter is responsible for maintaining homeostatic levels of extracellular glutamate and provides extracellular cysteine for the production of glutathione; a major antioxidant protects cells from oxidative damage. If the function of system xc is interrupted, it results in increased generation of reactive oxygen species and lipid peroxidation. Traumatic brain injuries and strokes cause release of glutamate through damaged cell membranes, making the system xc antiporter of interest because it relies on the extracellular glutamate concentration gradient to function properly. Although ferroptosis has been studied in cancer cells, we are interested in the mechanisms that may regulate this type of cell death in oligodendrocytes in the context of brain injury and stroke. In addition to elevated extracellular glutamate levels, bioactive sphingolipids may play a regulatory role in ferroptosis. Acid sphingomyelinase (ASM) is an enzyme activated by high extracellular glutamate responsible for making sphingosine, a bioactive sphingolipid, by cleaving sphingomyelin. ASM and its relationship to extracellular glutamate and the production of bioactive sphingolipids makes it likely to be involved in ferroptosis. Pharmacological inhibitors were used to confirm that ferroptosis, and not other forms of cells death, were occurring in these conditions. Additionally, preliminary data indicate that the concentrations of bioactive sphingolipids as well as the activity of ASM is increased when extracellular glutamate levels are elevated. This study will provide experimental evidence regarding the role that elevated extracellular glutamate, bioactive sphingolipids, and ASM play in system xc dependent ferroptosis in oligodendrocytes. The results may be useful in treatment of traumatic brain injuries and strokes to prevent or reduce further cell death because of secondary injuries.
This project was supported by NIH R01NS083544.

Andres M, Moore S, Duckworth C, Macy N, Tran V, Shanks RA, Lloyd SA
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Effects of adolescent prescription stimulants and stress on adult addiction susceptibility
Adolescence represents a time of physical change and psychosocial stressors with an increased incidence of risk-seeking tendencies. Notably, adolescence is characterized as a critical window of neurological development. This maturation of neurocircuitry combined with risk-seeking tendencies results in a period of vulnerability to harmful factors such as drugs of abuse, or even chronic stress. The increasing availability, over-prescription, and subsequent misuse or abuse of ADHD-psychostimulant medications, such as dextroamphetamine (Adderall®) or methylphenidate (Ritalin®), in adolescent populations, necessitates studies investigating the long-term effects of these drugs. To model adolescent ADHD psychostimulant misuse or abuse, male and female C57Bl/6J-mice were exposed to 1.0 mg/kg amphetamine (AMPH) or methylphenidate (MPD) from postnatal days 42 to 51 (P42-P51), which is the late adolescent timeframe. After postnatal day 90, adult mice were challenged with a subacute methamphetamine (METH) dose (0.5 mg/kg). To test the long-term effects of adolescent drug exposures, animal activity was assessed using an open field chamber. Increased open field activity is a common measurement of behavioral sensitization, which a hallmark of addiction produced by long-lasting alterations in the neurocircuitry of the brain. Late adolescent AMPH (male) and MPD (male and female) exposures induced cross-sensitization to METH in adulthood suggesting a sex-specific increased susceptibility to substance use. Unlike previous studies of early adolescent (P22-P31) drug exposures, late adolescent saline control treatments also induced adult cross sensitization to METH. To isolate the hypothesized effects of the drug dosing induced stress, additional controls were included. Preliminary data suggest that adolescent stress produces an additive or synergistic effect to drug exposure on adult addiction susceptibility. This study demonstrates differential treatment-, sex-, and potential development-specific alterations induced by adolescent psychostimulant exposure and stress which lead to detrimental behavioral alterations toward drug abuse that persist into adulthood.

Bagnell AM, Cheon S, Lizarraga SB
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Characterizing the Impact of the Gene ASH1L in the Regulation of Neuronal Gene Expression
Autism spectrum disorder (ASD) is a neurodevelopmental disorder associated with defects in neuronal connectivity and is highly heritable. A significant proportion of ASD cases are of a complex genetic-etiology which reflect the impact of gene environment interactions. The mechanism which facilitates these complex genetic and environmental interactions are not well understood. Genetic findings suggest that there is an overrepresentation of chromatin regulatory genes associated with autism which may exert their effect by modulating epigenetic mechanisms that alter circuitry development. We have found that that ASH1L, a chromatin remodeling gene, is strongly downregulated in human neurons treated with Valproate (VPA), a robust environmental ASD risk factor. ASH1L dimethylates Histone H3 on Lysine 36 (H3K36me2), a histone mark implicated in transcriptional activation and repression. Therefore, ASH1L could differentially modulate expression of genes relevant to A SD in response to the environment. How mutations in ASH1L lead to deficits in neuronal connectivity associated with autism pathogenesis is not well understood. This project aims to characterize the impact of ASH1L on neuronal development and pathogenesis of ASD. We are using genome editing and shRNA knockdown approaches to interrogate the function of ASH1L in stem cell derived human neurons. This study is important as ASD pathology could be associated in part with deficits in neuronal arborization that would lead to defects in neuronal circuitry.

Barboreck R, Combs K, Knick M, Halsell S
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Identifying the Molecular Components of Cold Nociception in Drosophila melanogaster
Nociception refers to an organism's perception and reaction to potentially damaging noxious stimuli. While this response is essential, humans suffer from chronic pain in which the pain signals abnormally persist months after trauma, injury or infection. This study aims to better understand the molecular mechanisms of pain by researching the potential role of eight individual Drosophila Innexin gap junction proteins in cold nociception. Similar to mammalian Connexins, some of these proteins are hypothesized to be involved in the electrical synapsis between neurons. The expression level of each protein is knocked-down by cell specific expression of innexin RNAi constructs in the class III dendritic arborization sensory neurons that mediate nociception.
Wild type third instar Drosophila larvae exhibit a characteristic "cringe" response when exposed to noxious cold. Knocked-down larvae are subjected to the cold behavior assay, and their behavior is videotaped and analyzed to quantify the "percent cringe" value in order to identify the number of "cringers" for statistical analysis. By comparing the proportion of cringers between the knocked-down, experimental larvae and the wild type, the involvement of the knockdown protein in the cold nociceptive signaling pathway can be inferred. Controls utilizing Oregon-R wild type larvae and larvae in which tetanus toxin is expressed specifically in da neurons will be described.
Two different RNAi constructs were used to test each of the eight Drosophila Innexins, with the exception of the ogre Innexin. To date, twelve of the fifteen constructs have been tested, and all Innexins have been tested using at least one construct. Eight of these constructs have shown significant change in cringing compared to wild type controls (Two-Tailed Homoscedastic TTest, $p>0.1$). The remaining constructs will be tested and a pan-da neuron driver will also be used to drive RNAi expression.

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The effects of cholinergic degeneration on septal facilitation of Long-Term Potentiation in rat perforant pathway
The septodentate (SD) pathway is comprised of projections originating from the septum, an important area in the brain contributing to attention and memory processing. The SD projects to the dentate gyrus (DG) of the hippocampus through dense fiber tracts composed of cholinergic, GABAergic, and glutamatergic neurons. Another source of input into the DG arises from the entorhinal cortex (EC) ipsilaterally through the perforant path (PP). The degeneration of the cholinergic neurons within the SD is a characteristic Alzheimer's disease (AD), which has led to the hypothesis that memory deficits in AD may be related to cholinergic loss in the cerebrum. Although cholinergic neuron degeneration has been confirmed, the exact mechanism by which the cholinergic degeneration leads to memory deficits remains unknown. In particular, the role of SD cholinergic neurons in mnemonic function has come under scrutiny. In this study, in vivo, heterosynaptic, paired-pulse neurophysiology was performed to determine if degeneration of cholinergic neurons within the SD affect septal facilitation of long-term potentiation (LTP; a neurophysiological model of learning and memory) in the PP in rats. In order to examine the role of septal cholinergic neurons in the facilitation of LTP, stereotaxic surgery was performed in order to create a lesion of septal, cholinergic neurons with a microinjection of the selective, cholinergic neurotoxin, 192 IgG saporin, into the medial

septum. Three weeks later, neurophysiological analysis of the septodentate and perforant pathways was conducted by inserting a recording electrode into the DG and two stimulating electrodes, one in the septum and one in the EC, to examine the dentate evoked response after SD or PP stimulation. In addition, scopolamine, a muscarinic receptor antagonist, was injected systemically after pre-tetany recordings were taken, but prior to tetany, to further reduce cholinergic activation of the DG. Presence of both the acetylcholinesterase-containing SD pathway and the choline acetyltransferase positive cells in the medial septum was then assessed to determine the loss of cholinergic neurons in the SD pathway. Preliminary results suggest that degeneration of cholinergic neurons in the SD may cause depression to occur in the DG after PP stimulation.

Barman N, Siecinski SK, Morgenlander WR, Zhao C, Corcoran D, Kwee LC, Arvai S, Gregory S
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The role of miR-149-5p in the T-cell mediated immune response of multiple sclerosis
Multiple sclerosis (MS) is a debilitating autoimmune disease characterized by the progressive destruction of the axonal myelin sheath in the central nervous system (CNS). While the exact mechanisms are unclear, recent studies have delved into the role of Th17 cells in the autoimmune response of MS and the role of Treg cells in preventing autoimmunity. MicroRNAs (miRNA) are a short non-coding class of RNA that regulate gene activity by targeted mRNA degradation, and are of increasing interest in the study of autoimmunity in MS. Our lab utilizes the experimental autoimmune encephalomyelitis (EAE) mouse model of MS to identify miRNA candidates that influence disease progression. Our initial miR-Seq profiling of PBMCs during the clinical progression of EAE discovered consistently increased expression of miR-149-5p ($p < .005$) relative to non-induced controls. miR-149-5p has previously been implicated in tumor regulation, cell proliferation, and apoptosis, but any association with T-cell mediated immune response would represent a novel function for the miRNA. Our work aims to statistically validate miR-149-5p upregulation in EAE and investigate its potential role in the stimulation and apoptosis of T-cells. To do this, we utilized the miRQuant software package to reanalyze the initial RNA-Seq data and verified increased expression of miR-149-5p during all stages of EAE clinical progression. We also used Jurkat cells, a lymphoid cell line similar to human T-cells, to conduct an in vitro investigation of how miR-149-5p expression may change in response to immune stimulation. Stimulation of Jurkats lasting 4 hours and 24 hours resulted in a 25% and 50% decrease in miR-149-5p expression compared to non-stimulated controls, respectively. This result was in contrast to our initial in vivo results showing upregulation in EAE clinical stages, and may hint that miR-149-5p expression is contingent upon other immune system components not see in the in vitro model. Our study is ongoing in that we will expand our Jurkat study to include earlier stimulation time-points to observe transient changes in miR-149-5p expression in case this was missed in the original experiment. We also intend to conduct knockdown studies of miR-149-5p in cultured Th17 and Treg cells from mouse splenocytes and measure the levels of apoptosis and proliferation in each subtype.

Barnes CB, Wallace C, Jacobowitz BS, and Fordahl SC
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The effect of dietary saturated vs. unsaturated fat on dopamine neurotransmission.
Obesity and high saturated-fat diets have been linked with systemic inflammation and insulin resistance. Recent evidence suggests that obesity and diets high in saturated fat also impair dopamine neurotransmission, which is involved in motivation, reward, motor control and executive function. This study examined whether unsaturated fats, in the form of flaxseed oil (FSO), affect dopamine neurochemistry in a similar manner. Mice were fed high-fat diets for 6 wks, containing saturated fat (HF; n=9), saturated fat plus FSO (HF/FSO; n=6), FSO only (n=6), or low-fat control (n=6). Body weight was significantly elevated in the HF ($37.7g \pm 0.9$), HF/FSO ($36.9g \pm 1.3$) and FSO ($32.6g \pm 1.1$) groups compared to control ($25.8g \pm 0.9$) ($p <0.001$). Insulin sensitivity, however, was only impaired in the HF and HF/FSO groups, as observed with reduced blood glucose clearance in an intraperitoneal glucose tolerance test. Using ex -vivo voltammetry to measure sub-second dopamine neurotransmission in the nucleus accumbens, we found that synaptic dopamine uptake (Vmax) at dopamine terminals was significantly reduced in the HF and HF/FSO groups compared to control (1.768 ± 0.08, 1.995 ± 0.17, and 2.214 ± 0.09, respectively; $p < 0.05$). In contrast, dopamine uptake was preserved in the FSO group similar to low-fat controls, indicating type of fat, not the amount of fat negatively impacts dopamine clearance. A Pearson's Correlation revealed a significant negative correlation showing that as body weight increased, dopamine clearance (Vmax) was reduced in the HF group ($r = -0.407$; $p < 0.05$); however, there was no correlation observed in the LF, FSO, HF/FSO groups. Overall, these data suggest that unsaturated-rich FSO does not alter dopamine neurotransmission like saturated fats, possibly through attenuating inflammatory damage and preserving insulin sensitivity.

Beck K, McDonnell M
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The effect of BaCl2 on the resting membrane potential of the superior flexor muscle of crayfish, Procambarus clarkii
Barium ions are known to block potassium leak channels which contribute to the resting membrane potential of most animals (Armstrong and Taylor, 1980). It was expected that barium chloride would block potassium leak channels thus increasing the crayfish resting membrane potential. Calcium chloride was replaced with barium chloride in a normal crayfish Ringer's solution and the resting membrane potential of the crayfish muscle in the control and barium solution was compared. Barium chloride was found to block potassium leak channels and produce an increase in resting membrane potential. To ensure that barium was specifically blocking potassium channels to increase resting membrane potential, and that this effect was not caused by the absence of calcium, barium chloride and calcium chloride were replaced with magnesium chloride in the Ringer's solution. There was no significant difference in resting membrane potential between the control and the magnesium solution supporting the hypothesis that barium blocks potassium leak channels and increases resting membrane potential. Future studies should look at other organisms or different concentrations of barium chloride to determine the amount of barium chloride needed to block the potassium leak channels.

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Learning Enhancement through Neurostimulation (LENS): The Effect of Transcranial Direct Current Stimulation (tDCS) and Social Training in Autistic Spectrum Disorder
Autism Spectrum Disorder (ASD) is a pervasive personality disorder that results in impaired social and cognitive functioning. The hallmark symptoms of ASD include social-interaction difficulties, communication challenges and a tendency to engage in repetitive behaviors. The symptomology of ASD is hypothesized to be a result of intracortical inhibitory dysfunction, specifically in the frontal cortex. Transcranial Direct Current Stimulation (tDCS), a non-invasive brain stimulation technique, has been used to modulate spontaneous cortical activity using a low-intensity direct current by means of electrodes placed on the scalp. In the present study, PEERS®, a teacher facilitated social skills class, was used to measure the impact of tDCS on the learning and retention of new information that is processed in the part of the brain suspected to be altered by ASD. PEERS® is adapted from an evidenced-based social skills program for high-functioning adolescents with ASD that focuses on making and keeping friends and managing peer rejection and conflict. This program is unique in that allows for the students with ASD to learn and practice their new skills in a classroom setting that emulates the one in which they will be using and applying these skills. This preliminary research study look at the safety and efficacy of administering tDCS to high-functioning autistic adolescents. tDCS is theorized to increase social functioning of high-functioning adolescents with ASD, and this will be shown through increased performance on the dot probe task. Eleven high functioning ASD adolescents participated in 14 weeks of PEERS® sessions during which they received 30 minutes of either active or sham tDCS. tDCS was administered to the dorsolateral prefrontal cortex in order to test the theory that increased activation will enhance learning. The sessions were divided into two parts, with half of the participants receiving tDCS during the first part of the lesson and then during the break the tDCS was switched to the other half of the group. Five subjects received active tDCS and six received sham. The effects of tDCS were measured through two different tasks versions of the dot probe task, which measures attentional bias and social awareness. The preliminary results suggest that tDCS over the dorsolateral prefrontal cortex enhances and reinforces the social skills taught during PEERS®.

Benz I and Grider MH
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ISRIB as a Potential Therapeutic Drug for Neuronal Injury.
An ischemic stroke causes the loss of oxygen and glucose (blood sugar) to the brain. We aim to investigate potential therapeutic drugs on the survival of neurons following removal of glucose and/or oxygen. In the current experiment, we tested the effects of glucose withdrawal on the survival of a neuronal cell line, PC12 cells. Recent studies have suggested a possible role of ISRIB, a drug that decreases the cell's internal stress response, in promoting survival of injured neurons. Therefore, we tested whether ISRIB could promote neuroprotection following glucose withdrawal. Using approximately 10,000 cells per well, we differentiated the PC12 cells to a neuronal phenotype with Nerve Growth Factor for 7 days and replaced the media every 48 hours. Following 24 hours of injury and treatment, the cell viability was determined using an MTT assay, and compared to control cultures. Our results confirm that glucose withdrawal leads to cell death in PC12 cells. Preliminary results suggest that addition of ISRIB attenuates cell death in response to glucose withdrawal, compared to injured cells with no treatment. However, further studies are required to statistically interpret the data. Future studies will include the examination of ISRIB as

neuroprotective to other injuries, such as oxygen withdrawal. Our current model is easy to conduct and inexpensive, permitting replicates to strengthen the data. This practical model's versatility will also allow for future testing with other potential therapies such as Argon or other chemicals that could potentially produce neuroprotection following stoke injuries.

Bland KM, Casey ZO, Handwerk CJ, Holley ZL, Vidal GS
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Exploring a role for integrin beta 3 in dendritic spine pruning in cerebral cortex
Synaptic pruning in the cerebral cortex is a normal developmental process. Early in postnatal development, the cerebral cortex normally overproduces synapses, and then undergoes a period in which synapses are pruned away, leaving only synapses that are important for neural function. Dysregulation of synaptic pruning in excitatory neurons of the cerebral cortex may lead to neurological disorders such as intellectual disability and autism spectrum disorder, and is thought to be detectable by examining dendritic spine pruning. Integrin subunits have been implicated in axonal and dendritic outgrowth, as well as dendritic spine plasticity. In particular, a strong positive association has been found between mutations in integrin beta 3 (Itgb3) and intellectual disability, but little is known about neuronal Itgb3 function in vivo. To examine the role of Itgb3 in shaping cortical circuits early in life, here we use an approach that targets layer II/III cortical pyramidal neurons to either simply label them (controls) or to label them and cause Itgb3 loss of function (mutants). Results show that, when comparing neurons across the entire cerebral cortex, dendritic spine density on Itgb3 mutant neurons is unchanged compared to controls, and spine pruning is undetectable in both conditions. However, regional analysis reveals that dendritic spine pruning can be detected in controls. In summary, these results point to specific ages and regions in which dendritic spine pruning may be observed to investigate the role of Itgb3 in neurological disorders.

Bodner KA, Riley MP, Hochschuler JL, Gendle MH
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Potential cognitive-enhancing effects of oral phosphatidylserine in middle-age adults
Phosphatidylserine (PS) is a phospholipid found in cell membranes and plays an important role in multiple cellular processes. PS is often marketed as a supplement to aid in cognition, and has been demonstrated to be orally bioavailable and is taken up in the central nervous system. Oral PS supplementation has been demonstrated to reduce cognitive decline in aged populations, however, little research has been done to verify the efficacy of supplemental PS in generally healthy middle-age adults. In this study, 13 adult participants (mean age 45.3 years) orally ingested 100 mg of PS three times daily (300 mg/day) or an equivalent placebo for 30 days. After the 30 days, participants were administered the Wisconsin Card Sorting Task (WCST) and the Stroop Color and Word Test to measure facets of cognitive performance. Performance of the two groups was compared using ANCOVA analysis, which controlled for body weight and self-reported biological sex. Results indicate no statistically significant difference between the PS group and control group for any of the outcomes in either task (all p's > 0.20). It is important to note that this is a preliminary analysis of an ongoing research project, so we did not necessarily expect to uncover statistically significant differences between the two groups given the small sample size. A greater number of participants is necessary to accurately determine the efficacy of oral PS.

Bolton PG, Arjune K, Phillips T, Zarubin V, Mickley Steinmetz KR
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Examining the N1, an event-related potential (ERP) waveform, and cortisol responses to motivational stimuli: A pilot study
In the current study, the N1 waveform was examined, which has been associated with an unconscious response to visual cues and selective attention. The N1 was investigated to determine the influences of highly-motivating stimuli on attention. Likewise, cortisol was examined to determine the influence of stress and arousal on the N1. First, we examined the effect of stimulus motivation level on the N1. Second, we examined cortisol on arrival (TP1) to determine if stress/arousal influence the N1. Third, we examined cortisol following the presentation of stimuli (TP2) to determine if individual differences exist in reactions to the stimuli. Event-related potentials (ERPs) were recorded via electroencephalography (EEG) while 52 participants viewed 48 positive/high-motivation (desserts, animals) and 48 neutral (rocks) images. Salivary cortisol levels were measured for a subset of 24 participants. First, results showed that there was not a significant difference in the N1 between image types, suggesting that the N1 is not modulated by stimulus motivation level. Second, there were no significant correlations between TP1 and N1 amplitude, suggesting that stress on arrival does not influence the N1. Third, a higher N1 amplitude was significantly correlated with increased cortisol levels, suggesting that cortisol levels were influenced by the

individual differences in participants' reaction to the stimuli. Higher cortisol in reaction to the stimuli also corresponded to a faster reaction time. The correlations found between cortisol levels and the N1 could be due to the excitement concerning the stimuli or to stress induced by the desire to perform well in the experiment. These correlations suggest that cortisol levels augment attention as evidenced by the N1.

Brown, TA
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Coping Skills in Freshmen Versus Seniors
In college, students are normally involved in many activities on their college campus along with dealing with homework and many other problems. These aspects can cause a lot of stress on students. How a student copes with their stress can determine how well a student may perform academically, mentally, and physically. The main purpose of this study is to examine the differences between freshman and seniors coping skills. It is hypothesized that freshmen will use less positive coping skills than seniors due to their recent transition to the more independent college life. In the present study, 61 Ferrum College students (31 freshmen, 30 seniors) were given the Ways of Coping questionnaire to determine the type of coping skills the students use the most. Independent samples t-tests were conducted. The study found that seniors are more likely to use better self-control coping skills and to seek more social support than freshman. As these are considered positive coping mechanisms, future research should explore why this difference exists between freshmen and senior students. Providing incoming freshmen with information about coping mechanisms and/or teaching opportunities on how to cope with stress may help ease their transition to college life.

Burgos Aguilar C, Ferris MJ, Sexton LL, Childers SR, Xiao R, Howlett AC
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mGluR2/3 Agonist LY379268 on G protein activation and CREB phosphorylation
Drug addiction is a growing issue without FDA-approved pharmacotherapy. Therefore, the concern on how to treat it is rapidly rising. The neuroadaptation of neurotransmitter release and signal transduction results in changes in cognitive processes that make it harder for users to stop using the addictive drug. It is believed that restoration of lost cognition due to substance abuse may be an effective treatment for addiction. Recent studies in our NIDA-supported "Center for the Neurobiology of Addiction Treatment (P50-DA006634)" indicated that the cognitive remediator LY379268, an mGluR2/3 agonist, is able to reverse self-administration of cocaine in rats (Karkhanis, Beveridge, Blough, Jones, & Ferris, 2016, Drug and Alcohol Dependence, 166, 51–60). We hypothesized that the mechanism of LY379268 is to alter cellular signaling in parts of the brain critical for enhanced cognition. To test this hypothesis, we examined changes in cellular signal transduction, determined as phosphorylation of the kinases extracellular-regulated kinase 1 and 2 (ERK1/2) and cyclic AMP response element binding protein (CREB) in specific regions of the brain related to drug addiction and cognitive processes. The rats were treated with acute and chronic dosing of LY379268, and after sacrifice, quantitated ERK and CREB phosphorylation in brain sections. Experimental groups of Sprague Dawley rats included: Control, acute, and chronic. The acute and chronic groups had a pump inserted subcutaneously that released 1mg/kg of LY379268 for 36 hours or 14 days, respectively, while the control group was subjected to the procedure but did not have a pump inserted. Immunohistochemistry was performed to look for changes in phosphorylation levels using antibodies coupled to infrared dyes. The stained brain slices were imaged and densities were analyzed for different parts of the brain related to reward, motivation, and executive function on each brain slice using LiCor Odyssey and GraphPad Prism software. We observed desensitization of the G protein-coupled receptor resulted after the chronic treatment with LY379268. We also observed an increase in CREB phosphorylation in chronic compared with acute treatment but there were no differences between groups in ERK phosphorylation.

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Behavior and Biochemical Aspects of Learning and Memory in Danio rerio
The goals of the current project are to examine the cognitive function of zebrafish (Danio rerio) using a unique approach to the Novel Object Recognition Test and to evaluate the contributions of Akt and GSK-3beta to zebrafish learning and memory. A NORT evaluates the ability of animals to recognize the presence of a novel object in the testing environment. The NORT protocol involves, first, showing two identical objects to the test subject, then, after a retention interval, replacement of one the familiar objects with a novel one. The animal should preferentially explore the novel object compared to the familiar object. Current NORT procedures entail removing the zebrafish from the testing aquarium during the retention interval. Our test employs a unique design in which the zebrafish remains in the aquarium during the retention interval. Behavior observations indicate that a long acclimation period

in the behavior arena is required prior to NORT testing. Additionally, the phosphorylation of Akt and GSK-3beta were assessed to determine the effect of novel environments on known biochemical aspects of learning. Preliminary biochemical data indicate that the Akt signaling pathway, as reported in other organisms, is present in zebrafish. Long-term goals for this project are to explore the effect of stress on memory in D. rerio.

Carter JS, Kearns AM, Weber RA, Reichel CM
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Long term impact of acute stress on cognition, anxiety, and reinstated heroin seeking in male and female rats
It has been suggested that withdrawal symptoms following substance use disorders (SUD) and post-traumatic stress disorder (PTSD) reciprocally exacerbate one another. Withdrawal symptoms, induced by cessation of substance abuse behavior, culminate as stressors and lead to persistent and compulsive relapse behavior. In addition, PTSD can be triggered by, and contribute to, withdrawal-induced stress responses further increasing relapse potential. Previous work has shown that rats potentiate substance-seeking behavior when exposed to scents associated with restraint stress, a model used to mimic PTSD in rodents. We examined the effects of restraint stress on heroin seeking and anxiety related behaviors during withdrawal in male and female rats. Rats in the stress group were restrained in a plastic tube that did not allow for mobility with exposure to a scent (Group: Stress). Unstressed rats were exposed to the odor in a neutral cage with no restraint (Group: NoStress). All animals underwent heroin self-administration (SA) and extinction followed by non-cued reinstatement testing with exposure to each scent. During SA, active lever presses and intake (mg/kg) did not differ between stress groups, but females had higher intake than males. On day 1 of extinction, females pressed the active lever more than males, without regard to stress group. For testing, the paired odor (or a novel odor) was placed into the operant chamber and lever responding was recorded. NoStress male rats responded above extinction values in response to paired and unpaired scents indicating a lack of discrimination between odors. Stress males differentiated between the odors by exhibiting the greatest reinstatement responding to the paired odor. There were no differences in responding to odors for females. A subset of rats went through an abstinence period before extinction, during which they experienced a battery of behavioral tests. Object recognition tests demonstrated that stressed males had a higher recognition index relative to all other groups. On an elevated plus maze, stressed rats spent more time on the open arms than their unstressed counterparts. Defensive burying trials showed that stressed rats had a shorter latency to bury and spent more time burying a paired odor-contaminated dish than unstressed rats, with males burying for more time than females. Future studies are necessary to examine the effects of heroin self-administration on behavior compared to rats taking saline.

Colbert S and Ackerman KM
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Effects of Nicotine on Zebrafish Retinal Development.
Smoking during pregnancy is associated with higher miscarriage rates, stillbirth, low birth weight, and sudden infant death syndrome. According to the CDC, 1 in 14 women continue to smoke during pregnancy despite the known risk of detrimental consequences for the infant (2016). Nicotine, the main addictive component in tobacco, is a likely contributor to these adverse outcomes as fetal brain and spinal cord are primary targets. Danio rerio (zebrafish) are a common animal model for developmental biology due to their easy raising, mapped genome, vertebrate characteristics, and rapid transparent development. Zebrafish may be an especially advantageous model system for the effects of nicotine on retinal development due to the similarities between the anatomy, circuitry, and development of the visual systems in zebrafish and in humans (Fadool & Dowling, 2008). Current literature indicates that nicotine disrupts normal development of the nervous system in zebrafish, but its effects on the visual system specifically has not received as much attention. Nicotine exposure results in path-finding errors among axons and delays differentiation of spinal neurons; reduces the overall size and survivability of embryos; and alters swimming behavior (Svoboda, Vijayaraghavan, & Tanguay, 2002). The broad goal of this experiment was to investigate the effects of nicotine exposure on eye development. Preliminary data from our laboratory demonstrates a change in eye size, but the overall size of the animal was not taken into account. Thus, this study will examine the effects of exogenous nicotine on eye size relative to overall body length. Zebrafish embryos were exposed to varying concentrations of nicotine (0 μM, 100 nM, 1 μM, 10 μM, 30 μM, 50 μM, and 100 μM) beginning at the 1-4 cell stage continuing through 3 days of development, a time point when the retina has fully differentiated. First, mortality rates were obtained one day after fertilization (24 hours post fertilization-hpf). These rates were pooled with data from other student researchers to provide an estimate of spontaneous mortality rates at different concentrations of nicotine. At 72 hpf, zebrafish were fixed overnight in paraformaldehyde and then transferred to PBS and glycerol series the following day. Representative images were obtained for each treatment using a Leica stereoscope. Body length and eye diameter were measured using ImageJ software. The experiment was replicated three times and the mean ratios for eye diameter to body length were compared to determine the effect of nicotine

concentration on eye size. This could have implications for how smoking during pregnancy impacts eye development in infants.

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Hemispheric Lateralization in Pun Processing
The purpose of this study was to better understand the way the brain processes specific kinds of speech information that could be interpreted in multiple ways. More specifically, previous literature has suggested that both the left and right hemispheres of the brain are necessary to accurately recognize a syntactic ambiguity as a pun (McHugh & Buchanan, 2016). For our study, we assessed the laterality of pun processing through a dichotic listening task, with greater performance in one ear reflecting a stronger use of the opposite hemisphere. Participants began by completing an Edinburgh Inventory to assess their degree of handedness to control for the influence of handedness on laterality. Following the inventory, participants then completed the dichotic listening task, with each trial consisting of either two neutral sentences or one neutral sentence and one sentence containing a pun. In the pun-present trials, puns were randomly presented in either the right or left ear, with an equal number of pun-present trials being presented to each ear over the course of the experiment. The participant's task was to identify whether or not a pun was present. For this test, the participant was first presented with a practice block of 12 trials, followed by two blocks of 192 trials each. Finally, an online questionnaire was used to collect relevant demographic and health information. D prime (d') values were calculated for both the left and right ears across participants. Our hypothesis was supported based on a paired-samples t test that yielded no significant difference between d' values from the left ear and d' values from the right ear. Therefore, we conclude that it is likely that the two hemispheres are contributing equally to the processing of spoken puns. These findings inform future research pertaining to syntactic ambiguities, as well as research investigating speech perception in numerous populations who experience deficiencies in speech comprehension.

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Genetic induction of CD163 in macrophages decreases pain and inflammation and promotes wound healing.
Chronic pain could develop in up to 50% of patients following major surgeries in which the degree of tissue damage is extensive. The prolonged period required for tissue repair results in a prolonged local inflammatory process. Surgical inflammation is initially driven by M1 macrophages. Macrophages also orchestrate tissue repair and the resolution of inflammation by acquiring an anti-inflammatory phenotype, namely M2 macrophages. In consequence, a prolonged transition from M1 to M2 macrophage phenotype is associated to the development of chronic postsurgical pain. Results from past experiments in our lab indicated that the scavenger receptor CD163 promotes the transition from M1 to M2 macrophages in vitro. Further experiments showed that local CD163 induction in vivo decreased pain-related behaviors in a rat model of postoperative pain. Although CD163 appears to speed up the resolution of inflammation and the resolution of pain, its effects in wound healing are not known. We now hypothesize that the induction of CD163 macrophages would not negatively affect the rate or quality at which an open wound heals. To test this, we utilized a 3D human wounded skin organotypic tissue ex vivo model, human primary macrophages, and nanotechnology to induce CD163 gene expression. Macrophages were randomly added to the wounds under three different conditions: macrophages with no treatment (n=4), macrophages transfected with an empty vector plasmid complexed with the nanoparticle mPEI (n=6), or a plasmid encoding CD163 complexed with mPEI (n=6). Tissues were collected at day 0, 1 and 3 for immunohistochemistry. The re-epithelialization over the wound, the cellularity and thickness of that new tissue were quantified. We found that wounded tissues with the intervention have significantly more re-epithelialization on day 3 of incubation than tissues with macrophages with the empty vector. There was no significant difference in the cellularity or the overall thickness of the new tissue among groups, which demonstrates that the new tissue induced in the CD163 group is healthy. Our findings indicate that the genetic induction of CD163 in macrophages may be beneficial for efficient tissue repair. The clinical implications of our studies are based on the fact that this nanotechnology has been already tested in humans for gene induction, and that our precise cell targeting gene therapy approach could be a non-narcotic therapeutic alternative to treat chronic postoperative pain.

DeZego P, Reed E, Kim S, Anderson A, McKinney G, Benedict C, Adams E, Gonzalez S, Lewis J, Noonan T, Saunders C, Silver W
Department of Biology, Wake Forest University
The sixth sense and the earthworm, Eisenia Hortensis
The earthworm is an extremely important indicator of healthy soil, yet little is known about how these annelids detect and avoid irritating chemicals in polluted soil. Thus, we designed and conducted experiments investigating the earthworm's ability to detect chemicals in their environment. The detection of irritating chemicals is known as chemesthesis. One known chemical repellent is allyl isothiocynate (AITC). AITC is known to activate the channel transient receptor potential subfamily A, member 1 (TRPA1) in a wide variety of animals. Thus, TRPA1 is likely present in Eisenia hortensis, the European nightcrawler. We tested for the presence of this channel through a simple behavioral assay using varying concentrations of AITC. These data show that earthworms detect and avoid concentrations of AITC as low as 1.0 mM. After treatment with a TRPA1 antagonist (H3-030031), the avoidance of AITC was significantly reduced. We also tested for the avoidance of denatonium benzoate (a bitter compound) and acetic acid (a sour compound) using the same assay. Worms often feed on bacteria, which can have a bitter taste. Although most organisms are averse to bacteria, our hypothesis was that worms would be attracted to the bitterness due to their diet. Contrary to our hypothesis, denatonium was shown to be aversive at concentrations as low as 40mM. Acetic acid is aversive to a wide variety of organisms; we hypothesized that the worms would be aversive to this compound because acidic receptors are highly conserved across species. Additionally, we tested ethyl hexanoate and ethyl pentanoate—two chemicals produced by fungi that earthworms consume —to determine if they attracted earthworms. To test for attractive behavior, we placed multiple worms in a small box with one Kimwipe soaked in the chemical and another Kimwipe soaked in water on the opposite side. After 24 hours, the number of worms on each side were counted; these results do not support the hypothesis suggested by an earlier study that fungal compounds are worm attractants. In summary, earthworms are capable of detecting and responding to dilute chemicals in their environment.

Doddapaneni D, Asede D, Bolton MM
Max Planck Florida Institute for Neuroscience
Properties of neurons in the anterior intercalated cell cluster
Characterization of amygdala circuits is important for understanding fear behavior. Whereas thalamic and cortical sensory inputs to glutamatergic neurons in the lateral amygdala (LA) are well studied, the contribution of GABAergic neurons to information processing in this system is still poorly understood. The ITCs are masses of GABAergic cells organized in clusters around the basolateral amygdala (BLA), ideally located to regulate amygdala output. Among these is a distinct anterior paracapsular ITC cluster (apITC). Although fiber tracts carrying fear-related sensory inputs (thalamic and cortical) overlap with the location of apITC, nothing is known about their properties and function in amygdala fear circuit. Here, we address two questions: (1) Are the intrinsic properties of apITCs different from other ITCs? (2) Are apITCs innervated by sensory inputs and what are their properties? Using whole cell patch clamp recording technique in brain slices from adult mice, we measured passive and spiking properties of identified apITCs and found that they display higher input resistance and membrane capacitance, compared to the medially located ITCs (mpITCs). In addition, the maximum firing rate of apITCs was lower than mpITCs, with larger action potential half width and fast after-hyperpolarization. These properties were modulated by Dopamine (D1) receptor activation. To determine whether apITCs receive fear –related inputs, we expressed Channelrhodopsin fused to a yellow fluorescent protein in the relevant cortical and thalamic areas. Optogenetic stimulation of terminals revealed that apITCs receive monosynaptic sensory inputs from the same thalamic and cortical regions that innervate the LA. These inputs are glutamatergic, and mediated by AMPA- and NMDA-receptors. However, excitatory drive from the thalamus was stronger than from cortex. Ongoing research seeks to understand apITC targets, and possible role in regulating the activity of LA principal cells during fear learning. This knowledge may ultimately help us better understand fear and anxiety-related disorders and the neuronal circuitry underlying their pathogenesis.

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The Developmental Impact of DDAH1 in Mural Cells
Stroke is one of the leading causes of death in America. Uncovering a potential genetic mechanism of or vulnerability to stroke would introduce the potential of more effective targeted therapies to lessen the effects of life-altering disabilities connected to stroke. In our lab it has been demonstrated that following ischemia, the blood brain barrier (BBB) does not recover its previous integrity, leaving the brain vulnerable to harmful molecules passing

through the blood. One method of this BBB breakdown involves the enzyme dimethylarganine dimethylaminohydrolase (DDAH). The DDAH1 isoform increases the effectiveness of enzyme nitric oxide synthase (NOS) to produce NO by inhibiting the endogenous inhibitor of NOS. The NO produced as a potential result of this pathway in pericytes (contractile cells found in vascular endothelium) seems to rapidly activate matrix-metalloproteinase 9 (MMP9), degrading the extracellular matrix during ischemia and increase volume of tissue affected by stroke. These findings led us to explore the role of DDAH1 in stroke. We hypothesized that genetically knocking out DDAH1 in pericytes could reduce stroke volume by preventing the NO-mediated rapid activation of MMP9. However, little is known about how a deficiency in DDAH1 would impact development of mural cells (pericytes, smooth muscle cells) and resulting BBB integrity. We used immunohistological protocols including CD13 antibody to compare the number of pericyte somas in the control versus knockout mice. We also performed two-photon (2p) microscopy to examine in vivo flow within microvessels of the cortex, and electron microscopy (EM) in order to look at markers of BBB integrity such as presence of pericytes and tight junction morphology in the DDAH1 knockout mice as well as controls. 2p imaging showed selective BBB leakage of 10kDa but NOT 40kDa contrast in only knockout mice. Preliminary data indicates that there is no significant difference in pericyte number or morphology. EM imaging shows no overt structural differences in tight junction morphology. These data indicate that a genetic knockout of DDAH1 could play a role in the actual development of the tight junctions. We are moving toward examining differences in the structural makeup of the tight junctions of control versus knockout mice at this time. The selectivity of leakage by the BBB in knockout mice could provide a mechanism for the lasting inflammatory effects of vascular injury including stroke.

Dougherty, Miranda
Ferrum College
Stress In Season and Out of Season
College student-athletes experiences many stresses academically and from their sport. The purpose of the study was to investigate stress of college student-athletes in and out of season. The hypothesis was that there will still be stress from the sport out of the season. The physical, mental and time demands are similar during the season as out of season. A stress survey was given to the men and women's soccer teams during their season and in the post-season ends. The scores from the questions were averaged. A correlated groups t-test was conducted to compare the survey averages from the in season results to the out of season results. The study demonstrated that student-athletes experience significantly similar amounts of stress in the areas of family relationships, interpersonal relationships, and romantic relationships in season and out of season. The stress and obligation of athletics continues to play a role in a student-athlete's life despite being out of season. There were non-significant findings in similarity in the areas of injuries, performance demand, training adaptations, family relationships, and academic requirements.

Duryee ML, Zens, MM, Sparrock L, Franssen RA, Franssen, CL
Department of Psychology, Longwood University
The Trials and Triumphs of a New Interdisciplinary Neuroscience Studies Minor
With the development of our Interdisciplinary Neuroscience Studies (or "NeuroStudies") Minor in 2015, Longwood University became a part of the rapid proliferation of undergraduate neuroscience programs both within Virginia and nationwide. Our efforts have been rapidly rewarded; over 70 students from a variety of disciplines – Biology, Communication Sciences and Disorders, Criminology, and Psychology - have declared a NeuroStudies minor. Our first class of 4 NeuroStudies minors graduated in 2017 and over 20 will graduate in Spring 2018. This torrid pace of growth is both exciting and instructive. We are recognizing a need at Longwood to continue development of interdisciplinary opportunities to keep up with the demands of current and prospective students. Our initial curriculum was designed with an intentional focus on connections between neuroscience and other disciplines across the liberal arts, as described in Wiertelak & Ramirez (2008). This interdisciplinary approach, incorporating social sciences and humanities with traditional STEM disciplines, is resonating well with our students, faculty, and administrators at Longwood. Here, we discuss the design, implementation, and growth of the minor thus far, with an eye toward the preparation that our minors have for life after Longwood. We discuss the beginning phases of evaluating and assessing the program. Further, we compare and contrast the design of our neuro-related curriculum with curricula of other Virginia schools and peer institutions with the goal of determining the benefits and drawbacks of our interdisciplinary NeuroStudies approach.

Eagle AK, Lever LC, Franssen CL
Department of Psychology, Longwood University
Spit Happens! Salivary Cortisol Responses of Wilderness Therapy Clients as an Efficacy Measure
Wilderness therapy, a subset of the broader field of outdoor therapy, is used to treat clients with a range of issues from addiction to emotional struggles (Russell, 2001). The efficacy of wilderness therapy is unclear, however, as current evidence of effect relies heavily on self-reported measures. Clients do report beneficial outcomes related to substance abuse, behavioral disorders, and mood disorders when programs incorporate elements of nature and exercise as well as interactions with a licensed clinical practitioner (e.g., Bettmann et al., 2013; Hoag et al., 2014; Russell et al., 2015). Amidst changing health insurance coverage, enhanced research support and improved efficacy measures for alternative mental health treatments are needed. Anxiety disorders are typically comorbid, if not a primary diagnosis, for many patients seeking treatment through wilderness therapy, indicating that assessing changes in anxiety profiles may be one measure of efficacy. To meet this need, we studied mental health patients' neuroendocrine profiles during a wilderness therapy experience. In this project, we partnered with Blackwater Outdoor Experiences, a wilderness therapy group based in Midlothian, VA. We collected saliva samples and behavioral data at several time-points before, during, and after a 22-day wilderness therapy trip. Samples were assayed for stress-related hormones, namely cortisol and DHEA. Our findings represent the first integrative analysis of wilderness therapy clients that includes neuroendocrine data.

Eceiza A, Lahue C, Kaur A
Neuroscience Program, UNC Asheville
Cloning Vomeronasal Type-2 Receptors for Expression and Analysis in a Cell Culture Model System
The vomeronasal organ (VNO) is a chemosensory organ present in amphibians, reptiles, and non-primate mammals. In mice, vomeronasal neurons express vomeronasal-1 receptors (V1R) or vomeronasal-2 receptors (V2R), both of which are G protein-coupled receptors involved in pheromone detection. V2Rs are expressed by the basal neurons of the VNO. They differ from V1Rs in their sequence length and G-protein linkage, and are of special interest because they are used to detect protein pheromones, the Major Urinary Proteins (MUPs), which induce intermale aggression, female responsiveness to mating, and territory marking behaviors. Because V2Rs use combinatorial coding instead of a labelled line coding strategy, linking pheromone responses to the correct V2R has so far been difficult as each cell expresses a different V2R and multiple cells could respond to a single MUP. We designed primers for each V2R to amplify separate individual sequences from a cDNA library, which could then be transfected into cells using vectors. This cell culture method would allow for deorphanization of V2Rs by creating entire cell populations which only express a single V2R. With V2Rs deorphanized, mapping of pathways can begin from a bottom-up method, instead of the more difficult top-down approach. Here we show how to isolate and clone V2Rs for individual eventual expression in mammalian cells using DNA purification, Zero Blunt TOPO cloning PCR kit and ligation into mammalian vector. V2Rs 83 and 121 were successfully cloned into a bacterial vector with a complete sequence. The blunt end cloning did result in correct directionality when the V2Rs were transferred into mammalian vectors. Our results demonstrate progress towards setting up a cell culture based V2Rs expression system. This system will allow for further research to deorphanize individual V2Rs by matching them to their corresponding ligands. Stimulation of V2Rs reproducibly activate specific neural circuits in mouse brains, allowing for reliable study of how external environmental cues can direct behaviors. Cloning V2Rs is the first step to identify receptor-ligand interactions, which can be utilized for future research.

Elder NH, El Bejjani R
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Engineering a Luminopsin Tool to Study the Caenorhabditis elegans Nervous System
Luminopsins (LMOs) are self-activating optogenetic tools that have the potential to be utilized in a wide array of C. elegans experiments. Luminopsins consist of a channelrhodopsin (ChR) tagged with an N-terminus Gaussia luciferase (GLuc) and a C-terminus fluorescent protein. LMO proteins retain ChR's functional response to an external light source, but they also can also open when GLuc metabolizes coelenterazine (CTZ) to produce blue light. The GLuc-produced fluorophore in turn provides the nearby channelrhodopsin with the energy required to open the ion channel. LMOs offer the unique capability, therefore, of being either chemically or optically activated. Chemical activation of LMO provides it with the advantage of being used in broad screens. Additionally, titration of the concentration of CTZ would allow for interrogation of effects of neural firing over a range of excitation strengths. LMOs could also be used to compare the effects of rapid light-induced neuron firing and chronic chemically-induced firing on processes such as axon regeneration and synaptic plasticity. The nematode Caenorhabditis elegans and its well characterized nervous system have provided an ideal model system in which to study neurobiology. Due to the organism's rapid generation time and tractable genetics, numerous forward genetic

screens have utilized C. elegans to identify a range of pathways and genes involved in processes such as synaptic transmission and axon regeneration. Their naturally translucent bodies have also made them an ideal system in which to develop and use optogenetic tools, such as channelrhodopsins. Channelrhodopsins (ChR), non-specific cation channels that are gated by blue light, are frequently used to interrogate specific aspects of C. elegans neural circuitry. However, due to ChR's need for an external light source, they are not easily scalable to large screens. Here, I present my attempts to build a functional luminopsin for expression in the C. elegans nervous system and the differences between a putative novel LMO and existing luminopsin constructs. I will also outline experiments to functionally validate a C. elegans optimized LMO in the future and its potential applications.

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Effects of Estrogen on Sensory Neuron Participation in Axon Regeneration Following Peripheral Nerve Injury
Peripheral nerve injuries affect hundreds of thousands of people each year in the United States alone. Despite the fact that peripheral nerves have the ability to regenerate, functional recovery is often very poor due to insufficient axon regeneration. Previous studies have shown that treatment with estrogen can significantly enhance the regeneration of motoneurons following peripheral nerve injury; however, this has not yet been studied in sensory neurons. Both sensory and motoneuron regeneration are necessary in order to obtain full functional recovery; therefore, the present study examined the role of estradiol signaling in sensory neuron participation and hypothesized that systemic estrogen treatment will increase the rate of axon regeneration in sensory neurons following peripheral nerve injury. Male and female mice were anesthetized and the common fibular branch of the right sciatic nerve was cut and repaired using fibrin glue. Immediately after transection surgery, mice were treated for two weeks with either estradiol-filled or unfilled (blank) capsules inserted in the nape of their neck. Upon completion of estradiol or control treatments, animals underwent a second surgery during which a retrograde tracer was applied 1.5mm distal to the original repair site in order to label the sensory neurons whose axons had regenerated at least that distance. Three days following retrograde labelling, all animals were sacrificed, and regeneration was quantified by counting the number of neurons containing fluorescent tracer dye. Systemic estradiol treatment significantly increased the number of labelled axons, and therefore indicates an increase in sensory neuron participation in axon regeneration. These findings demonstrate the ability of sensory axons to regenerate in a similar manner as motoneurons, and show that estrogen treatment can be used to increase the likelihood of full functional recovery following peripheral nerve injury.

Evans D, Trevisani C, Marshall SA
Fred P. Wilson School of Pharmacy, High Point University
Detecting The GFAP-DREADD Gene Using PCR And Gel Electrophoresis
G-coupled protein receptors (GPCRs) are found on various cells and can be activated by a number of different ligands. However, DREADDs (designer receptors exclusively activated by designer drugs) are synthetic GPCRs activated exclusively by CNO (clozapine-N-oxide). DREADDs are artificially introduced by either using transgenic lines or through virus. GFAP-DREADD transgenic mice express DREADDs in the GFAP+ astrocytes without the need for an infection by a virus. Since the long term interest of this lab is alcohol abuse, and the wildtype (C57) is known to consume alcohol, our mice are bred by backcrossing GFAP-DREADD transgenics and C57 mice. Before being used in a study, we need to know that the mice being used has the GFAP-DREADD gene. To do this, we take a DNA sample by snipping the mouse's tail. DNA is extracted from it then amplified using PCR. This is done by using a forward primer for the DREADD gene and a reverse primer for the fluorescent tag, Tg Tet R, that is co-expressed with the GFAP-DREADD gene. After amplification, the sample is loaded into a gel electrophoresis chamber where the DNA samples are separated based on size. These bands are compared to a known DNA ladder to determine whether the GFAP-DREADD and Tg Tet R genes are present. Results have shown that we can successfully breed GFAP-DREADD/C57 mice at High Point University. Future studies will use these mice to determine the role of astrocytic GPCR signaling in behaviors such as alcohol consumption, anxiety, and drug abuse.

Fisher C, Nazemi A, Kaur A
Neuroscience Program, UNC Asheville
Inducing Gene Expression of Major Urinary Proteins (MUPs) in the female mouse liver cell line Hepa1-6"
Mice, Mus musculus, are a primarily nocturnal species and therefore rely heavily on their olfactory system to detect changes in their environment. Specifically, mice rely on protein pheromones, the Major Urinary Proteins (MUPs), non-volatile molecules which are detected by the vomeronasal organ (VNO). MUPs are synthesized in the liver, excreted in the urine, and serve as genetically encoded pheromones which direct social behaviors such as countermarking, aggression, or mate preference. Mice can also use MUPs as a way to detect sex, status, and identity

of the emitting individual. MUP expression is thought to be controlled by a set of hormonal axes consisting of testosterone, growth hormone, and thyroxine. The mouse genome encodes 21 MUPs, yet, each adult male mouse will express a unique set of 4-12 MUPs. The mechanism by which MUPs are chosen for expression is not well understood, but does not appear to be random, as some MUPs are chosen for expression more often than others. This study looks to understand how individual MUPs are chosen for expression by utilizing a cell culture model system. The female murine liver cell line Hepa1-6, is being used because it does not endogenously show expression of any MUPs, but previous studies have shown that female mice are capable of producing MUPs at male levels if they are exposed to testosterone. A combination of hormonal and drug treatments consisting of methylation inhibitors and deacetylation inhibitors are being used to induce MUP expression in these cultured cells. Following treatment of cells, they are harvested for RNA isolation, and the resulting cDNA library is examined for MUP expression. The results of this study using the chosen concentrations and treatment periods are not sufficient to induce MUP expression. As such, a working protocol for the induction of MUP expression is yet to be established. The creation of a working protocol will in the future contribute to the greater understanding of gene expression.

Fruchterman TC, Elliot CN, Sparrock LS, Franssen RA
Department of Biological and Environmental Sciences, Longwood University
Individual variation in maternal response tied to differential expression of oxytocin and estrogen in several brain regions
Over the past few years, our lab has been working to understand why some maternal rats are more responsive to a given litter of pups than others. Specifically, we presented thirty primiparous rats with six different combinations of pups: 8 of her own pups (own) and 0 pups from another mother (alien), 4 own/4 alien, 3 own/5 alien, 2 own/6 alien, 1 own/7 alien, and 0 own/8 alien pups. We found that some mothers would quickly collect and care for all pups in a litter regardless of what percentage were her own (Good Moms) whereas others would be slow to care for pups – if at all – regardless of group (Bad Moms). To determine the underlying neural basis of these behavioral differences, we counted the number of neurons responding to oxytocin or estrogen in the prefrontal cortex, amygdala, hippocampus, nucleus accumbens, and medial preoptic area. Our findings suggest that both oxytocin and estrogen are correlated with Good and Bad maternal behavior in all of the studied brain regions except for the hippocamus. These results have broad application for labs conducting clinical tests using maternal rats as well as providing a target for remediation of Bad maternal behaviors.

Gaudin VA, Cleland CL
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Escape responses from looming stimuli in Phidippus audax
Animals use withdrawal and escape responses to retreat from threats. Looming stimuli, which represents the approach of a predator, evokes an escape response in jumping spiders that is mediated primarily by visual cues. The majority of studies have focused on the spider jumping in response to prey; only limited studies have explored the escape response from a predator. The goal of our research was to determine the strategy used by jumping spiders, Phidippus audax, to escape from looming stimuli. Looming stimuli was created by using a controlled projection of a 3” black polyurethane ball, 1 m/s at a 45 degrees angle, against a white background, towards the spider, without actually hitting the spider. The direction of “attack” was varied in 45-degree increments, totaling in 8 angles, around the spider. The resulting response was captured with high speed video (300 fps) and automated software particle was used to quantify the location and orientation of the spider throughout the escape response. Looming stimuli consistently evoked a side-step, or turn followed by walking. Our results (n=9) showed that turning response angle depended significantly on stimulus direction. Preliminary findings (n=9 spiders) showed that the spiders uniformly escaped from the looming stimulus. In most (~80%) of the trials the spiders translated without turning and in ~20% of the trials both turned and translated. Typically, spiders showed multiple temporal components to their response. Surprising, 2 spiders jumped away from the looming stimulus, a response previously reported only associated with prey capture. These preliminary results suggest jumping spiders may use novel strategies to escape from looming stimuli

Gonzalez EN, Amat S, Christie JM
Max Planck Florida Institute
Using Viral Vectors to Target Cell Types in the Cerebellum
The cerebellum is the part of the brain that is responsible for motor learning which includes coordination, balance, and posture. How exactly the cerebellum does this is not yet fully understood. Experimental manipulations can show how different cerebellum cell types interact and how they individually function. Viral vectors are used to carry genetic information and promoters within these vectors transduce expression in specific cells. The distinct cell types

in the cerebellum each have unique characteristics that can be targeted with promoters that genetically access the cells alone or in combination.

Greengrove EL, Dorn LE, McIntosh SE, Marshall SA
Fred P. Wilson School of Pharmacy, High Point University
Increase in hippocampal microglia after non-dependent ethanol binge
The impact of excessive drinking on the neuroimmune system is an emerging issue as the neuroimmune response has the capacity to affect both brain damage and behavior. It has previously been shown that microglia are activated following dependent alcohol consumption in clinical and preclinical studies, but the effects of the non-dependent binge alcohol consumption on the neuroimmune system is relatively unknown. Moreover, many people who binge drink do not become alcohol dependent; therefore, it is important to understand the implications of binge drinking independent of brain damage and prior to dependence. Using the "Drinking in the Dark" (DID) model, the current study specifically examines this window of alcohol misuse. This project focused on the hippocampus as binge drinking is associated with lapses in memory, and memory dysfunction is related to neuroimmune system dysregulation. Immunohistochemistry labeling of ionized calcium binding adaptor molecules 1 (Iba1) was used to determine the effect of alcohol on microglia. C57BL6/J male mice were divided into two groups and underwent either a one-week of ethanol (EtOH) or sucrose consumption. Using immunohistochemistry, Iba1+ cells, or microglia, were identified and counted in the Dentate Gyrus (DG), Cornu Amonis 1 (CA1) and Cornu Amonis 2/3 (CA2/3) regions of the hippocampus. Results indicated a significant increase in microglia for animals who consumed EtOH compared to the sucrose groups in DG and CA1 regions. However, there was no effect found in the CA2/3 region for microglia or Iba1 cells in EtOH compared to sucrose groups. These findings provide further evidence that non-dependent alcohol consumption affects the neuroimmune response within the hippocampus. Future studies should determine if the microglial responses observed here alter memory or behavior.

Grifasi IR, McIntosh SE, Marshall SA
Fred P. Wilson School of Pharmacy, High Point University
Alterations in glial activation following binge-like ethanol consumption
Consistent bouts of alcoholic binge drinking increases the likelihood of eliciting hyper reactive responses of the neuroimmune system. Glial cells, key players in the neuroimmune response, play a prominent role in ameliorating neuronal damage. However, independent of neuronal damage, ethanol leads to altered glial cell function. Many people who binge drink show no neurodegeneration, therefore these experiments study the effects of ethanol on glial activation in a non-dependent, non-damaging alcoholic binge model. Binge drinking was modeled with male C57BL/6J mice in the Drinking in the Dark (DID) paradigm in order to effectively simulate nondependent binge consumption. Punches of the hippocampus from snap frozen brain were taken immediately following the DID, 1-day post-ethanol, or 10-days post-ethanol ingestion. RNA was extracted to perform qRT-PCR analysis of microglia and astrocyte mRNA expression. The microglia specific markers, integrin alpha M (Itgam), and allograft inflammatory factor 1 (Aif1) were used. Itgam is up regulated by microglia activation, whereas Aif1 is expressed in all microglia. An astrocyte specific marker, glial fibrillary acidic protein (GFAP), was used to determine if ethanol induced astrocyte activation. A one-way ANOVA analysis indicated a 3 fold increase in GFAP mRNA and a 4 fold increase in Aif1 mRNA on the fourth and final day of ethanol consumption. However, ethanol did not trigger any significant changes in Itgam mRNA. Moreover, there were no significant changes in mRNA after 1-day or 10-days post-ethanol consumption. These results indicate that changes in the mRNA are only prevalent during intoxication in non-dependent animals and that there are no persisting alterations during abstinence. We presume acute binge drinking in nondependent individuals only produces short-term effects on the glial responses, but the effects of repeated binge exposures are likely to produce more long-term effects. Future studies will examine whether repeated cycles will evoke long-term effects on glial activation.

Guerrero R, Brown Q, El Bejjani R
Department of Biology, Davidson College
A novel role for Notch in mechanosensory neuron connectivity in C. elegans
Notch receptors are conserved transmembrane proteins that regulate key developmental processes and promote stem cell proliferation and renewal. Notch signaling remains active in the nervous system from birth to adulthood. Notch pathway genes are highly conserved across many species, including humans, and extensive work to decipher Notch signaling was initially done in Caenorhabditis elegans. While these animals' mechanosensory neurons have been well characterized behaviorally, little is known about the late stage development of the connections between them. We show that animals lacking the Notch metalloprotease sup-17/ADAM10 have significantly higher rates of the ALM-AVM nerve ring not developing than wild type animals. Significantly higher rates of incorrect development

were also found in Notch receptor and gamma-secretase complex mutant animals, confirming that the Notch pathway is involved in the ALM-AVM connection at the nerve ring. We have recently found that Netrin pathway mutants also display a similar nerve ring defect to the Notch pathway mutants suggesting a possible mechanism.

Hathaway S., Cely C., Mulloy S., Tibbetts E., Fahrbach S.
Department of Biology, Wake Forest University
Impact of social experience on synaptic density in the mushroom bodies of the paper wasp Polistes fuscatus
Polistes wasps establish a social hierarchy among nestmates that guides division of labor. In Polistes fuscatus, individuals can recognize nestmates using visual cues provided by facial and abdominal markings. Visual information is processed in the mushroom bodies of the insect brain, which may accordingly contribute to the recognition of individuals via visual cues. Wasps were reared in their normal social environment to preserve their facial recognition ability, or raised in social isolation to reduce this ability. Brain sections of adult wasps were immunolabeled with the synapsin antibody anti-SYNORF1 for imaging on the Zeiss 880 Laser Scanning confocal microscope. The density of the microglomerular synaptic complexes in the lip and collar of the mushroom body were analyzed blind to rearing condition using Imaris analysis software. Statistical analysis of the density of microglomeruli in the collar region of both groups showed no difference between the two rearing conditions. The unchanged synaptic density of the socialized individuals compared with the isolated individuals indicates that synaptic plasticity in the mushroom body neuropils may not be required for facial recognition, or that such plasticity may only be evident earlier in adult life. This study provides a foundation for the use of P. fuscatus for further exploration of the neural mechanisms of learning and memory.

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Alcohol Studies Center, UNC-Chapel Hill
An inexpensive and customizable system for measuring consummatory behaviors in mice.
Our lab seeks to understand the neurobiological underpinnings of various consummatory behaviors including alcohol consumption. In order to collect reliable data that accurately measures drinking behaviors in rodents, we required an inexpensive system capable of detecting and recording licking behaviors at multiple water spouts simultaneously. To accomplish this goal we turned to open-source computer science, with economical micro-controllers and single board computers that can be used to cheaply replicate otherwise expensive behavioral equipment. An Arduino and Raspberry Pi were used to create Lick-o-Meters that can count the number of licks from up to four single-mouse cages simultaneously and in real time display a time-series showing licks per minute. This system is both significantly cheaper than commercially available products, and is far more customizable because the code running the system can be optimized to suit any number of behavioral tasks. Projects such as this can be instrumental in making experimental equipment more freely available for the wider neuroscience community.

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Combination of cues in 3D vision: Interaction between binocular rivalry and motion parallax
As we make head movements, the relative motion of objects at different distances (motion parallax) is a powerful cue for depth perception. Motion parallax makes it possible to perceive the 3D layout of a scene, including the relative distances of different objects. Binocular rivalry is a well-known perceptual phenomenon, in which two different images are shown to the two eyes (e.g. left and right diagonal), and perception alternates between the two images. Here we investigated the conditions where it is possible to perceive both depth from motion parallax and binocular rivalry simultaneously, in the same display when they are spatially superimposed. We used diagonal sinusoidal gratings for rivalry and moving vertical sinusoidal gratings for motion parallax, varying the spatial frequency of the components (vertical: 1.2, 2.64 cpd; diagonal: 1.2, 2.64 cpd). CrystalEyes LCD shutter glasses were used for stereoscopic display to present binocular rivalry. To simulate motion parallax, observers made side-to-side head movements. A system using an electromagnetic head tracker was used to synchronize the motion of images on the computer to the head movements. Four observers performed a depth task or rivalry task in separate trials. In the rivalry task, observers pressed one key when they perceived the left diagonal, and another key when they perceived the right diagonal, continuing to make key presses over a period of 60 seconds. The time between key presses gave a measure of how fast the rivalry alternations occurred. In the depth task, observers indicated whether the top or bottom half of the image was in front or behind. There was a strong interaction between binocular rivalry and motion parallax: it was difficult to perceive both simultaneously. The rivalry alternations were very slow and hard to perceive, while depth perception performance was poor. The interaction was reduced if binocular rivalry and motion parallax were shown with different spatial frequencies: it was possible to perceive both rivalry and depth simultaneously. These results have implications for models of 3D vision, indicating that motion parallax

and binocular rivalry are both important cues involved in depth perception. Furthermore, binocular rivalry and motion parallax are processed in different spatial frequency channels. These results are similar to those found previously with stereopsis and binocular rivalry (Buckthought & Wilson, 2007).

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Examining the role of serotonin agonism at the rostromedial tegmental nucleus towards behaviors of cocaine-averse rats
Substance abuse and drug addiction research is currently dominated by a focus on the rewarding properties of such substances. However, many drugs produce a period of aversion following this rewarding phase. This biphasic mechanism of drugs is critical to understanding the variation in individual drug users' decisions to continue using a drug, as users need to decide whether or not the rewarding effects outweigh the aversive, and whether continued use would be worth the cost. Current research indicates dopamine (DA) as the primary motivational neurotransmitter released to produce the rewarding effects experienced by drugs. The recently discovered rostromedial tegmental nucleus (RMTg) inhibits the key DA regions involved in the motivation to pursue further use of substances such as cocaine, and acts as a "braking" mechanism contributing to cocaine's aversive phase. However, previous research had only partially elucidated the mechanisms by which cocaine activates the RMTg to produce aversive effects. Recent data from our lab shows evidence of serotonin 2c receptor (5-HT2Cr) expression on cells at the RMTg, suggesting a novel mechanism by which cocaine (which increases both dopamine and serotonin) produces aversive or anxiogenic effects due to serotonergic influences, and, in turn, helps to prevent continued cocaine use that may eventually result in addiction. To better understand serotonin's influences on cocaine aversion, we investigated the effects of 5-HT2Cr agonism at the RMTg in rats on two types of aversive behaviors: anxiety-like behaviors as measured by an elevated-plus maze (EPM) apparatus, and cocaine avoidance as measured by a runway task. Rats exhibiting high cocaine avoidance on the runway task also exhibited high cocaine-induced anxiety on the EPM. Microinfusions of a 5-HT2Cr antagonist into the RMTg significantly reduced cocaine-induced anxiety one hour later and returned rats to anxiety levels not significantly different from baseline recordings taken before cocaine involvement. Hence, microinfusions of the 5-HT2Cr antagonist into the RMTg of high avoider rats altered EPM behavior in a way consistent with a reduction of cocaine-induced anxiety levels, supporting the hypothesis that serotonin agonism is involved in the formation of aversion to cocaine. In summary, these findings provide evidence of serotonin's direct influence on the RMTg, and corroborate a mechanism to be exploited to prevent cocaine addiction. Funding NIH-NIDA 1R01DA037327.

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Cellular Activity in the Primary Somatosensory Cortex Underlying Animal's Perception and Decision-Making
The rodent Vibrissae system is an excellent model for investigating somatosensory perception. This is because the neurons of the primary somatosensory cortex are organized into specific barrels, each corresponding largely to an individual whisker. Using this model, we investigated the neural correlate of sensory perception and decision making. This was done by examining cellular activity in the primary somatosensory cortex that was underlying the animal's perception and decision making. We used two-photon imaging to investigate the barrel cortex in awake, behaving C57BL/6 mice. This technique enabled us to image cells over long periods of time with high resolution. We trained mice with a detection task that gave stimulation on one go-whisker. A water reward was available after stimulation. This task was useful because detection is the main aspect of sensory perception. Success of the task was measured by the ability to pair the stimulus on the go-whisker with water reward.

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Early Developmental, Low-Dose BPA Exposure does not Significantly Impact Zebrafish Locomotor Activity
Bisphenol-A (BPA) is a foundational component of polycarbonate plastics and epoxy resins used to make water bottles, food containers, pipe sealants, and receipt paper. Developmental exposure to BPA has been associated with increased rates of aggression, depression, and hyperactivity. Researchers suspect that these symptoms are caused by BPA mimicking estrogen and disrupting hypothalamic neurogenesis. The hypothalamus is a brain region that regulates numerous physiological functions. This research investigated the effect of early developmental, low-dose (<1 uM) BPA exposure on zebrafish locomotor activity levels by exposing embryos to 0.01, 0.1, and 1 uM BPA for 12-72 hour windows in the first 72 hours post fertilization (hpf). A locomotor assay measured total distance and dart distance (longest distance captured between any two measurement points) zebrafish moved in response to a tactile

stimulus. In contrast to published results, my locomotor assay did not detect hyperactivity at 72 and 120 hpf. To determine the amount of BPA absorbed by zebrafish during 24 – 36 hpf I used a super sensitive BPA enzyme linked immunosorbent assay (ELISA). The results of the BPA ELISA suggested that the zebrafish did not absorb BPA. My research contributes to the growing understanding of how low-dose BPA and similar estrogen mimicking synthetics may affect neurological development.

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Influence of Antibiotic Cocktail on Gut Microbiota in Adolescent and Adult Male Rats
The gut-brain axis is a bidirectional communication system that connects the central nervous system (CNS) to the gut, including the microbial flora that flourish symbiotically in the intestinal lumen and mucosa. A healthy gut microbiome may be necessary for normal CNS function. In fact, gut dysbiosis appears to correlate with a variety of neuropsychiatric disorders, such as depression, anxiety and panic disorders, and schizophrenia. Yet the influence of gut-brain interactions on substance use disorder has just begun to be explored. Moreover, although the developmental stage of adolescence is a period of high rates of drug use among humans, the potential influence of gut-brain interactions on substance use among adolescents remains completely unexplored. Therefore, the present study tested the ability of oral antibiotic intake to reduce the bacterial species in the gut of adolescent and adult male rats, in preparation for future studies on the effects of gut dysbiosis on intravenous cocaine self-administration. Adolescent and adult male Wistar rats began antibiotic intake on post-natal days 29 and 77-81, respectively, with a low, medium or high concentration of antibiotic cocktail (neomycin, pimaricin, bacitracin) in the drinking water. Fecal samples were collected prior to and during antibiotic treatment. To test whether antibiotic administration altered cocaine metabolism or stress reactivity, rats were given a single injection of cocaine (20 mg/kg, i.p.) and blood samples were taken to measure the cocaine metabolite, benzoylecgonine, as well as the stress hormone, corticosterone, by ELISA. Preliminary data indicate that rats given the low and medium concentration antibiotics maintained consistent water consumption and bodyweight. However, the high concentration group had inadequate water intake, leading to a significant decrease in bodyweight. Using quantitative PCR on bacterial DNA from fecal samples, results suggest that the low and medium antibiotic cocktail concentrations failed to show a consistent reduction in the gut microbiota. Plasma analysis and testing of various antibiotic cocktails on gut microbiota are ongoing. Future directives include examining the effects of gut dysbiosis on cocaine self-administration.

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Slitrk1 Knockdown Reduces Rohon-Beard Neurons in the Developing Zebrafish Spinal Cord
The Slitrks are a novel gene family thought to play important roles in the central nervous system due to their structural similarities to the well known Slit and Trk proteins families. Slitrks influence with neurite outgrowth, neuronal survival, and synapse formation. Mutations in Slitrk genes have been associated with neuropsychiatric disorders, such as Tourette's syndrome and OCD spectrum disorders. Both the structure and function of Slitrk1 make this gene particularly interesting to study. Slitrk1 is the only member of the Slitrk family that lacks tyrosine phosphorylation sites in the intracellular component of its transmembrane protein. In addition, overexpression of Slitrk1 promotes neurite outgrowth, whereas overexpression of other Slitrks inhibits neurite outgrowth. Thus, Slitrk1 may have distinct roles in neuritogenesis and synaptogenesis. Slitrk1's robust expression pattern in the young zebrafish spinal cord suggests its importance in spinal cord development. Furthermore, Slitrk1 is localized to Rohon-Beard (RB) neurons at 48 and 72 hpf (hours post fertilization). RB neurons are transient mechanosensory neurons that innervate the trunk, are some of the first neurons to sense information from the environment, and mediate initial escape responses. To determine Slitrk1's involvement in RB neuron development and function, knockdown experiments were conducted by injecting antisense morpholino oligonucleotides (AMOs) to inhibit translation of Slitrk1 in early zebrafish development. After multiple variations of the fixative and immunohistochemical protocol, a procedure was developed to visualize RB neurons after incubation with mouse anti-acetylated α-tubulin antibody. Slitrk1 knockdown significantly reduced the number of RB neurons in a defined region of the anterior spinal cord at 48 hpf. This study will provide insight into potential roles of Slitrk1 in vertebrate central nervous system development.

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Cellular mechanisms associated with RAB3GAP1 dysregulation and relevance to Warburg Microsyndrome pathology

Intellectual disability affects close to 6.5 million people in the US. The study of the etiology of intellectual disability has benefited from the study of rare neurogenetic disorders. Warburg Microsyndrome is a rare neurodevelopmental disorder characterized by severe intellectual disability and postnatal microcephaly. However, the mechanisms that underlie the pathogenesis of Warburg Microsyndrome are largely unknown. Genetic findings suggest that Warburg Microsyndrome is caused by mutations in genes associated with vesicle trafficking. Proper regulation of vesicle trafficking during development is essential for the establishment of neuronal circuitry. In particular, RAB3GAP1 has been identified as the most commonly mutated gene associated with Warburg Microsyndrome. We find that RAB3GAP1 interacts with the axon elongation factor-DOCK7; and with the golgi-trafficking regulator-TMF1. Therefore, RAB3GAP1 could be contributing to the development of human neuronal circuitry through its interactions with DOCK7 and TMF1. However, how mutations in RAB3GAP1 lead to deficits in neuronal connectivity associated with intellectual disability and postnatal microcephaly is not known. Using overexpression and knockdown approaches we begin to interrogate the molecular and cellular pathways associated with dysregulation of RAB3GAP1 in human cells.

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The Role of Complement in Retinal Ganglion Cell Survival and Regeneration after Optic Nerve Crush
Currently, there is no effective neuroprotective treatment to improve the survival of central nervous system (CNS) neurons after injury or disease, creating a significant need for therapeutic intervention. Complement proteins are traditionally regarded as part of the immune response to pathogens, but recent studies have demonstrated novel roles for complement after CNS injury and disease. Complement proteins C1q, C3, and C5a are neurotoxic in ischemia and genetic glaucoma models, as complement causes apoptosis and synapse loss, but these same proteins can be neuroprotective under certain conditions. The mouse optic nerve crush model mimics both glaucoma and traumatic CNS injuries by resulting in rapid death of the retinal ganglion cell neurons (RGC) whose axons were injured. The role of complement proteins C1q and C3 in RGC survival after optic nerve crush has not yet been studied. We hypothesize that complement proteins are important for RGC survival after optic nerve crush. In this study, we used C1q, and C3 knock-out mice and wild-type control mice to test this hypothesis, and demonstrate that while C1q does not affect RGC survival, complement C3 promotes survival 14 days after optic nerve crush with pro-survival treatment.

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Ex vivo activation of cholinergic receptors inhibits GSK-3β in the telencephalon of adult zebrafish (Danio rerio).
Alzheimer's disease (AD) is characterized by progressive cognitive decline and several hallmark pathologies such as overproduction of beta-amyloid protein, intracellular aggregation of tau protein and progressive brain atrophy due to the loss of acetylcholine-producing neurons. Activating the M1 subtype of acetylcholine receptor (M1 AChR) reverses the cognitive and molecular pathologies of AD in rodent models, and M1-mediated neuroprotection is linked to the phosphorylation of glycogen synthase kinase-3 beta (GSK-3ß). Accordingly, pharmacological tools are being developed to enhance M1 receptor activity in AD patients. Our lab has developed a unique ex vivo approach to screen neuro-active drugs in adult zebrafish (Danio rerio). In the current study, it was determined that ex vivo incubation in carbachol, a non-selective cholinergic agonist, induces inhibition of GSK-3β in the telencephalon of adult zebrafish. Specifically, total GSK-3β levels decreased, and the ratio of phosphorylated (inactive) GSK-3β/total GSK-3β increased after carbachol incubation relative to vehicle-treated controls. These data indicate the coupling of cholinergic receptors to inhibition of GSK-3β is conserved between rodents and zebrafish, and they support the use of adult zebrafish brains ex-vivo to test compounds targeting the cholinergic system. In future experiments, we hope to elucidate the sub-type(s) of cholinergic receptor that couples to the inhibition of GSK-3β and test compounds enhancing this pathway.

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The effect of noxious stimulation on the nociceptive tail anf foot witdrawal response of unrestrained rats
The nociceptive withdrawal response (NWR) is a protective movement of a body part away from a noxious stimulus. Research in restrained rats, either in boxes or tubes, has shown that when the tail receives a heat stimulus, the rats move their tails away from the stimulus. However, no research has been conducted in rats to evaluate the movement of unrestrained rats when provided similar type of stimulus. The specific aim is to determine whether an unrestrained rat will move its body when provided a tail stimulus to its tail or feet. Rats were anesthetized to mark five points on the body with one at the urethra, another under the chin, a third between the shoulder blades, fourth at

the hollow opening of the rib cage, and the last one between the rib cage and the urethra. There were 12 evenly spaced points marked on the ventral side of the tail, along with five points on the lateral surface which were used as the locations for tail stimulation. Each of the individual paws were marked with three points on the hind feet and one centralized point on the front paws. The animal was placed on a 3"x3" glass table with two LED lights and a standard camera (60 fps) placed underneath. A heat stimulus was randomly delivered to either one of the five points on the lateral side of the tail for tail stimulation or one of the centralized points on the paws using an infrared laser (980nm) with a 2 to 5 min interval before the next stimulus was delivered. A total of 60 trials were conducted for each stimulation type (tail or foot) with 30 trials taking place on a single day. Movements recorded using the video camera were analyzed and tracked in software. In addition to tail or foot withdrawal, we observed that concomitant body movement always occurred. The direction of body movement consisted of both forward translation and rotation away from the stimulus. The timing of body movement during tail stimulation, based on initial foot movement, lagged tail movement by only 167 ms (median), however, the timing was not dependent on the stimulus location ($p=0.37$). The initial foot movement did depend on tail stimulus location; when the base of the tail was stimulated, the back left foot moved first, but when the tip was stimulated the front right paw moved first. These results indicate that while the tail and foot movement occurs similarly to previous studies, body movement also plays an integral part in their nociceptive withdrawal responses.

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The Effect of Combination Therapies on Task switching in Autistic Adolescents
Autism is a highly heritable and pervasive neurodevelopmental disorder, involving fixations, social interaction, and cognitive deficits. There is currently no cure for the core social and cognitive deficits associated with autism, thus, researchers are working toward preventative and therapeutic measures to decrease the prevalence of these behaviors. At this time, the main therapy used for the treatment of autism is UCLA Program for the Education and Enrichment of Relational Skills (PEERS). This therapy teaches social skills including peer interactions to acclimate individuals to different social environments. Due to the success of this therapy on social deficits, researchers are beginning to focus their attention on whether PEERS can also alleviate the cognitive deficits associated with autism. For this particular study, we opted to use a combination of PEERS and transcranial direct current stimulation as a form of treatment for the adolescents with autism. Transcranial direct current stimulation was used to allow researchers a way to target the dorsolateral prefrontal cortex, which is the area known for its executive function on our cognitive abilities. We used a task-switching paradigm to illustrate the cognitive abilities with and without combination therapy in adolescents with autism to investigate whether the combination of transcranial direct current stimulation along with PEERS caused an increase in cognitive abilities. Ten autistic adolescents received PEERS intervention for 90 minute weekly sessions for 14 weeks. During these 14 weeks, the autistic adolescents received either sham or active transcranial direct current stimulation over the dorsolateral prefrontal cortex for 30 minutes during their PEERS session. This study will illustrate that the use of combination therapy compared to PEERS alone resulted in a decrease in cognitive deficits in autistic adolescence. These findings would support the hypothesis that combination therapies can provide a significant means for autistic individuals to reduce their core cognitive and behavioral symptoms.

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The effect of chemogenetic activation of prelimbic cortical neurons projecting to the nucleus accumbens core on relapse to cocaine-seeking.
Substance use disorder (SUD) is a highly prevalent issue in modern society, leading to staggering loss of life and considerable burden on society. A major setback in treating SUD is the high vulnerability for relapse following the presentation of drug-conditioned contexts and cues despite prolonged periods of abstinence. Using rodent models of cocaine self-administration (SA), researchers have identified the prelimbic (PrL) subdivision of the medial prefrontal cortex as a major instigator of relapse via downstream dysregulated glutamate (Glu) release in the nucleus accumbens core (NAcc). Normalizing Glu receptor phosphorylation in the PrL cortex with an intra-PrL cortical infusion of brain-derived neurotrophic factor immediately after the final cocaine SA session in rats leads to a long-term normalization of Glu release in the NAcc, thus preventing relapse to cocaine associated contexts and cues. We then hypothesized that preferentially activating PrL-NAcc Glu neurons immediately after the final SA session would sufficiently suppress relapse post-abstinence (PA). To investigate this hypothesis, we used an intersectional viral chemogenetic method. Rats received an intra-NAcc CAV2-Cre or a retrograde AAV (rgAAV) microinjection, which

show preferential retrograde transport and express Cre recombinase in neurons projecting to the NAcc. Next they received an intra-PrL cortical Cre-dependent Gq-coupled Designer Receptors Exclusively Activated by Designer Drugs (DREADDs, AAV5-hSyn-DIO-hM3Dq). Controls received intra-PrL cortical Cre-dependent mCherry viral construct (AAV5-hSyn-DIO-mCherry). Immediately after the final cocaine SA sessions, all rats received an injection of Clozapine-n-Oxide (CNO, 3 mg/kg i.p.), the otherwise inert ligand for hM3Dq. Results showed non-significant trends of CNO-mediated activation of PrL-NAcc neurons on PA context-induced relapse, cue-induced reinstatement, and cocaine prime-induced reinstatement after extinction. Further analyses found time points where hM3Dq rats showed suppressed cocaine seeking in all relapse tests. We hypothesize the overall lacking effect is due to PrL neurons activating two distinct cell types in the NAcc. Specifically, recent experiments found that activating D1-expressing neurons in the NAcc accentuates, whereas activation of the D2 counterpart attenuates, relapse to cocaine-seeking. Future studies should investigate the degree PrL cortical neurons projecting to the NAcc innervate D1 vs. D2 neurons.

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"Light" Sleeping: The Effects of Chronic Sleep Deprivation on Zebrafish Brain Biochemistry
Stress is a well-known cause of cognitive dysfunction, but the cell signaling mechanisms underlying this phenomenon are unclear. Here, we explore the behavioral and biochemical effects of chronic disruption of zebrafish (Danio rerio) circadian rhythms. The zebrafish tanks are enclosed within two light-sealed boxes connected to electrical timers, which are continually altered to induce sleep deprivation and stress for four days. The control box is outfitted similarly but maintains a natural zebrafish light cycle (14 hours on and 10 hours off). Dissected brain samples are homogenized and prepared for Western Blotting in order to obtain data on targeted biochemical effects of chronic sleep deprivation. Homogenates are probed for activated Akt1, a protein known to play a role in the establishment of long-term potentiation (the neural basis for learning and memory). Samples are also probed for deactivated GSK-3β, a protein known to inhibit long-term potentiation. We hypothesize that stressed fish will show a decrease in activated Akt1, and an increase in activated GSK-3β. Thus far, preliminary data do indeed show a decrease in active Akt1 and an increase in active GSK-3β in treated fish compared to controls, especially in the telencephalon (an integral area in learning and memory processes). These data suggest that this signaling pathway, known in other models, is also present in zebrafish. Long term goals for this project include testing the effects of this stressor on learning and memory via novel object preference testing, as well as preventing a novel environment related increase in active Akt1 in pre-stressed fish.

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Male Physiological Response to Female Voices During High and Low Fertility
Previous research found an increase in male's attraction to the voices of high fertility females compared to the same females at low fertility (Shoup-Knox & Pipitone, 2015). Though humans are likely naive to changes in individual voices across the menstrual cycle, increased attraction to the voice of a fertile female would provide a reproductive benefit to males. This preference could either increase the likelihood of choosing a fertile partner over a non-fertile partner, or increase the likelihood of copulation when a long-term partner is fertile compared to other times across her menstrual cycle. The current study is an extension of Shoup-Knox and Piptone's (2015) study that determined how the voices of women recorded at different phases of their fertility cycle affect male physiology. The purpose of the current study is to examine whether the content of female speech in a dating scenario affects male physiology differently when recorded at high versus low fertility phases. Audio was recorded of females accepting a date, rejecting a date, and making a neutral statement during the follicular phase (high fertility) then again during the luteal phase (low fertility) of her cycle. Male participants listened to these recordings and rated them for both attractiveness and emotional response. Physiological responses including galvanic skin response and heart rate were also recorded. It is hypothesized that males will display a greater change in sympathetic nervous system arousal in response to the voices of females recorded at high fertility compared to low fertility. We expect these differences to be greater when a female is accepting a date versus rejecting a date, as it presents a potential mating opportunity with a fertile female.

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Establishing a Damage Paradigm to Examine Retinal Neuron Regeneration at a PUI.
Zebrafish (Danio rerio) is a powerful model system in the field of regenerative biology. In contrast to mammals, zebrafish possess the innate capacity to regenerate a multitude of tissues/organs including brain, fin, heart, kidney, retina, and spinal cord. The teleost retina is a model for studying cellular and molecular mechanisms underlying retinal regeneration because following neuronal death, MŸller glia undergo cell division to yield neuronal progenitors that continue to proliferate, migrate, and differentiate into the lost retinal cell. While avian and mammalian MŸller glia exhibit limited proliferation, they cannot regenerate significant numbers of neurons and restore vision. A multitude of damage paradigms exist to damage the zebrafish retina including: chemical lesions (NMDA excitotoxicity or ouabain injection), physical (optic nerve crush, needle ablation, or surgical removal), genetic methods of ablation (Xops:nitroreductase), and light damage. To specifically cause photoreceptor loss, there are three light damage paradigms detailed in the literature: 1.) constant intense light, primarily damaging rod photoreceptors, 2.) exposure to extremely intense ultraviolet (UV) light for a short time period, targeting both rods and cones and recently 3.) a new focused-light lesion paradigm where light is focused through a microscope onto the retina of an immobilized fish. The number of colleges and universities with undergraduate neuroscience programs have significantly risen the last five years, including programs at Primarily Undergraduate Institutions (PUIs). Thus, the goal for this project was to provide a protocol for PUI laboratories to be able to study neuronal regeneration. Due to their low cost, short reproductive cycles, and well-mapped genome zebrafish are an ideal organism for use in smaller class settings. In these projects two retinal damage paradigms were developed to allow for simple and effective study of the regeneration process: 1.) one novel project consisting of a five-day chronic nicotine-exposure and the other 2.) a modified focused-light lesion damage. After each damage paradigm was completed, the fish were sacrificed, enucleated, and tissue was fixed in 9:1 ethanolic formaldehyde before frozen cryosections were obtained. Damage was assessed through DAPI-labeling to visual cell nuclei and will continue with immunofluorescent-labeled proliferating progenitor cells (PCNA). We propose these models as an inexpensive and manageable way to study regeneration at PUIs.

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The Role of Executive Functions in Verbal Fluency: An Examination of the Controlled Oral Word Association Test
Executive functions (EF) are frontal lobe-mediated, complex constructs that allow us to effectively adapt to our environment, including working memory, inhibition, novel problem solving, planning, and verbal fluency. One way to assess verbal fluency is through the Controlled Oral Word Association Test (COWAT). The COWAT requires participants to state as many words as they can that begin with a certain letter over a 60 second time constraint. The traditional scoring procedure requires summing the total words and supplemental scores (clustering and switching) over the whole 60-second trial. It has been suggested that time-based scoring procedures may be used to assess different cognitive functions, as performance during each epoch (0-30 secs) vs. (31-60 secs) differentially reflects automatic vs. controlled processes respectively. The present study sought to address the reliability of this novel scoring procedure. A previous model of score interpretation predicts that higher correlations should result between measures of EF and COWAT scores generated from responses in the 2nd epoch relative to the 1st. Participants (70 undergraduate students) completed a test battery administered across 2 sessions, and the assessments included measures of relevant neuropsychological variables to examine for construct validity. The test-retest reliability of the COWAT for correct words, based on the traditional procedure, was r=0.82. Reliability estimates for correct words generated during separate epochs were around r=.70. In contrast, the temporal stability coefficients for supplemental scores were very poor (r=0.4-0.5) for scores traditionally derived, and lower for scores generated during separate epochs (r=0.2-0.4). Correlations between temporally based COWAT indices, select WAIS-IV subtests and measures of EF were also examined. The resulting correlations were low and mostly non-significant. In no cases were higher correlations observed between EF measures and COWAT scores from the 2nd epoch. Scores based on performance during the second epoch were hypothesized to be purer indices of EF and this was not observed. The temporally based measures suffered from low reliability, which likely attenuated validity coefficients. It remains unclear whether supplemental score indices represent a deliberate strategy of executive control, or if they are more a passive artifact of responses generated that simply reflect organization of the semantic network.

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Examining the role of Lysophosphatidic Acid as a Potential Axon Guidance Molecule in the Chicken Visual System
In order for developing axons to reach their synaptic target, they must be guided by environmental chemical cues known as axon guidance molecules. Retinal ganglion cells (RGCs) are central nervous system (CNS) neurons in the eye that project their axons to the tectum in birds. In vitro evidence has suggested lysophosphatidic acid (LPA) to be a potential repulsive axon guidance molecule. In this study, we examined the role of LPA by inhibiting its production in the developing chicken visual system. We sought to deplete the enzyme autotaxin (ATX), which converts the precursor molecule Lysophosphatidylcholine (LPC) to LPA, via ATXsiRNA retroviral injection. To examine the effects of LPA depletion in the brain, CTB labeling was used to trace RGC projections to the tectum. We first confirmed successful labeling of RGCs in the retina using confocal microcopy. We then used light-sheet microscopy to analyze the RGC axon pathway from the optic nerve through the optic chiasm and up to the tectum. Analysis of the projections from ATX siRNA treated embryos and control treated embryos showed no difference in axon trajectories, suggesting a lack of requirement of LPA. However, one concern is that the ATX siRNA is not sufficiently depleting ATX enough to inhibit LPA production. Therefore, we are uncertain whether LPA has an effect on retinal axon guidance.
Acknowledgement: This work was funded by a WFU pilot research grant and a grant from the WFU Center for Molecular Signaling

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The IMPULSE neuroscience journal: an educational tool for undergraduates of all disciplines
Since the founding of IMPULSE: The Premier Undergraduate Neuroscience Journal in 2003, undergraduate research journals have become increasingly popular and widespread. However, only a small number of journals are specifically dedicated to publishing undergraduate neuroscience research. IMPULSE allows undergraduates to become familiarized with the processes of writing and reviewing scientific papers for publication within the field of neuroscience. Unlike many undergraduate journals, IMPULSE accepts submissions from students of all disciplines, as well as from all undergraduate institutions. The important factor is that the submitted manuscript reflects research or review within neuroscience. Since 2014, IMPULSE has published a total of 17 articles from students at 13 undergraduate institutions with 11 submissions from 11 institutions currently under review. Prior to publication, all manuscripts undergo an extensive peer-review process, which is accomplished by undergraduate reviewers from multiple institutions around the world. The peer-review process is overseen by faculty at Reviewer Training Sites (RTS), where student reviewers at a particular institution collectively meet to discuss reviews. Articles in the 2016 issue were reviewed by over 100 reviewers at 16 RTSs, as well as students from schools without an established RTS; satellite reviewers represent 33 institutions in seven countries and five continents. Following the peer-review process, the Associate Editor at a RTS, an undergraduate student, compiles individual reviews and submits the RTS's review to the Executive Editor (EE) and his or her Associate EE, also undergraduates. The RTS reviews are compiled into a comprehensive summary of edits, which is sent back to the author(s) for further revision. Two other undergraduates, the Editor-in-Chief and Managing Editor, complete the editorial board, overseen by faculty. While involvement with IMPULSE provides undergraduates with uniquely valuable experience in analyzing scientific literature, the benefits of IMPULSE extend well beyond the field of neuroscience. The use of IMPULSE as an educational tool, as well as an academic journal, has been shown to provide a lasting impact on students' writing, reviewing, and leadership skills, regardless of major. IMPULSE may serve as an effective teaching tool for undergraduates of all disciplines as it provides students with opportunities to gain experience in various aspects of scientific publishing.

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Bodily Self Disturbances as a Specific Predictor for Schizophrenia
"One of the core experiences of schizophrenia is a disrupted sense of bodily self, central to conceptualizations of the disorder since Bleuler coined the term "schizophrenia" from two Greek words that translate to "splitting" of the "soul, spirit, mind". People diagnosed with schizophrenia routinely report experiencing bodily self disturbances that are present from prodromal stages, and predict outcome. However, little is known about mechanisms underlying these experiences such as interoceptive impairments. The goal of this study was to examine interoceptive function in individuals at risk for schizophrenia to test whether interoceptive difficulties are associated with prodromal signs before the onset of illness. We investigated interoception, the perception of physiological sensations in one's body, in relation to self-report measures of schizophrenia prodromal signs, schizotypal personality, and hypomania in

healthy students. Interoception was assessed with a standardized heartbeat task. Participants were instructed to sit with their palms facing up and count the number of times their heart beat during intervals of 33, 25, 41, and 17 seconds. While they counted, their actual heartrate was recorded using a Bluetooth heartrate monitor attached with a chest strap. After each trial, the participant relayed how many times their heart beat and how confident they were in their answer, on a scale from 0-10. Participants completed the Prodromal Questionnaire-Brief Version (PQ-B; Loewy & Cannon, 2010) which assesses risk for psychosis. Based on past research (Loewy et al., 2011), those with a PQ-B distress score higher than 6 were included in the "high-risk" group. Those with a PQ-B distress score less than 6 were designated "low-risk." The Hypomanic Personality Scale (Eckblad & Chapman, 1986) and the Schizotypal Personality Questionnaire-B (SPQ-B; Raine & Benishay, 1995) were also administered.
The high-risk group was less accurate than low-risk participants on the heartbeat task; however, they were not impaired on interoceptive awareness. We found no significant correlation between interoceptive accuracy and hypomania or schizotypal personality. This supports earlier research showing that self-disturbances appear before the onset of psychosis in at-risk individuals. Interoceptive accuracy was not associated with SPQ or hypomania scale scores, suggesting that in the healthy population, interoceptive accuracy is not associated with these traits. "

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Contributions of Aδ Nociceptors to the Nociceptive Withdrawal Response in Intact Unanesthetized Rats
The nociceptive withdrawal response (NWR), characterized by rapid withdrawal of stimulated body parts, can be evoked by stimulation of two classes of nociceptors: Aδ and C-fiber. Previous studies revealed conflicting results concerning the factors that determine the direction and magnitude of the NWR. Some showed that the direction of the NWR depends upon stimulus location. In contrast, studies from our laboratory showed that the direction of the NWR does not depend on stimulus location but rather depends on posture. However, it is likely that the heat stimuli delivered in our studies stimulated a mixture of C-fiber and Aδ nociceptors. The effect of C-fibers may have obscured the effect of Aδ nociceptors because their receptive field size differs. C-fibers have large receptive fields, sometimes encompassing the whole paw of the rat, while Aδ nociceptors have smaller receptive fields. Consequently, we hypothesized that stimulus location would affect the Aδ but not the C-fiber evoked NWR. Our specific aim was to determine if Aδ-evoked NWRs depend on stimulus location by preferentially stimulating Aδ nociceptors. Sprague-Dawley rats (n=14, n=414 trials) were placed on a mesh or glass surface, and the plantar aspect of the hind left foot was stimulated in five locations with brief electrical (200μs) or heat pulse (100ms) stimuli that are known to preferentially stimulate Aδ nociceptors. Upon stimulation, the rat rapidly withdrew and then replaced its paw on the surface. The initial and final positions of the foot were recorded using a camcorder placed underneath the surface. The difference between the initial and final positions represented the NWR response vector. Consistent with previous studies, for both electrical and heat stimulation, we found no dependence of stimulus location on the direction and magnitude of the NWR in rostral-caudal and lateral-medial axes ($p>0.24$, ANCOVA), even though only Aδ nociceptors were stimulated. However, the direction and magnitude could be explained in part by the initial position of foot prior to movement (average $r2=0.19$, $p<0.001$, correlation); for example, when the foot was initially rostral the movement was caudal and when initially caudal the movement was rostral, thus avoiding disruption of the rat's balance. Our results falsify the hypothesis that Aδ-evoked NWRs vary with stimulus location and suggest that over evolution the NWR has traded off optimal withdrawal movement direction for maintaining postural stability.

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What is the role of PTPRZ1 in astrocyte development?
Astrocytes are complex, non-neuronal cells in the brain and spinal cord that perform many critical functions, including promoting the growth and survival of neurons, and regulating the formation and function of synaptic connections between neurons. Many functions of astrocytes are disrupted in human brain disorders, such as autism, schizophrenia, and neurodegenerative diseases. Thus, understanding astrocyte biology is of fundamental importance for understanding both normal brain development and disease. Surprisingly, very little is known about how astrocytes develop and mature into complex cells. Recently, the Eroglu laboratory found that a cell-surface protein named Protein Tyrosine Phosphatase Receptor Type Z1 (PTPRZ1) is a critical regulator of astrocyte development, both in cultured astrocytes and in the developing mouse brain. PTPRZ1 itself is associated with a variety of neurological and psychiatric disorders including schizophrenia, glioblastoma, and multiple sclerosis, and is expressed at high levels in astrocytes, yet its function in astrocytes is largely unknown. This led us to wonder how PTPRZ1 regulates astrocyte development. To answer this question, I first performed immunohistochemistry and confocal microscopy to examine PTPRZ1 expression in the developing mouse brain and found that PTPRZ1 is

strongly expressed in astrocyte branches. To determine how loss of PTPRZ1 alters astrocyte development, we used postnatal astrocyte labeling by electroporation (PALE) to introduce PTPRZ1 shRNA and a fluorescent label (mCherryCAAX) into astrocytes in the mouse cortex. I examined the morphology of PTPRZ1 knockdown astrocytes using confocal microscopy and found that loss of PTPRZ1 substantially alters astrocyte morphology. Since astrocytes are critical regulators of neuronal synapse formation and function, I wanted to determine whether loss of PTPRZ1 alters synapse development. To do this I quantified the number of excitatory intracortical synapses in the synaptic zone of the mouse visual cortex in PTPRZ1 knockout (KO) mice. Compared to wild type littermate controls, PTPRZ1 KO mice had significantly reduced synapse numbers. Future studies will determine whether knockout of PTPRZ1 specifically in astrocytes causes a similar reduction in synapse number. Ongoing studies are focused on further characterizing the effects of PTPRZ1 deletion on astrocyte morphology and determining the impact of PTPRZ1 deletion on neuronal circuitry.

Schein H, Mans KA
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The effects of vibration stress on zebrafish brain biochemistry
The goal of this research project was to explore biochemical outcomes that occur due to stress using the model organism zebra fish (Danio rerio). Fish were subjected to vibration stress as follows. One set of subjects was exposed to a 60Hz vibration for approximately 5 minutes per day for four days, using a percussion apparatus. A second set was placed in the vibration chamber but not exposed to vibration (control), and a third set (internal control) remained in the housing unit as a home-tank control. Following the experiment, fish were anesthetized and brains were dissected out. Using Western blot analysis, we investigated the activation status of pro-survival proteins Akt and GSK-3ß. The phosphorylation of Akt (forming pAkt) is associated with long-term potentiation (LTP) while GSK-3ß produces long-term depression (LTD), both of which are processes important in memory formation. We hypothesized that both pAkt (activated) and pGSK-3ß (inactivated) expression decrease in response to stress. We blotted for tubulin, an abundant cytoskeletal protein, as a loading control. Data indicate that this signaling pathway, as shown in other vertebrates, is present in zebrafish. Furthermore, preliminary observations indicate that Akt activity is diminished and GSK-3ß activity is enhanced in fish that have been subjected to environmental stress.

Shook EN, Kuchera M
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Exploring brain-like representations in recurrent neural networks trained on a spatial navigation task.
"Machine learning and neuroscience are fundamentally intertwined. Neuroscience studies biological systems that underlie intelligent behavior, while the goal of machine learning is to design intelligent artificial systems. A long history connects the two fields -- many of the original machine learning algorithms were designed as networks of neuron-like units that mimic the brain. Recent work in the field of computational neuroscience has shown that artificial neural networks trained for real-world tasks converge to representations similar to that of the brain[1,2,3]. Specifically, it has been shown that under certain conditions artificial neural networks that incorporate time (recurrent neural networks) show place cell and grid cell like tuning[4,5]. Here we build on this line of work by investigating what conditions produce place and grid cell representations. Specifically, we simulate a rat's trajectory in a square open field and train a long short-term memory network (LSTM)[6] on noisy estimates of head direction and velocity. The task of the network is to predict the location in space of the rat at the next time step. The network is able to learn the task and we see unit selectivity in responses to location in space, specifically border cell-like tuning. In future work we further study this model and the learned representations with the goal of parameterizing the conditions under which specific tuning patterns arise.

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The Influence of Aging on Glutamatergic Concentrations in a Model of Alcohol-Induced Brain Damage
The purpose of this project was to determine the effects aging has on ethanol's effects on the glutamatergic tone within the hippocampus. Glutamate is a neurotransmitter in the brain that is associated with excitation. It has previously been shown that binge alcohol consumption decreases glutamate concentrations, and it is believed that alcohol-induced glutamate depression causes lapses in learning and memory. Aging can also depress glutamatergic tone and is often associated with memory lapses. Unfortunately, as people age they continue to binge drink. In order to study this comorbidity, aged (13 months) and adolescent (2 months) rats were subjected to a model of alcohol dependence and their brains were extracted. The hippocampus was homogenized, and glutamate concentrations were determined using a glutamate colorimetric assay. Glutamate concentrations were normalized to the total protein levels found using a BCA kit. A two-way ANOVA (age x treatment) indicated no significant interaction or main

effect on age however ethanol treatment (p= 0.01) significantly impacted the glutamatergic tone. All together these findings suggest that age had no effect on alcohol-induced glutamatergic tone in the hippocampus, but ethanol alone reduces concentrations of glutamate. The lack of influence by aging was surprising, but future studies will use older rats (18 months+) as well as determine the functional implications of changes in hippocampal glutamate levels in memory studies.

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How to Irritate an Earthworm: A molecular Investigation into the presence of TRP channels in E. hortensis
The earthworm fulfills an important ecological function in breaking down waste, providing key nutrients for plants, and promoting soil health worldwide. Despite their indispensable role in agriculture, little is known about the chemical senses of earthworms, especially how they detect noxious compounds - a sense known as chemesthesis. The goal of this investigation was to examine a specific group of cellular channels, transient receptor potential (TRP) channels, specifically TRPA1 and TRPV1, in the European nightcrawler, Eisenia hortensis. TRP channels are cation channels that play a critical role in the detection of chemical irritants as well as thermal and mechanical stimuli in most animals. The search for these TRP channels was conducted using degenerate reverse transcriptase polymerase chain reactions (RT-PCR) and RNA sequencing (RNA-Seq). RT-PCR was used to uncover fragments of TRP channel sequences in E. hortensis using genetic information from closely related organisms. If TRPA1 and TRPV1 channels are present in E. hortensis, then their DNA sequences should closely resemble known TRP channel gene sequences in other animals. Using RT-PCR, I have identified several E. Hortensis DNA fragments that are similar to the TRP channels responsible for detecting irritants in other species, including leeches, clam worms, and oysters. Additionally, using RNAseq data the presence of E. hortensis TRPA1-like channel sequences was verified. Using this data, future explorations will aim to identify and amplify this entire sequence for cloning and signaling assays. These are a few of the first steps in elucidating the chemosensing capabilities of earthworms. We also hope to determine olfactory and taste receptors that are present in earthworms. This information will help us understand how earthworms are attracted to or repelled from particular soil areas, and could allow earthworm abundance to serve as a bioindicator of soil health.

Sprouse A and Grider MH
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Relative contributions of apoptosis and necrosis in PC12 stroke model.
Stroke is the fifth leading cause of death in the United States, the most common type being ischemic, or the blockage of blood flow to the brain. There are currently no therapeutic treatments specifically aimed at promoting survival of neurons following ischemia. We selected an in-vitro model of ischemia that allowed more control of experimental conditions. We culture neuron-like PC12 cells under oxygen-glucose deprivation (OGD) conditions to model a lack of blood flow to neurons following a stroke. Additionally, in order to better elucidate the relative contribution of oxygen deprivation OR glucose deprivation to neural injury, we include injury groups deprived of only oxygen or only glucose. We seek to identify the specific pathways through which these injuries damage the cells. For example, cell death in response to glucose deprivation has traditionally been demonstrated to occur by apoptosis, programmed cell death. However, our data suggests that, compared to controls, glucose deprivation for 24 hours also significantly increases necrosis, the unregulated destruction of cell components. We use various cell survival assays such as LDH, MTT, and flow cytometry to quantify the relative participation of necrosis or apoptosis in response to each of the previously listed injuries. Through this technology, we aim to develop therapeutic treatments directed towards the specific apoptotic and necrotic pathways, as appropriate to each aspect of an ischemic stroke. Future studies will aim to continue this specific study in other neuronal and neuronal-like models and observe possible treatments for ischemic stroke, including argon gas.

Stefanowska-Cieslak M, Driscoll G, Birgbauer E
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Investigating Semaphorin 3A as a Possible Repulsive Axon Guidance Molecule in the Chick Visual System.
During the development of the visual system, retinal ganglion cells (RGCs) must project their axons to the synaptic targets in the brain. A variety of different molecules, known as axon guidance cues, play an important role in this process. At the end of each axon there is a growth cone, a finger like projection that detects these molecules in the environment and reacts to it. Some axon guidance cues are repulsive and cause a growth cone collapse and retraction. One such axon guidance molecule is semaphorin 3A (Sema3A), which has been shown to cause dorsal root ganglion cells (DRG) growth cone collapse in vitro. In the visual system, previous studies indicate that Sema3A leads to RGC growth cone collapse in mouse and Xenopus, but not in chick. We have analyzed this species

difference and, contrary to previous literature, we have found that Sema3A treatment leads to a dose-dependent growth cone collapse of retinal axons as well as DRGs in embryonic chick. Therefore, Sema3A might play an important role during the development of the visual system.

Teklezghi A, Schmidt KT, McElligott Z
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Ethanol Withdrawal Affects Activity of Noradrenergic Neurons
Alcohol use and abuse is known to interact with stress to drive drinking behavior. Clinical studies have shown that during alcohol withdrawal there are increased in norepinephrine (NE) metabolites in the CSF of the patients. NE is a neurotransmitter associated with stress responses and this increase in NE could be seen as a factor contributing to alcohol withdrawal responses. Here, we used transgenic mice expressing a green fluorescent protein reporter in the noradrenergic neurons of the locus coeruleus, ventral medulla, and nucleus of the solitary tract to identify which region(s) activate during alcohol withdrawal. These mice underwent a chronic intermittent ethanol exposure paradigm in which they were exposed to either ethanol or air vapor chambers 16 hours per day, 4 days per week, for 4 weeks. Either 8 or 24 hours following their final ethanol exposure, brains were extracted and processed using immunohistochemistry to identify activated neurons. We found at 8 hours following ethanol exposure, noradrenergic neurons in the locus coeruleus were activated, but these changes did not persist. These results highlight a potential link between noradrenergic activity and alcohol use and abuse.

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Escape Behavior of the Grammostola rosea Tarantula and Phidippus regius Jumping Spider in Response to Heat Stimuli
Insects often respond to aversive stimuli such as wind, looming, and heat by escaping in a direction opposite the stimuli. Spiders, because they have eight legs, have potentially a greater repertoire of escape responses. However, there are few published studies on the escape response of spiders, especially regarding the effects of stimulus location or direction. The specific aim of this project was to determine the relationship between the stimulus location and direction of response in Phidippus regius (Regal jumping spider) and juvenile Grammostola rosea (Chilean Rose tarantula) for heat stimuli delivered to the tarsi of the spider's eight legs. Chilean Rose tarantulas were chosen because they are docile and readily obtained while jumping spiders have complex predatory strategies. To evoke an escape response, the tarsi of each of its 8 legs was stimulated in random order at 5 minute intervals with an infrared laser (980 nm). The resulting escape response was captured with high-speed video (300 fps). Following the experiment, movement was tracked (Proanalyst), allowing quantification of the animals' location and orientation over time. Jumping spiders (n=9) and tarantulas (n=5) displayed similar responses in which they withdraw the stimulated leg and then translated their body directly away from the stimulus. In contrast to responses of crickets and cockroaches to wind and looming stimuli, the spiders only minimally turned. In addition, the translation direction depended significantly on the location of stimulated tarsi, suggesting that both the stimulus location on the body and the location of leg are used to program the escape. These results demonstrate that tarantulas and spiders, like insects, have a well-organized response to aversive stimuli.

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Investigating the Function of the Rostromedial Tegmental Nucleus (RMTg) through Optogenetic and Chemogenetic Methodology.
Learning is mediated through the dopaminergic mesolimbic pathway, which connects the ventral tegmental area (VTA) to cortical areas in the forebrain. This circuit, also known as the reward pathway, incentivizes certain behaviors and discourages others by varying dopamine levels. Recently, a novel area known as the rostromedial tegmental nucleus (RMTg) was found to send a inhibitory GABAergic projection to the reward pathway. Therefore, the RMTg has been indicated as a potential "brake" mechanism for this well-researched circuit. Past research shows that inactivation of the RMTg through Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) increases self-administration in rats in the presence of negative stimuli, indicating that the RMTg is necessary to process aversion. However, the DREADD mediating drug clozapine-n-oxide (CNO), when administered peripherally in non-DREADD animals, had a similar effect, likely due to the production of its metabolite, clozapine. Also, data from our lab indicates that Sox2 gene expression is highly concentrated within the RMTg, potentially providing a new, more specific target for our research. Therefore, we hypothesized that the deactivation of the RMTg through DREADDs and localized intracranial CNO injections will cause an increase in self-administration

without the clozapine effects. We also predicted that activating the RMTg optogenetically (using a Cre-driven Sox2 promotoer) would result in conditioned place avoidance (CPA) in a mice model. Sprague-Dawley rats were placed into a cocaine self-administration paradigm combined with a progressive shock to determine if inactivation of the RMTg caused an increase in self-administration. Male Cre-Sox2 mice were administered a channelrhodopsin viral vector into the RMTg and optical fibers were placed into the VTA. A CPA paradigm was then used to determine if RMTg activation causes avoidance of a aversive environment. Our results indicate that DREADD inactivation does cause an increase in shock tolerance in the cocaine self-administration paradigm. We also saw significant CPA in animals presumed to have correct injection sites and optical fiber placements. These results indicate that the RMTg plays a role in the inhibition of the dopaminergic circuit and could be used as a novel treatment target for human drug addicts. It also implicates Sox2 as a novel target for stimulation of the RMTg that can be used to increase specificity of future experiments.

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Factors influencing age of first sexual encounter
Early sexual activity can lead to many negative consequences such as unwanted pregnancies and STIs. Parents and educators work hard to educate young adults about the consequences of early and/or unsafe sexual activity, but is an impossible task when the factors that influence an individual's decision to engage in sexual activity are unknown. Previous studies have suggested many different factors that may influence this decision including race, family structure, parents' education, and socioeconomic status. Many studies have concluded that race is one of the main factors that influences the age of first intercourse. Studies have looked at the effects of these other variables, but only within race. These studies have concluded that these other variables do influence the age of first intercourse, but not as much as race. The purpose of this study was to look at race, family structure, parents' education level, and socioeconomic status individually and determine which one(s) have the strongest correlation with age of first intercourse. The researcher predicted that family structure, parents' education level, and socioeconomic status would have a stronger correlation with age of first heterosexual, vaginal intercourse than race. A survey was given out to Ferrum College students that asked them to identify their race, family structure, parent education, socioeconomic status, and age of first heterosexual, vaginal intercourse. The data found no significant correlation between age of first intercourse and race, family structure, or socioeconomic status; however, it did find a positive correlation between age of first intercourse and parents' education.

Wagner SW
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Developing Sensory Behavioral Assays for Zebrafish Autism Model
Individuals of all ages can suffer from a wide variety of symptoms and disabilities that could be diagnosed as Autism Spectrum Disorder (ASD). Individuals diagnosed with ASD share many similar disabilities and symptoms such as hyperactivity to social, visual, auditory stimuli and hyporesponse to olfactory stimuli. Neural circuit-based alterations are widely considered as a cause for these behavioral aberrations. We have created behavioral assays against zebrafish larvae to study hyper-response towards social or visual stimuli, hyper-response with tactile/touch-based stimuli and remarkable hypo-response towards olfactory stimuli in autistic patients. We hypothesize that zebrafish autistic models will exhibit similar phenotypes. A zebrafish larva will be placed in a six-welled plate. The well will contain an inner container that holds a visual/social, tactile, or olfactory stimulus. The visual/social stimulus will contain 3 or more zebrafish larvae in the inner container, acting as a social stimulus. The tactile stimulus will contain brine shrimp in the inner container and will diffuse through the small holes creating a chaotic stimulus, triggering their gustation and tactile sensory receptors. The olfactory stimulus will have tissue from another dead zebrafish placed in the outer container. The outer container will have larger holes poked through the plastic to allow diffusion of the dead fish odor and pheromones to the zebrafish. The larvae will be exposed to the stimulus once every day for five days to see if they learn the stimulus and if the behavior changes once learning has occurred. Using a DanioVision instrument and EthoVision software, I will record the distance moved, duration spent, and movement pattern in the zones and analyze the statistical significance of the data for control and autistic zebrafish larvae. It is expected in the control zebrafish that there will be decreased distance moved when exposed to the stimulus, but increased distance moved after the zebrafish has learned the stimulus. In autistic zebrafish it is expected that there will be increased distance moved when exposed to the stimulus; however, it is not determined if they are capable of learning after being exposed to the stimulus multiple times. Modifications that help regulate the autistic zebrafish larvae behavior and responses can potentially be translated to human autistic patients. This could lead to improved treatment and medications for individuals with ASD.

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Enduring effects of saturated fat on dopamine neurotransmission are not reversed by replacement with omega-3-rich flaxseed oil
Recent studies link saturated fat, inflammation, and insulin insensitivity with lowered dopamine levels in the nucleus accumbens, a brain nucleus that assigns food value and drives eating behaviors. Therefore, we used a saturated fat-induced mouse model of obesity to investigate whether anti-inflammatory omega-3 fatty acids (flaxseed oil (FSO)) may rescue detrimental effects of dietary saturated fat. Mice were fed diets containing low (n=6) or high (n=9, 60% fat) saturated fat (LF and HF, respectively) for six weeks. After six weeks, separate HF-fed groups were switched to a calorically matched FSO (HFxFSO, n=6) or a 1:1 combination HF:FSO (HFxHF:FSO, n=6) diet for three weeks to see if omega-3s could restore dopaminergic deficits caused by HF. We conducted intraperitoneal glucose tolerance tests to assess insulin sensitivity and ex-vivo slice voltammetry to measure dopamine release and uptake kinetics at dopamine terminals. Overall, HF elevated fasting blood glucose (184.9 mg/dL HF vs. 118.3 mg/dL control) ($p < 0.05$), reduced insulin sensitivity, and reduced dopamine uptake compared to low fat controls (1.76 ± 0.08 uM/s HF vs. 2.21 ± 0.09 uM/s LF) ($p < 0.01$). When the HF group was crossed over to a blend of HF and FSO (HFxHF:FSO) or just FSO, we observed no improvements in fasting blood glucose (200.0, and 177.3 mg/dL; HFxFSO, and HFxHF:FSO, respectively). Additionally, glucose tolerance testing revealed HF, HFxFSO, and HFxHF:FSO had slower glucose clearance compared to LF, indicating reduced insulin sensitivity. With respect to neurochemistry, no significant improvements in dopamine release or uptake were observed when the HF group was crossed over to FSO or the HF:FSO blend; however, FSO displayed the ability to partially rescue impaired dopamine uptake. Moreover, body weight was negatively correlated with Vmax only in HF animals ($r = -0.407$, $p < 0.05$), showing that heavier animals in the HF group tended to have reduced dopamine uptake. Overall, we found that switching from HF to FSO or HF:FSO diets did not rescue deficits in dopamine uptake. These data suggest that high levels of saturated fat may have enduring effects on dopamine neurotransmission and insulin sensitivity despite replacement with unsaturated fats.

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Activation of the p75 Neurotrophin Receptor in Degenerating Dopaminergic Neurons Subjected to Oxidative Stress
The p75 neurotrophin receptor is a transmembrane protein that promotes the death of neurons affected by various injuries or pathological conditions. Tissue damage can lead to upregulation of pro-neurotrophins, ligands that bind and activate p75NTR. Upon activation, p75NTR is cleaved by TNF-α Converting Enzyme (TACE) and the gamma-secretase complex, thereby releasing the intracellular domain of the receptor to activate downstream pro-apoptotic mediators. From previous analyses of sympathetic neurons, we discovered that cleavage of p75NTR is induced by oxidative stress, a cellular condition associated with numerous types of pathological conditions. Surprisingly, however, this mechanism of receptor activation did not require neurotrophins. Thus, ligand-independent activation of p75NTR in neurons subjected to oxidative stress may underlie the ability of the receptor to promote degeneration of cells affected by a wide variety of pathological conditions. Whether oxidative stress-induced p75NTR activation contributes to neurodegeneration in the central nervous system has not been explored. In the present study, we evaluate the contribution of p75NTR to oxidative stress-induced degeneration of midbrain dopaminergic neurons, a population of cells particularly susceptible to Parkinson's disease. To overcome limitations of primary cultured dopaminergic neurons for protein analysis, these investigations were conducted using LUHMES cells, a population of conditionally-immortalized human mesencephalic cells that can be differentiated to post-mitotic, electrically active cells with morphological features and protein expression profiles resembling mature dopaminergic neurons. We have discovered that these dopaminergic cells abundantly express p75NTR and are susceptible to oxidative stress-associated death induced by 6-hydroxdopamine (6-OHDA), a compound commonly used to mimic the effects of Parkinson's disease. Exposure of differentiated LUHMES cells to 6-OHDA resulted in proteolysis of p75NTR. Our preliminary analyses also indicate that oxidative stress-induced cleavage of p75NTR in dopaminergic cells does not require neurotrophins. Thus, ligand-independent stimulation of p75NTR proteolysis may contribute to oxidative stress-associated degeneration of dopaminergic neurons. Future studies will further elucidate the mechanisms through which p75NTR is activated by oxidative stress, as well as evaluate the contributions of the receptor to dopaminergic neurodegeneration.

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Neuroprotection following Injury: The role of Integrated Stress Response Inhibitor (ISRIB).
In response to trauma to the nervous system, neural cells increase reactive oxygen species (ROS) through a stress response, leading to cell death. Currently, there are no approved treatments to attenuate neuronal damage following an injury. However, recent studies indicate a drug, Integrated Stress Response Inhibitor (ISRIB), has the potential to inhibit a key molecule in the stress response pathway and therefore, attenuate neuronal cell death. Here, we test whether addition of ISRIB can promote survival of vertebrate forebrain neurons and/or neuronal PC-12 cell cultures, following an ROS injury. Preliminary results indicate that there was no significant difference in cell viability between injured vertebrate neurons and injured neurons treated with 2mM ISRIB. This is likely due to insufficient viable neurons for the treatment to target following the robust peroxide injury (900uM H2O2). I am currently investigating ISRIB treatment in less severe ROS injuries and in neuronal cell cultures.

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The effects of dopamine D3R partial agonists and antagonists on drug seeking, cognition, and analgesia in female cynomolgus monkeys self-administering oxycodone
Opioid addiction and the prevalence of substance use disorders (SUD) have become a national problem, resulting in the current overdose epidemic. Although powerful in treating pain, opioids have been shown to have high abuse liability and affect cognitive performance. In addition, sex differences have been identified in opioid pharmacology, yet few preclinical studies have studied opioid self-administration in female subjects. Most drugs of abuse alter dopamine concentrations and recent studies indicate the dopamine D3 receptor (D3R) as a potential therapeutic target for SUD. The present study examined, in female cynomolgus monkeys (N=4), oxycodone self-administration, analgesia, and cognitive disruptions, alone and in combination with two D3R-selective compounds: VK4-116, a partial agonist, and VK4-40, a receptor antagonist. Baseline oxycodone self-administration dose-response curves, as well as the effect of naltrexone, a known mu-opioid antagonist, have been completed in all subjects; analysis of the effect of D3R compounds is underway. Oxycodone self-administration was characterized as an inverted U-shaped function of dose. When tested with the oxycodone dose that maintained peak rates, naltrexone decreased responding; when tested with a dose of oxycodone on the descending limb, naltrexone increased response rates, shifting the oxycodone dose-response curve to the right. Preliminary results show modest reductions in response rates at the peak dose of oxycodone and a dose on the descending limb when tested with VK4-116. For two monkeys, VK4-40 dose-dependently decreased oxycodone self-administration at the peak oxycodone dose and when the oxycodone dose was on the descending limb. For one monkey, VK4-40 had no effect up to a dose of 10 mg/kg, while in a fourth monkey, increases in oxycodone self-administration were noted. Reasons for these individual differences are being investigated. The effect of D3R compounds on oxycodone-induced reinstatement, a model of relapse, is currently being tested. Future research aims to provide an understanding of opioid self-administration and possible strategies to maintain analgesia while blocking abuse, and ideally, opioid-induced consequences on cognitive performance. DA017763, DA06630

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Antennae pointing and the escape response in the cricket Acheta domesticus
The escape response in animals varies widely depending on the animal and main sensory organ. In the cricket, Acheta domesticus, the escape response (e.g., turning, running, jumping) is mostly directed by cercal detection of incoming wind stimuli, though previous experiments show that vision and other sensory modalities may play a role. It is known that the angle of incoming stimulus has a large effect on the angle and magnitude of the escape response. The escape response is often accompanied by the pointing of the antennae toward an incoming object. To date, little research has been done on antennae pointing and its possible relationship to escape in crickets. The goal of this research focuses on determining the primary sensory modality responsible for antennae pointing in Acheta domesticus. We hypothesize that the presence of antennae pointing in crickets will be most affected by vision rather than cercal detection.
Looming stimuli were presented by a 3" black polyurethane ball (1 m/s; 45 degrees to vertical), against a white background, toward the cricket from eight directions centered every 45° around the body. The cricket was constrained in a clear acrylic cylinder and placed into a rotatable arena made of white canvas and roof flashes. Escape responses were recorded using a high-speed camera (650 fps), three times for each of the eight directions. Video was manually tracked through motion analysis software.

Preliminary findings show stimuli presented from the posterior end of the cricket most often result in running or jumping with very little movement from the antennae. Stimuli presented from the anterior mostly resulted in a turn and run, with more frequent antennae pointing than the posterior end. We have noticed that when antennae pointing does occur there is an attempt by the cricket to maintain the pointing throughout the escape response. Further results may identify the primary sensory or motor factors responsible for antennae pointing and offer insight into the function of pointing.

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Using Dual Luminescense-Based Reporter Gene Assay: Luciferase and beta-Galactosidase to explore the functions of tau and alpha-synuclein
Neurodegenerative diseases are often characterized by aggregated proteins that build up in the cell. For example, Parkinson's Disease (PD) is diagnosed during an autopsy by noting the presence of alpha-synuclein aggregates called Lewy bodies, while Alzheimer's Disease (AD) is defined neurofibrillary tangles made up of hyper-phosphorylated tau protein. Although these proteins are synonymous with having the diseases, the cellular role of these proteins is not entirely understood. Current literature has noted that both alpha- synuclein and tau are found in the nucleus and function to bind DNA. It is hypothesized that the tau and alpha-synuclein proteins may assume the function of transcription factors. Reporter gene assays can be used to better understand the role of tau and alpha-synuclein in gene expression. These assays use synthesized promoters to determine where tau or alpha-synuclein may bind and function. These synthesized promoters direct firefly luciferase expression. In these assays a second viral promoter which directs expression of beta-galactosidase (b-gal) must be used as a normalization control. The Dual-Light Reporter Gene Assay System (Applied Biosystems) allows for the measurement of both luciferase and beta-galactosidase activities on a machine called the H1 Synergy. My research has involved setting up this assay system for our laboratory so that it can be used to further explore the function of tau and alpha-synuclein in the lab.