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Drs. Kristin Ackerman and Mike Grider

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Effect of In-Vivo Nicotine Treatment on Adult Zebrafish Brain.

Allgood CS*, McCauley MK*, Mans KA
Georgia Southern University, Armstrong Campus

The use of nicotine, a known anxiolytic with highly addictive properties, has been prevalent for decades; however, the multitude of effects it causes in the brain are not fully elucidated. Furthermore, studies investigating the effects of nicotine use on the brain have been limited to developmental models. The current study specifically investigates the effects nicotine has on protein kinase B (AKT) and glycogen synthase kinase-3β (GSK-3β) in the brain of adult zebrafish. AKT plays a role in cell growth, division, survival, and metabolism. AKT is activated when it becomes phosphorylated on Ser473 or Thr308 by insulin signaling, growth factors, and increased neuronal activity. AKT phosphorylates and inactivates GSK-3β, a constitutively active protein, on Ser9. This is important in neurophysiology, because unregulated GSK-3β is associated with cell death, dysregulated metabolic function, neuro-inflammation.
The protocol followed during this experiment includes the use of three fish: home tank (naive) control, vehicle control, and 15uM nicotine treatment. The vehicle treatment and nicotine treatment fish were treated for five minutes, immediately followed by dissection of the telencephalon and optic tectum. The brains of naive fish were harvested with no treatment. Tissues were homogenized and prepared for western blot for Akt, GSK-3β, pSer473-Akt, pSer9-GSK-3β, and tubulin (a loading control). Bands were visualized using chemiluminescence, and images were analyzed using optical density.
We hypothesized that the anxiolytic effects of nicotine via nicotinic acetylcholine receptors in the brain would result in an increase in pSer473-Akt and pSer9-GSK-3β with respect to control treatments. Preliminary results suggest that a longer treatment of nicotine may be necessary to observe the desired effect.

Volunteering, Major Depressive Disorder, and the Default Mode Network.

Anderson GE, Ballard PJ
Department of Family and Community Medicine, Wake Forest University School of Medicine

Previous studies have shown that volunteerism can be beneficial to mental health (Ballard, Hoyt, & Pachucki, 2018). While causal pathways are unknown, it is possible that volunteering can decrease depression, for example, by changing the focus of an individual from himself/herself to others. If this is true, then volunteering might affect neural functioning specifically in the Default Mode Network (DMN), which is activated during self-rumination (Borgonovi, 2008). Previous studies have shown that activity in the DMN is elevated during rest in individuals with depression than when compared with non-depressed samples (Gudayol-Ferré, et al, 2015). This study is utilizing fMRI methods specifically looking at the DMN to analyze potential changes in functional connectivity before and after volunteering in the community. Participants are adolescents (aged 14-20) from the Winston-Salem, NC community who have recently been diagnosed with mild major depression disorder (MDD) and are actively seeking treatment. Participants are volunteering at an organization of their choice for a total of 30 hours, approximately 3 hours per week over the course of 10 weeks. Participants' depressive symptoms will be measured before intervention, midway through intervention, and after intervention. fMRI data collection will be collected pre-intervention, midway through intervention, and post-intervention. In addition, data will be collected on qualities of the volunteer experience. The goals of this study are (1) to determine the feasibility of incorporating volunteering into treatment for depression and (2) to analyze

functional connectivity changes in the DMN of adolescents recently diagnosed with mild MDD while in treatment and participating volunteering.

Autonomic Nervous System Dysregulation and Vagal Activity in Postpartum Depression and Anxiety .

Ataei S, Kimmel MC, Rackers HS
Department of Psychiatry, UNC Chapel Hill

Postpartum depression (PPD), a subset of major depressive disorder (MDD), occurs in up to 10-20% of women around the world and significantly impacts maternal and fetal health. Presently, however, there are substantial gaps in the understanding of etiology, prevention, diagnosis, and treatment. Studies have demonstrated a relationship between autonomic nervous system (ANS) regulation and psychopathologies such as anxiety and panic disorders. Recent literature has also suggested that ANS dysfunction is related to depression, and that changes in hypothalamic-pituitary-adrenal (HPA) stress reactivity can serve as a marker of vagal tone via measures of heart rate variability (HRV). The current study's objective is to compare the effects of HPA axis stress reactivity in women with history of perinatal mood and anxiety disorders (PMAD) versus non-PMAD women. Two groups of pregnant women were recruited: 1) women with a history of MDD or anxiety disorders (n=60), and 2) healthy controls (n=30). The participants attended three study visits: one in the first trimester, one in the third trimester, and one at eight weeks postpartum. During the first two visits, participants completed questionnaire measures of depression and anxiety, and during the last visit they completed the Trier Social Stress Test (TSST) to test for HPA stress reactivity. Outcome measures were cortisol (CORT) and salivary amylase (sAA) response after the TSST as markers of ANS dysfunction, and blood pressure and heart rate response during the TSST. Preliminary results will compare experimental and control groups using the area under curve analyses of HRV. Such findings can be utilized to advance the understanding and treatment of PMAD worldwide, positively impacting mother and infant health and relationship.

Identifying the molecular components of cold nociception in Drophila melanogaster.

Barborek R, Combs K, Neighbors A, Ward M, Nettemeyer N, Tolvay H, and Halsell SR
Department of Biology, James Madison University

Nociception refers to an organism's perception and reaction to noxious stimuli. While nociception is a beneficial behavioral response to harmful stimuli, humans suffer from chronic pain in which pain signals abnormally persist months after any form of trauma, injury or infection. This study aims to better understand the molecular mechanisms of pain by researching the potential role of eight Drosophila Innexin gap junction proteins in cold nociception. These invertebrate proteins are evolutionarily similar to mammalian Connexins. To screen for a possible role of the Innexin proteins in cold nociception signaling, the expression level of each protein is knocked down by cell-specific expression of innexin RNAi constructs in all of the dendritic arborization sensory neurons (da neurons), and in separate trials expression is knocked down in just the class III da neurons.

Wild type third instar Drosophila larvae exhibit a characteristic "cringe" response when exposed to noxious cold. Larvae are subjected to a cold plate, and their behavior is videotaped. Subsequently, the larval images are processed using Image J software to quantify the "percent cringe" value for statistical analysis. By comparing the percent cringe of the protein-lacking, experimental larvae to 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

(positive for wild type cringe response) and larvae in which tetanus toxin (TNTE; cringe inhibition control) is expressed specifically in da neurons will be described.

To date, all of the Innexins have been knocked down in class III da neurons, some Innexins have been knocked down in all the da neurons, and the resulting larvae's response to noxious cold has been quantified. Down-regulation of several Innexins in class III and all da neurons significantly inhibited cringing (Two-Tailed Fisher Exact Test).

This work was supported by a 4-VA grant to Susan Halsell, the Jeffrey Tickle scholarship and General Biology scholarship to Rachel Barborek, and a research stipend from the JMU Biology Department to Althea Neighbors.

ISRIB as a Potential Therapeutic Drug for Neuronal Injury.

Binz, Ian and Grider MH
Department of Biology and Program in Neuroscience, High Point University

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.

Gut Microbiome Effects on Cocaine Seeking Behavior in Adolescent and Adult Male Rats.

Brock N, Anthony B, Kasiah J, Suess G.J, Williams B.F, Chassaing B, Frantz K.J.
Department of Neuroscience, Georgia State University

The gut-brain axis is a bidirectional communication system that connects the gastrointestinal flora with the central nervous system (CNS). In fact, a healthy population of microbial species in the gut may be necessary for normal CNS function and disrupted homeostasis in the gut microbiota (dysbiosis) appears to correlate with a variety of neuropsychiatric disorders, such as depression, anxiety and panic disorders, and schizophrenia. Substance use disorder is often comorbid with these psychiatric illnesses, but whether it relates to gut dysbiosis remains unknown. Additionally, the adolescent period of life has shown high rates of recreational drug use among humans, but the potential influence of gut-brain interactions on substance use in adolescents remains completely unexplored. Therefore, the present study examined whether antibiotic-induced gut dysbiosis heightens cocaine-related reward and reinforcement, using the animal model known as intravenous drug self-administration. Adolescent and adult male

rats were given a two-week, oral antibiotic cocktail treatment, fecal samples were collected, DNA was extracted, and qPCR was conducted to assess changes in the gut microbiota. Simultaneously, rats acquired lever-pressing in operant conditioning chambers using a white noise training procedure, followed by cocaine self-administration under a fixed and progressive ratio schedule of reinforcement. Following forced abstinence, animals underwent extinction and cue-induced reinstatement testing. Based on qPCR results, bacterial abundance decreased in rats that took antibiotics, regardless of age group. Gut dysbiosis did not alter cocaine intake during lever pressing acquisition, but several animals on antibiotics reached high levels of lever pressing during progressive ratio testing. Moreover, adult rats on antibiotics showed higher levels of cue-induced reinstatement of cocaine-seeking in a model of relapse, an effect not observed in adolescent-onset groups. These results suggest that gut dysbiosis could contribute to cocaine vulnerability, and that gut dysbiosis may be a target to treat those affected by substance use disorder.

Associations Between Lifetime Use of Classic Psychedelics, LSD, and Psychological Distress.

Caudill, CV, Crawford, MS, Thorne, CB, Hendricks, PS
Department of Psychology, University of Alabama in Birmingham

Lifetime classic psychedelic use is associated with a lower likelihood of experiencing serious psychological distress (SPD) in the past month (Hendricks, 2015); however, little is known about the use of individual classic psychedelics and their relationship to SPD. LSD has been shown to reduce end-of-life anxiety (Gasser et al. 2014), but the relationship between LSD and SPD remains unexplored. Combining the most recent 11 years of data (2007-2017) from the National Survey of Drug Use and Health, we created three independent groups: group one which isolated respondents who had used LSD but no other classic psychedelic, group two which combined those who had used LSD and at least one other classic psychedelic, and group three which combined those who had used at least one classic psychedelic excluding LSD; groups were created based on responses to questions about lifetime classic psychedelic use. Using a variable denoting presence or absence of SPD based upon responses to the K6 Distress Scale, we analyzed each group to determine the relationships of LSD and classic psychedelics with SPD. Based on multivariate logistic regression with survey weights, isolated use of LSD is not associated with a decreased likelihood of experiencing past month SPD (OR=1.08, 95%CI=.0.96, 1.20, $p>0.05$) while classic psychedelic use including LSD is associated with an 21% decreased likelihood of experiencing SPD in the past month (OR=0.79, 95%CI=0.71, 0.88, $p<0.001$). Classic psychedelic use not including LSD was a associated with a decreased likelihood of experiencing SPD in the past month by 18% (OR=0.82, 95%CI=0.731, 0.91, $p<0.001$). Due to the slightly different mechanisms of action, experiences, and duration with each classic psychedelic, it is important to determine the relative benefits of each specific classic psychedelic on mental health outcomes; these results suggest that while a decrease of SPD is seen with general classic psychedelic use, LSD alone is not associated with a decrease of SPD in this observational population-based study.

Adolescent nicotine exposure has long-term effects on dopamine release in the nucleus accumbens in rodents.

Christensen BA, Pitts EG, Ferris MJ
Department of Physiology & Pharmacology, Wake Forest School of Medicine

Nicotine use in adolescence increases the likelihood and severity of adult substance use disorders. Animal studies support a causal connection finding that adolescent nicotine exposure increases cocaine and ethanol consumption during adulthood. With the current precipitous rise of nicotine vaping in adolescence, it is crucial to understand the mechanisms mediating this vulnerability. The purpose of this study was to examine the long-term neurochemical effects of adolescent nicotine exposure using ex vivo fast scan cyclic voltammetry in the nucleus accumbens (NAc) core. We found that adolescent, but not adult, nicotine self-administration causes long-term decrease in NAc dopamine release and differentially impacts nicotinic acetylcholine receptor modulation of striatal dopamine. Our study shows that nicotine exposure during adolescence results in long-term neurochemical consequences.

The Effect of Semaphorin 3A on Chick Embryo Retinal Growth Cones.

Cisse FN, Reed A, Birgbauer E
Department of Biology, Winthrop University

Semaphorin 3A (Sema3A) is a crucial axon guidance cue in the nervous system during embryonic development. Axon guidance molecules interact with growth cones, extensions of growing or regenerating axons supported by microfilaments looking for their synaptic target. Inhibitory axon guidance molecules are known to cause growth cone collapse in vitro, and Sema3A is one of them. When growth cones collapse, they cease to move and then retract, often turning away from the inhibitory molecule. Sema3A has been shown to be important for axon guidance in the spinal cord. Luo et al. (1993) demonstrated that Sema3A causes growth cone collapse of chick embryo dorsal root ganglion cells (DRGs), but they stated it did not cause growth cone collapse of chick retinal ganglion cells (RGCs). We have found preliminary evidence that Sema3A does cause growth cone collapse of embryonic chick retinal ganglion cells, which is inconsistent with the Luo et al. (1993) finding. We are investigating this inconsistency; one hypothesis is that the treatment times in the previous study were not optimal. We are further testing the effect of Sema3A on RGCs using time-lapse microscopy to examine growth cone responses to Sema3A. Furthermore, we are employing a quantitative growth cone collapse assay to investigate the concentration dependence as well as treatment times to verify and extend our preliminary data.

The Effect of a Stand-Alone Mindfulness Exercise on Anxiety.

Clark AM, Gill V, Sivakumar A, Blumenthal, TD
Department of Psychology, Wake Forest University

Engaging in mindfulness with a trained instructor can decrease symptoms of anxiety and depression. However, more individuals are now employing online sources to treat these same symptoms. As the population increasingly turns to internet-based relaxation exercises, psychologists have become interested in the effectiveness of this approach. Can a quick mindfulness exercise, done without an instructor, decrease anxiety? Our study sought to answer this question by examining the effect of a stand-alone mindfulness exercise on participants' state and physiological anxiety. We employed the eye-blink startle response as a physiological measure of anxiety, as previous research has suggested. We also used questionnaires to measure self-reported levels of mindfulness and anxiety. We were particularly interested in the role of trait anxiety and hypothesized that high levels would inhibit the ability to practice mindfulness. Data are being scored now and results of this study will be available by April 13th. When a treatment group undergoes a mindfulness exercise, we

expect self-reported state anxiety and startle reactivity to significantly decrease compared to that of a control group. Mindfulness treatment participants should also demonstrate significantly increased state mindfulness compared to controls. We further predict that this effect will be manifested physiologically in the startle response. Startle response magnitude should decrease across time for all participants, due to habituation, but the mindfulness treatment group should demonstrate more reduced startle responses than the control group. Overall, trait anxiety will mediate these interactions. This study's results will help inform internet consumers about the efficacy of their chosen mindfulness techniques.

Studying pain neural circuits with viral vector rAAV2-retro in rat brains.

Cunnane KA, Alvarado-Vazquez PA, Gwak Y, Peters C, Romero-Sandoval EA, Martin J, Eisenach J
Wake Forest School of Medicine Department of Anesthesiology

rAAV2-Retro has been shown to selectively transfect projection neurons in a retrograde fashion, however this has not been documented for many circuits in rat brain. This rAAV variant could be used to restore maladaptive neuroplastic changes associated with pain. For example, prefrontal cortex (PFC) activity could modulate amygdala function and alter cognitive performance or the emotional-affective dimension of pain. Thus, we hypothesize that rAAV2-Retro could be used to target projections between these regions using Cre and Flex technology in rat brains. Four weeks after rAAV2-Retro-CAG-GFP (1.8 x1012 viral particles) injection in the PFC we observed that 25% (0.25 ± 0.13 – 0.29, median ± 25-75% CI respectively) neurons expressed GFP in the PFC, 17% (0.17 ± 0.01 – 0.26) in the medial agranular cortex, and 65% (0.65 ± 0.32 – 1.3) in the BLA. Then, we injected rAAV2-retro-CAG-Cre in PFC and rAAV8-FLEX-CAG-tdTomato in the BLA. We confirmed Cre expression in BLA 4 weeks after viral injection. Furthermore, tdTomato was robustly expressed in BLA (1.5e4 ± 9.8e3 – 4.3e4 pixels of positive) when compared to its negative control (389 ± 171 – 1.1e3; $P<0.05$ by Mann-Whitney). We conclude that rAAV2-Retro could be efficiently used to target brain projections to the prefrontal cortex using Cre-Flex approaches for circuit-specific modulation.

Synaptic Plasticity in the crossed temporodentate pathway in female rats.

Doyle, H, Ghosh, A, Soden, G, Barlis, K, Ramirez, JJ
Department of Psychology, Neuroscience Program, Davidson College

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder characterized by memory loss. One of the principal early targets of AD is the entorhinal cortex (EC), a primary cortical input to the hippocampal formation. When the hippocampus is deafferented because of EC degeneration resulting from AD, several remaining afferents to the hippocampus undergo axonal sprouting. An established model to explore this concept in rats involves making a unilateral lesion of the EC, which elicits a sprouting response in fibers from the intact EC to the denervated contralateral dentate gyrus (DG) of the hippocampus, the so-called crossed temporodentate (CTD) pathway. Greater synaptic efficacy of lesion-induced, CTD sprouting has been found to occur as early as 6 days postlesion. To date, this model has been used almost exclusively in male rats, so it remains unclear whether the female brain evidences a similar kind of plasticity. The present study explored the nature of synaptic plasticity in the CTD in female rats 12 days postlesion. Male and female Sprague-Dawley rats received either unilateral entorhinal cortex lesions or sham operations, a craniotomy over the EC. A stimulating electrode was placed in the contralateral intact EC 12 days after a lesion or sham

operation, and evoked field excitatory postsynaptic potentials (fEPSPs) were recorded in the DG ipsilateral to the lesioned EC. The paired pulse paradigm involved one pulse to the EC, known as the "conditioning pulse," followed by a second "test" pulse at a range of interpulse intervals (IPIs; 10 to 500 ms). Additionally, estrus cycles of female rats were recorded by lavages on both operation days. Acteylcholinesterase Naik stain helped determine extent of sprouting in the septodentate pathway (SD), a hippocampal afferent that also projects to the denervated DG. Electrophysiological data show a lesion effect in the female CTD ($p = 0.001$) and in the males ($p = 0.009$). Interestingly, histological data show a significant synaptic response at ventral ($p = 0.007$) but not dorsal levels ($p = 0.519$), for both sexes, and females demonstrated higher levels of plasticity than males at dorsal level ($p = 0.012$). The electrophysiological findings of a lesion effect on synaptic efficacy in both sexes and the histological results that suggest females exhibit a more robust sprouting response dorsally at 12 days postlesion than males do prompt further investigation into the nature of synaptic plasticity in female rats.

In an in-vitro model of hypoxia, Cannabidiol (CBD) has not demonstrated neuroprotective effects in neuronal cells.

Dunn JM and Grider MH
Department of Biology and Program in Neuroscience, High Point University

Ischemic strokes are the third leading cause of death in the United States. However, there is currently no FDA-approved drug to promote neuroprotection following injury. Recent studies have identified potential neuroprotective effects of cannabidiol (CBD), a non-psychoactive cannabinoid from the cannabis sativa plant, in neonatal pig models of hypoxia. However, CBD-mediated neuroprotection in animal models may be mediated by CBD metabolites or by interactions of CBD with the vasculature, immune system, or glial cells. In the current experiments, we investigate the ability of CBD to protect against a hypoxic insult in a pure neuronal population utilizing the neuronal cell lines RN33B, PC12, as well as chick forebrain neurons. Concurrent with a 24-hour injury, cells are exposed to varying concentrations of CBD or a vehicle control. Neuronal viability is measured using an MTT assay or LDH assay. We found that CBD did not protect against the injury and, at slightly higher concentrations, may be neurotoxic itself. Further studies are required to elucidate the mechanisms of CBD-mediated interaction.

Effects of C-terminal Mutations in Fused in Sarcoma on the Structure, Function, and Viability of Motor Neurons.

Garris RL, Henken HJ, Kaur A
Department of Neuroscience, University of North Carolina at Asheville

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease primarily affecting motor neurons. It is characterized by insidious onset, death within six years of symptom onset, and the aggregation of misfolded proteins. Dozens of mutations in key proteins like Superoxide Dismutase 1 (SOD1), TAR DNA-binding protein 43 (TDP-43), and Fused in Sarcoma (FUS) have been implicated in promoting neuron death in ALS. However, the mechanisms behind the cell death associated with TDP-43 and FUS remain largely unknown. FUS a nuclear RNA/DNA binding protein, has been associated with ALS, specifically familial ALS. Mutations in the C-terminus, which contains a non-classical nuclear localization signal, are associated with aggressive, juvenile-onset variants of the disease. Two mutations were generated (P525L and 1557_1557deletion) by site directed mutagenesis and mutation

was confirmed by Sanger Sequencing. Confirmed mutants were subcloned into mammalian vectors and transfected into motor neurons differentiated from cell line NSC-34. These cells were then analyzed for how the mutations affected structure, function, and viability. These findings allow for greater understanding of the molecular mechanisms behind FUS mutation driven neurodegeneration in ALS.

The Effects of Cholinergic Degeneration in the Septodentate Pathway on LTP Induction in the Perforant Path of Rats.

Ghosh A, Doyle H, Soden G, Barhorst K, Ramirez JJ
Davidson College

Alzheimer's disease (AD) is a form of dementia characterized by devastating loss of memory. The cholinergic hypothesis indicates that this memory loss is a result of mass cholinergic degeneration. The implicated hippocampal circuit includes the septum, the entorhinal cortex (EC), and the dentate gyrus (DG) of the hippocampus. The fiber projection from the septum to the DG, called the septodentate (SD) pathway, is composed of at least three neurotransmitter systems: cholinergic, GABAergic, and glutamatergic fibers. Prior studies have indicated that a lesioned SD may have effects that are as detrimental to memory as a directly lesioned hippocampus. The EC projects to the DG in a unilateral input called the perforant path (PP). This study used a heterosynaptic model of AD by examining the effect of SD cholinergic neuron loss on the induction of long-term potentiation (LTP; a possible neural substrate of learning and memory) in the PP. Male Sprague-Dawley rats were given an intraseptal injection of either 192 IgG-saporin, a cholinergic neurotoxin, or a saline vehicle. After a 21-day wait period, stimulating electrodes were placed in the medial septum and the EC. Evoked, field excitatory post-synaptic potentials (fEPSPs) were recorded in the DG following stimulation of the EC, both unpaired and paired with stimulation of the septum at a range of inter-pulse intervals (IPIs; 30, 60, 100, and 500 ms). Recordings were collected before and after the induction of LTP through a high-frequency tetany protocol. Shortly after, an intrahippocampal injection of the muscarinic antagonist scopolamine was made to render any remaining cholinergic inputs inert. The cholinergic neuronal markers choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) were labeled in the medial septum and DG, respectively, to determine the extent of deafferentation caused by IgG-saporin. The degree of AChE labeling in the DG indicated there was one successful case, 1925, of cholinergic SD neuronal deafferentation. Electrophysiological analysis of this case indicated that there was greater depression of the PP than the unsuccessful sham control after a tetanization protocol. The electrophysiology of 1925 is comparable to another deafferented case from a prior study, 1718, where there is depression of the PP after SD cholinergic loss. These results may indicate that SD cholinergic cells are critical in LTP induction ability; however, more data is required to confirm these patterns.

Adolescent Δ9-tetrahydrocannabinol (THC) and stress exposure predicts alcohol dependence in adulthood.

Gilman HK, Smiley, L, Gass JT
Department of Biology and Program in Neuroscience, College of Charleston; Department of Neurosciences, Medical University of South Carolina

Chronic Δ9-tetrahydrocannabinol (THC) exposure in adolescents is detrimental to neurodevelopment and brain maturation, and can lead to cognitive and behavioral impairments in adulthood. Adolescent THC use is linked with increased vulnerability to

comorbid drug use in adulthood and known to exacerbate the development of anxiety-related disorders. Previous studies have shown that stress or a traumatic event often elicits drug-seeking behaviors. The comorbidity of THC, alcohol and stress in adolescents may further exacerbate drug-seeking behavior in adulthood. This study will first determine how exposure to THC and alcohol in adolescent Wistar rats affects fear-conditioning and fear-memory extinction. Further, we are currently examining how comorbid THC, alcohol and stress exposure in adolescent rats alters the alcohol consumption, extinction of alcohol-seeking behavior, and reinstatement of alcohol seeking when tested in adulthood. We hypothesized that adolescent animals exposed to THC and alcohol would react to a fear stimulus faster, and extinguish fear-memories slower, than the control rats. Additionally, it was expected that exposure to THC and alcohol in adolescence would affect alcohol related behaviors in adults by increasing alcohol seeking and reinstatement, and decreasing behavioral extinction. To test this, adolescent animals were exposed to THC vapor followed by a two-bottle choice paradigm to assess their motivation to drink ethanol. The THC exposed animals drank significantly more alcohol than the non-THC controls. Animals were then placed in a fear-conditioning paradigm (foot shock) to mimic the effects of a stressful event inducing a fear memory. Data indicate that adolescent animals exposed to THC and alcohol are more sensitive to the fear stimulus and significantly more resistant to fear-memory extinction than animals only exposed to THC or no THC. Once animals reached adulthood, they underwent a standard self-administration protocol followed by extinction. Data from these animals are in the process of being analyzed. Our current findings suggest that the combination of adolescent THC and alcohol increase stress responsiveness. Preliminary data indicate these animals also have an increase in drug-seeking behavior as adults. These findings serve to increase our understanding of addiction and may provide insight into mechanisms of relapse.

The effects of ethanol on Periplaneta americana (American cockroach) general locomotor behavior.

Gurba JD, Triblehorn JD
Departments of Psychology and Biology and Program in Neuroscience, College of Charleston

Ethanol is a common drug of abuse that affects the nervous system, causing cognitive, motor, and behavioral impairments. Although some mechanisms by which ethanol exerts these effects are known, these effects are complicated and not well-understood. One paradigm for studying the effects of ethanol on motor behavior is by examining dose-dependent effects on general locomotor behavior. In vertebrate studies, ethanol exhibits a biphasic effect, with increased locomotion at lower doses and decreased locomotion at higher doses. Both effects occur through a complicated interaction between excitatory (i.e. glutamate and dopamine) and inhibitory (i.e. GABA and glycine) neurotransmitter systems. Invertebrate models have been useful in understanding complex mechanisms in vertebrate systems due to the tractability of invertebrate nervous systems. In this study, we begin investigating whether Periplaneta americana (American cockroach) can serve as an invertebrate model for investigating the effects of ethanol on the nervous system. As the first step to establish P. americana as such a model, we investigated dose-dependent effects of ethanol on P. americana general locomotor behavior. Male P. americana were injected with 0.1 mL 1%, 3%, 5%, 7%, 10% ethanol or saline control (0% ethanol) and placed under a container inside a 24.5 cm circular arena for five minutes to allow the ethanol to take effect. After removing the container, locomotor activity was recorded for five minutes. Video analysis software

(Tracker) calculated total distance travelled, average velocity, and average acceleration. We also recorded other behaviors such as walking and stationary bouts, climbing attempts, and grooming occurrences. If ethanol affects the general locomotor behavior of P. americana similar to vertebrates, this is the first step in establishing P. americana as a new model system for studying the effects of ethanol on the nervous system. Future experiments would investigate the underlying mechanisms and neurotransmitter systems involved (and compare them to those in vertebrates) as well as effects on sensory processing. If the effects are different, then we can investigate why ethanol does not have similar effects on P. americana as in vertebrates.

Characterizing the Interaction between Annexin Family Members and α-Synuclein, a Parkinson's Disease Associated Protein.

Hendrick SC*, Glidden EA*, Cantor AG*, Banks SML
Florida Southern College

Approximately 10 million people worldwide have been diagnosed with Parkinson's Disease (PD), a progressive neurodegenerative disorder. PD symptoms, such as tremor, bradykinesia, stiffness of gait, and postural instability, appear only after extensive neuronal loss in the substantia nigra region of the brain. One of the hallmarks of PD is the aggregation of mutated or overexpressed α-synuclein. α-Synuclein is known to interact with highly curved membranes and is normally localized to synapses. There is some evidence to suggest α-synuclein functions normally during synaptic vesicle trafficking, but the exact mechanism is unclear. Several recent studies suggest that the presence of excess α-synuclein disrupts synaptic vesicle trafficking. More specifically, it inhibits clathrin mediated endocytosis of the plasma membrane following stimulation and exocytosis of neurotransmitter at the active zone. Mutations in proteins involved in clathrin mediated endocytosis such as synaptojanin and Auxilin, have been linked to PD, suggesting studying the effects of excess α-synuclein on this cellular process may be important for understanding the molecular biology that underlies the disorder. However, the effects of excess α-synuclein on the function of these known proteins is not sufficient to explain all of the synaptic defects observed under conditions that mimic PD. Therefore, it is possible that excess α-synuclein may be interacting with and affecting the function of another synaptic vesicle trafficking protein. Preliminary data suggests α-synuclein may interact with members of the Annexin protein family, which are calcium-dependent membrane binding proteins that regulate the endocytic and exocytic processes of synaptic vesicle trafficking. Experiments were performed to characterize the interaction between α-synuclein and Annexin family members under conditions that mimic PD. As both α-synuclein and Annexins are known to interact with membranes, interaction experiments were also performed in the presence of synaptic vesicle membranes. Characterizing a possible interaction between α-synuclein and Annexin-A2 may provide more insight regarding how synaptic vesicle trafficking is disrupted under PD conditions.

Resting-state functional connectivity abnormalities in patients with amnestic mild cognitive impairment and the role of the posterior cingulate cortex.

Hohmeister MR, Benitez AM, Joseph JE
Department of Biology and Program in Neuroscience; Departments of Neuroscience and Neurology, Medical University of South Carolina

The intrinsic functional connectivity exhibited among regions of the brain has provided interesting insights into neurodegenerative diseases like Alzheimer's Disease (AD). Using

resting-state fMRI, studies have shown that functional connectivity between the posterior cingulate cortex (PCC) and other networks is impaired in patients with amnestic mild cognitive impairment (aMCI); a condition considered to be a preclinical stage of AD. Alterations to neural connectivity at such an early stage in the disease suggests that the pathology of AD develops well before any cognitive manifestations begin to occur. Thus, understanding how this pathology progresses and causes impairments to functional connectivity can help us to establish early diagnosis and proper tracking of AD. Evidence has shown that functional connectivity varies between subregions of the PCC, suggesting that this key node contains an ability to form a wide range of neural connections and perform a variety of functions. In particular, the PCC sees a dorsal to ventral shift in functional connectivity with the dorsal PCC participating in networks associated with spatial processing and the ventral PCC with networks associated with memory retrieval. This understanding of the PCC suggests that one area of the PCC may be more prone to the development and subsequent transmission of AD pathology. The present study utilized resting-state fMRI to observe how early stages of AD pathology seen in aMCI patients affects functional connectivity in the dorsal vs. ventral subregions of the PCC, in hopes of identifying subregions that are significantly more impaired than others. A time series obtained from a resting-state fMRI scan was collected from patients diagnosed with aMCI. This data were then subjected to model dependent seed based analysis to create a connectivity map that would look for correlations in functional connectivity between the dorsal or ventral subregions of the PCC and the rest of the brain. While analysis of the data is ongoing, the ventral PCC is predicted to display significantly more impairment to functional connectivity based on its role in in memory retrieval. Regions anticipated to show particularly impaired connectivity with the ventral PCC are the angular gyrus, medial temporal lobes, and middle temporal lobe due to the unique connectivity these regions display with the ventral PCC and default mode network (a system affected in the early stages of AD).

Forced Abstinence from Cocaine Self-Administration in Rats Alters Prelimbic Cortical Astrocyte-Neuron Interactions.

Hooker KN, Siemsen BM, Scofield MD
Department of Biology and Program in Neuroscience, College of Charleston; Departments of Neuroscience and Anesthesiology and Perioperative Medicine, Medical University of South Carolina

Chronic cocaine users represent a significant portion of the population diagnosed with substance abuse disorder, characterized by high relapse rates even after prolonged abstinence. Preclinical models of self-administration (SA) have provided a method for studying the neuroanatomy implicated in addiction. Specifically, rodent models of cocaine SA have established a role for the prelimbic cortex (PLC) projection to the nucleus accumbens core (NAcore) in that dysregulated cortical glutamatergic transmission facilitates cue-induced craving, promoting relapse after abstinence. Excitatory synapses are found in subcellular structures referred to as dendritic spines, and synaptic plasticity is shaped by the interaction of the nonneuronal cells, astrocytes, with pre and post-synaptic compartments. Recent studies have shown that cocaine SA and abstinence increases dendritic spine diameter in PLC-NAcore neurons, while a period of extinction decreases astrocyte morphological features and association with synaptic markers in the NAcore, but not the PLC. In this study, we examined the effects of cocaine SA and abstinence on spine morphology and the extent to which the spines in the PLC-NAcore colocalize with synapses and astrocytes. In male rats, viral

techniques were used to selectively label PLC-NAcore neurons and astrocytes prior to undergoing cocaine SA or receiving yoked-saline infusion followed by seven days of forced home cage abstinence. Animals were then transcardially perfused and immunohistochemistry performed on coronal sections of PLC. Confocal microscopy and 3D reconstruction were used to visualize morphology and colocalization with Synapsin I (non-specific synapse marker) and PLC-NAcore dendrites and spines. We observed that while cocaine SA followed by one week of abstinence decreased the astrocyte association with Synapsin I, the same conditions increased PLC-NAcore dendritic spine head volume as well as the degree to which astrocytes associate with these spines. Thus, astrocyte association with PLC-NAcore neurons is enhanced after abstinence from cocaine SA, whereas the association with non-specific synapses is decreased. While an improved understanding of the modifications occurring after drug administration and abstinence may provide a more focused target for reducing the likelihood of drug-seeking behaviors and relapse, future studies will examine in more detail potential modifications in related pathways. NIH R00DA040004,T32DA007288,F31DA041021

Activation of norepinephrine neurons in nucleus tractus solitarius in response to acute morphine exposure.

I.M. Bravo, K.T. Schmidt, E.S. Cogen, Z.A. McElligott
Department of Psychiatry - Bowles Center for Alcohol Studies, UNC Chapel Hill

Although dopamine is commonly believed to be the main neurotransmitter involved in drug use and addiction, it has been shown that norepinephrine plays a key role in the rewarding effects of morphine. Most forebrain noradrenergic innervation arises from the locus coeruleus (LC), however there is also a small population of noradrenergic neurons that originate in the nucleus tractus solitarius (NTS) that are critical for morphine reward-learning. This study was conducted to determine if these noradrenergic neurons originating in the NTS are activated by morphine exposure. To do so, mice were given a single dose of morphine (10 mg/kg) and sacrificed 90 minutes later. Their brains were collected and imaged to look for colocalization of fluorescently tagged norepinephrine neurons with cFos activity, a protein expressed by activation of neurons. We have quantified the activation of norepinephrine neurons in the NTS and LC in response to acute morphine exposure.

Analysis of CLARITY and Tert-Butanol Optical Clearing Methods on Fluorescence Preservation in the Chicken Visual System.

Jeffrey Parham, Dr. Birgbuer
Winthrop University

Investigation into the anatomy of the human body is one of the oldest ways to diagnose diseases and observe how they are affected by therapy. Similarly, microscopic exploration of tissue and its pathological changes have proven equally useful in our understanding of anatomy at the cellular level. For example, the nervous system is composed of a tapestry of thin fibers called axons. These axons carry electrical information from individual nerve cells to other neurons that receive the signals. To understand the inner workings of the nervous system, neurobiologists need ways of deciphering how this neural tapestry is organized. However, standard 2D imaging of tissue lacks information on 3D structure, and 3D images are often limited by penetration of light into the tissue. The advent of optical tissue clearing methods (OTC) along with improvements in 3D imaging addressed both these issues. OTC is comprised of three steps, each differing based on the specific characteristics of the tissue. First, clearing of the opaque tissue to produce transparent specimens. Second, Refractive

index matching to reduce light scattering. Finally, imaging using multiple light microscopy modalities such as advanced confocal or light sheet microscopes. The aims of this study are to assess the level at which passive and active CLARITY along with tert-butanol BABB variant methods preserve indigenous fluorescence in the chicken visual system. We labeled retinal ganglion cells (RGCs), whose axons form the optic nerve and tract, with Green florescent protein or TD tomato and then optically cleared the brains using each respective method and compared them.

Effects of Sublethal Imidacloprid on House Cricket (Acheta domesticus) Neuron Firing Activity and Chirping Behavior.

Jenna Joyner, Jennifer Mozolic, Langdon Martin, David Coffey
Warren Wilson College

The use of pesticides to enhance crop yield is becoming increasingly prevalent as the human population continues to rise. Imidacloprid, a pesticide within a class of insect neurotoxins known as neonicotinoids, is the prevailing pesticide for treating common crop pests such as aphids and whiteflies. Neonicotinoids are designed to chemically mimic nicotine and bind to the same nicotinic acetylcholine receptors (nAChRs) within an insect's nervous system that nicotine would bind to. Lethal doses of the toxin can result in apparent intoxication, tremors, paralysis, and death of an insect, but sublethal doses have been shown to have unusual and unintended effects on non-target organisms. Honey bees, for example, have been shown to have a reduced ability to navigate to their hives after being exposed to sublethal amounts of the pesticide. Crickets, another example of a potential non-target organism, may be susceptible to undesirable effects as well. Due to the observed partial paralysis causing effects of the neonicotinoid, it was theorized that sublethal doses of imidacloprid could affect important aspects of a male cricket's chirping behavior, a characteristic vital for the reproductive success of the species. In this study, sublethal doses of imidacloprid were administered to crickets to investigate potential effects on chirp quality, for example, changes in the intra-chirp intervals which have been shown in previous studies to affect a female's recognition of the mating call. Changes in neuron firing activity were also directly observed to assess the extent to which neuronal activity was being impaired by the neurotoxin. Preliminary findings from this study suggest that imidacloprid both affects neuronal response to a stimulus and duration of chirping.

Interleukin-1 receptor type 1 exercises a critical role in neurodegeneration following magnetic nanoparticle-induced focal cerebral ischemia.

Katta A
Neuroscience Undergraduate Program, The Ohio State University

Cerebral ischemia, more commonly known as stroke, afflicts approximately 15 million people worldwide and is the leading cause of long-term disability in the United States. Cerebral ischemia is characterized by a local buildup of plaque in the brain's circulatory system leading to ischemia–a decrease in hemoglobin circulation. Consequently, the lack of oxygen circulation to cerebral regions causes cerebral infarctions– severely damaging components of strokes– which activate the neuroimmune system and induce the release of pro-inflammatory cytokines, such as Interleukin-1 beta (IL-1b). Previously established, cerebral ischemia-related damage significantly decreases with IL-1b deficiency in mice; however, the role of its cognate receptor, Interleukin-1 receptor type 1 (IL-1R1), in cerebral ischemia was not investigated. Our lab has shown IL-1R1 is expressed in many cell types throughout the CNS;

however, each IL-1R1-expressing cell type responds to IL-1b in a unique manner and their respective roles in the context of ischemia are unknown. Using a genetic mouse model, our lab can restrictively express or selectively delete IL-1R1 in a specific cell type utilizing the Cre-Lox system. Additionally, a novel method of focal ischemia-reperfusion utilizing cranial magnet implantation and intravenous magnetic nanoparticles was used to emulate cerebral ischemia and subsequent reperfusion. We found induction of ischemia in wild type (WT) animals reduced NeuN+ cell bodies and activated glia in the ipsilateral cortex, thus verifying that the model accurately mimics focal ischemia. Mice lacking IL-1R1 (IL-1R1r/r) were found to have smaller infarct sizes, suggesting Il-1R1 plays a critical role in neurodegeneration following ischemia. Further studies are required to detail the roles of cell-type specific IL-1R1. Since endothelial cells are known to express the highest levels of IL-1R1, along with mediate release of neurotoxic reactive oxygen species (ROS), we hypothesize endothelial IL1-R1 may contribute to the neuronal damage and infarct volume.

Structural and Biophysical Characterization of the Human Neuropeptide Galanin.

Kraichely KN, Hendy CM, Clinkscales S, Giuliano MW
Department of Chemistry and Biochemistry and Program in Neuroscience, College of Charleston

Neuropeptides are important regulators of nervous system activity, often being co-localized with classical neurotransmitters and modulating a variety of physiological functions. Human galanin, hGal(1-30), is a 30 amino acid neuropeptide implicated in normal and pathological processes such as epilepsy, pain perception, learning, the progression of Alzheimer's disease, and neurite growth. Galanin exerts these effects through inhibitory action in the spinal cord, hippocampus, basal ganglia, and hypothalamus. Its receptor distribution, post-synaptic hyperpolarizing effects, and galanin's role as a trophic factor make galanin receptors attractive targets for the development of anticonvulsant and neuroprotective drugs. However, a high-resolution 3D structure of hGal(1-30) or of its pharmacologically active N-terminus has yet to be determined, though it could provide useful data for eventual drug design. In this work, we used 2D 1H-1H Nuclear Magnetic Resonance Spectroscopy to observe and quantify the spatial interactions of hydrogen atoms in hGal fragments. Using these interactions as distances in simulated peptide folding calculations, we calculated and compared the structures of N-terminal galanin fragments hGal(1-12)KK, hGal(2-12)KK, and hGal(1-17)K. We synthesized these fragments with lysine (K) amino acid residues to increase their solubility in solution. We discovered the structure of the N-terminal fragments in aqueous solution is dictated by the clustering of hydrophobic side chains in residues 2-9. Additionally, we identified a weak but significant alpha helix in fragments containing the glycine(1) residue. Most interestingly, the positions of residues shown to be important for receptor binding are conserved between fragments, a finding that could represent a 3D motif necessary for receptor binding. Structural study of a C-terminal fragment, hGal(17-30), showed fewer through-space correlations, indicating that the structure is far less ordered than in the N-terminus. Based on the evolutionarily conserved hydrophilic character in the C-terminal galanin sequence, we propose that the C-terminus does not play a structural role, but instead allows the neuropeptide to remain in aqueous environments in which the N-terminal hydrophobic cluster can form. In the process of drug design targeting galanin receptors, mimicking the binding motif and biophysical properties of galanin observed in this study could increase the affinity and selectivity of the drugs.

Heart Activity and Anti-Saccade Performance in Women with the Broad Autism Phenotype.

Lateef AU
Department of Communication Science and Disorders

Executive functioning skills are comprised of working memory, planning, flexibility, and inhibition. In this study, we zeroed in on inhibition control as a way to study executive function in mothers of children with autism, who may show features of the broad autism phenotype. To measure inhibition control, this study used the antisaccade task. Some prior studies suggest that poor inhibition control is linked to suboptimal physiological regulation. Research Question: (1) Do mothers with children who have autism, when compared to control women, show impairments in inhibitory control, as assessed by the antisaccade task? (2) Does the antisaccade performance of the women with autism relate to respiratory sinus arrhythmia (RSA), a measure of physiological function? Methods: Participants included 18 mothers of children with autism and 19 neurotypical control women, similarly aged 26-65 years ($p = 0.165$). First a block of 60 prosaccade tasks were performed, followed by a block of 40 antisaccade tasks, with a minute break in between. A difference score for average latency and percent correct was computed. Heart activity was recorded during the antisaccade task, and mean RSA and heart rate in each condition was estimated, in addition to a change score reflecting flexible the parasympathetic system is in response to environmental stressors. Results: The latency difference between the groups was significantly different [$F(1,37) = 4.51$, ($p < 0.041$)]. The mothers of children with autism took longer to inhibit the prepotent response, yielding higher change scores. A significant interaction between group and heart rate change was detected in predicating the latency change score [$F(1,37) = 4.21$, ($p < 0.0420$)]. RSA change predicted both the latency change score [$F(1,37) = 5.02$. ($p<0.0329$)], and percent correct change score [$F(1,37) = 9.87$, ($p < 0.0039$)] across both groups. Conclusion: The broad autism phenotype may be associated with inhibition deficits, as indicated by longer latency to inhibit a prepotent response. The relationship between physiological regulation and inhibition performance also differed across mothers of children with ASD and control mothers, suggesting that the broad autism phenotype may be characterized by difficulties with arousal modulation that could be linked inhibition deficits.

The Role for Complement in Optic Nerve Regeneration.

Leung, K. S., Benowitz, L. I., and Peterson, S. L.
Department of Neurosurgery, Boston Children's Hospital, Harvard Medical School

Cell survival and axon regeneration are often limited in the central nervous system (CNS) following injury or disease, creating a significant need for novel therapeutic interventions that improve regeneration. The mouse optic nerve crush model mimics neurodegenerative diseases and traumatic CNS injuries by inducing progressive death of retinal ganglion cells (RGCs), the neurons whose axons were severed and do not regrow. Complement proteins of the innate immune system have been found to have neurotoxic, neuroprotective, and neurodegenerative roles in various injury and disease models, though their roles in cell survival and regeneration following optic nerve crush are largely unknown. Zinc plays a crucial role in a variety of cellular functions. Mobile zinc accumulates in amacrine cells and RGCs after optic nerve injury, and its removal by zinc chelation (TPEN or ZX1) increases RGC survival and axon regeneration after optic nerve injury. We tested the hypothesis that complement is required for these effects by assessing RGC survival and axon regeneration in C1q knock-out, C3 knock-out, C3 receptor CR3 knock-out, and wild-type control mice 14 days

after optic nerve crush plus zinc chelation treatment (TPEN). Our data demonstrate that while neither C1q, C3, nor CR3 affect RGC survival, C1q, C3, and CR3 are all required for RGC axon regeneration. Further investigation into the role of the innate immune system in CNS axon regrowth may lead to the development of interventions to improve neuronal regeneration following injury.

Probing Cognitive Control Neurocircuits: A Concurrent TMS-fMRI Investigation of State Dependence.

Lopez JW, Mithoefer OJ, Dowdle LT, Badran BW, Summers PM, George MS, McTeague LM
Department of Psychology and Program in Neuroscience, College of Charleston; Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina

One in five adults in the United States experience a mental health issue in a given year. Unfortunately, medication and psychotherapy do not always relieve all patients of their symptoms. This leads to the need for new and novel approaches to treating mental disorders. One approach explored and utilized has been Transcranial Magnetic Stimulation (TMS). TMS has typically been used as a therapeutic tool for the treatment of depression, using repetitive patterns of stimulation (rTMS). In contrast, single pulse TMS (spTMS) combined with functional neuroimaging (fMRI) is used to map out circuit-specific responses. There has been a growing interest in examining how cognitive or emotional engagement; otherwise known as state dependence, may affect the therapeutic outcomes of rTMS. However, little is known about how context alters neural responses. We tested the effects of viewing emotionally arousing scenes on brain activation in response to TMS. We also hypothesized TMS/fMRI responses will show target specificity in regards to the networks activated. Healthy individuals (n=24) viewed 20 second blocks of a fixation screen (baseline condition), neutral, and emotionally arousing pictures from the International Affective Picture System (IAPS) while in the MRI scanner. Concurrent with picture or fixation viewing, TMS was delivered to the left dorsolateral prefrontal cortex (dlPFC) or the primary motor cortex (M1). Picture and fixation blocks in the absence of TMS were also presented intermixed with TMS blocks. TMS delivered to the dlPFC during emotional picture processing, as compared to fixation, led to increased brain activation in bilateral fronto-parietal regions. TMS delivered to M1 led to increased BOLD responses in cerebellar regions of the somatomotor network. In the absence of TMS, emotional images led to activation in the visual cortices and amygdala. This activation was also present during concurrent TMS, suggesting TMS did not disrupt the viewing task. This study provides evidence that state dependency, regardless of valence, leads to an overall increase in TMS induced activation. More specifically, increased emotional arousal when targeting the dlPFC and M1 led to greater activation of the cognitive control network (i.e., fronto-parietal) network and somatosensory networks, respectively. These data suggest that priming neural circuits during rTMS may result in improved clinical outcomes due to the increased engagement of targeted networks during TMS.

The neuroprotective potential of Cannabidiol in an in vitro model of peroxidase injury.

Lyons S and Grider MH
Department of Biology and Program in Neuroscience, High Point University

Following injury, excessive production of reactive oxygen species can lead to neuronal death. We tested whether cannabidiol (CBD), a compound derived from the cannabis sativa plant, could protect neurons against a reactive oxygen species injury. A neuronal cell line, RN33B cells, was incubated in media containing 150 – 900 uM hydrogen peroxide for twenty-four

hours, concurrent with 1uM CBD treatment. Cell viability was examined via multiple assays (MTT, LDH) and presented as percentage of control. We are continuing our investigation into the neuroprotective effects of CBD in various injury models and in different cell lines.

Nicotine as a gateway drug: Enhanced sign-tracking by nicotine leads to greater cocaine demand in rats.

Majors CT, Harryman DC, Smith AL, Day TC, Pham M, Kosky MM, Stillwell E, Palmatier MI
Department of Psychology, East Tennessee State Univeristy

Nicotine is often considered a 'gateway' drug because people typically experiment with tobacco before illicit drugs such as cocaine and amphetamine. We have shown that nicotine increases approach to reward-associated stimuli, this is referred to as 'sign-tracking', and that this effect persists after nicotine is discontinued. Individuals who are high in sign-tracking also show increased cocaine self-administration. The goal of this experiment was to determine whether nicotine enhanced sign tracking could result in greater cocaine self-administration. Rats were randomly assigned to one of 2 groups (NIC or SAL), and injected with their assigned solution (0.4 mg/kg base or placebo, respectively) 15 min before conditioning sessions. During conditioning sessions, a lever/light stimulus was inserted into the chamber for 15 s and immediately followed by sucrose delivery. Approach to the sucrose receptacle was recorded by monitoring head entries and defined as goal tracking. Contact with the lever was recorded and defined as 'sign-tracking'. After XX conditioning sessions, the rats were instrumented for cocaine self-administration and were shaped to respond for cocaine on the same lever that served as the CS. After 10 days of acquisition of cocaine self-administration (0.16 mg/inf), demand for cocaine was tested over 6 days using a within session procedure that increased cocaine price every 10 min. We showed increased sign-tracking, but not goal tracking in the NIC group relative to the SAL group. The NIC group also showed increased demand for cocaine during the price manipulation, but the essential value of cocaine did not differ, relative to the SAL group. Our results support a gateway interpretation of substance use – when both the gateway drug (nicotine) and drug-associated rewards (the lever/light) occur together, they can promote future self-administration of illicit drugs such as cocaine.

The Effects of p75NTR Signaling on Oxidative Stress-induced Dopaminergic Neurodegeneration.

Nelson K, Escobedo C, Waugh C, Schemanski M, Kalantar A, Kraemer B
Department of Biological Sciences, Eastern Kentucky University

The p75 Neurotrophin Receptor (p75NTR) is a multifunctional transmembrane protein of the tumor necrosis factor receptor superfamily that is expressed by neurons and glia of the central and peripheral nervous system. Activation of p75NTR leads to a variety of cellular functions, including regulation of neurite outgrowth and myelination. Among its established functions, the receptor also serves a critical role in regulating the survival of neurons that have been affected by neuropathological conditions. Tissue damage can lead to production and secretion of neurotrophins, ligands that bind to and activate p75NTR. Neurotrophin-induced activation of p75NTR leads to cleavage of p75NTR by the metalloprotease TNF-α-Converting Enzyme (TACE) and the γ-secretase complex. During this process, TACE cleaves p75NTR within its extracellular domain, producing a membrane-bound c terminal fragment, and the γ-secretase complex subsequently cleaves the receptor within its transmembrane region, thereby releasing the intracellular domain of the receptor. Fragments of p75NTR can

associate with a variety of intracellular interactors to regulate cell survival. Previously, we discovered that oxidative stress promotes p75NTR cleavage in sympathetic neurons, leading to their neurite degeneration and apoptosis. Because oxidative stress is associated with numerous neurodegenerative diseases, this mechanism of receptor activation may underlie the ability of the receptor to regulate neurodegeneration associated with a variety of pathological conditions. Here, we set out to determine whether oxidative stress promotes proteolytic cleavage of p75NTR in dopaminergic neurons, a cell population that is susceptible to oxidative stress and neurodegeneration associated with Parkinson's disease. Immunostaining revealed that p75NTR is expressed within neurites and cell bodies of midbrain-derived dopaminergic cells. Subjection of the dopaminergic cells to oxidative stress resulted in a significant accumulation of p75NTR fragments. Our preliminary analyses indicate that oxidative stress-induced cleavage of p75NTR results from neurotrophin-independent stimulation of metalloprotease and γ secretase activity. Interestingly, our preliminary results also indicate that blockade of p75NTR cleavage is associated with increased oxidative stress-induced apoptosis of dopaminergic cells, suggesting that p75NTR fragments may promote survival in this cell population.

Use of 3D Printed Capacitive Touch Objects for Object Recognition Tasks.

Potts K., Strauss J., Drye G., Stevanovic K., Cushman J.D.
Neurobehavioral Core, Neurobiology Laboratory, NIEHS

Object recognition is a critical behavioral assay in evaluating and understanding cognition especially learning and memory. Object recognition typically involves familiarizing mice with a set of objects and then presenting a novel object or displacing an object to a novel location. Learning and memory is inferred by the amount of object investigation of the novel/displaced object. These tasks are in widespread use, but unfortunately there are large discrepancies in findings between labs. Two major contributors to these discrepancies are the lack of consistency in the method of measuring object investigation and the lack of standardization of the objects that are used. Current video-based automated algorithms can often be unreliable, lack temporal precision and can be costly, whereas manually scoring object investigation is time consuming, tedious and more subjective. To resolve these issues, we sought to design and implement 3D printed objects, so that objects can be standardized across labs and we utilize capacitive sensing to better measure object investigation. Utilizing a 3D printer with conductive filament and low cost off-the-shelf components we demonstrate that 3D printed capacitive touch objects are a reliable and precise way to perform object recognition tasks. Ultimately, this approach will lead to better standardization, greater consistency and thus prove invaluable to evaluating and understanding cognition in a variety of diseases including mental illnesses, drug addiction, and neurodegenerative diseases.

Cholinergic stimulation of the adult Zebrafish brain induces phosphorylation of GSK3-beta and ERKin the telencephalon of adult zebrafish.

Powers GE, Hinton KD, Payne CH, Scheuermann NL, Saint-Jean M, Mans RA
Department of Biology, Georgia Southern University - Armstrong Campus

The zebrafish (Danio rerio) represents a widely used model organism for the study of vertebrate development, and it has recently gained in prominence for the study of brain function and disease. While it is known that zebrafish share approximately 70% genetic similarity with humans, the physiology of the zebrafish brain remains largely uncharacterized. In mammals, the proteins GSK3beta and ERK1/2 are integral for cell survival and synaptic

plasticity, which are key aspects of studying neurodegenerative disease. In mammals, the activity of GSK3beta and ERK1/2 are regulated by acetylcholine receptors (AChRs) coupled to intracellular signaling kinases, but it is unknown if this same relationship exists in the brains of zebrafish. The current study was conducted to determine if zebrafish AChRs influence the phosphorylation states of GSK3beta and ERK1/2 in a manner similar to mammalian AChRs. To this end, adult zebrafish brains were isolated and maintained ex vivo in oxygenated artificial cerebrospinal fluid (aCSF) before being subjected to pharmacology and Western blot analysis. It was determined that incubation in aCSF supplemented with carbachol (CCh), a non-specific agonist for AChRs, increased phosphorylation of GSK3beta and ERK1/2 in the zebrafish telencephalon. Blockade of muscarinic AChRs (mAChRs) using scopolamine attenuated the effect of CCh, indicating mAChRs contributed to the increased phosphorylation observed in carbachol treatments. Ongoing experiments are testing if oxometromorine-M, a specific activator of mAChRs, mimics the effect of CCh. These results provide evidence for functional conservation between the cholinergic systems of zebrafish and mammals. Further characterization will lead to better understanding of the extent by which zebrafish brains may be used to model mammalian brain function and disease.

A Quantitative Comparison of Hippocampal and Cortical Cultures Within Microfluidic Devices.

Rogers J, Tharkika N, Taylor A
Biomedical Engineering, UNC Chapel Hill

Microfluidic devices have been shown to offer a viable platform for culturing CNS neurons for various applications and experiments. As the devices allow for neurons to extend axons into adjacent compartments, the neurons that extend across the microgrooves may be individually analyzed for various experiments regarding neuronal activity. The devices may be used for culturing any CNS neurons, for example, cortex and hippocampus. While the heterogeneous nature of the cortex is known to be true in the in vivo model, here we quantitatively analyze the characteristics of the cortex and the hippocampus within microfluidic devices and decide based upon their features whether they are comparable or dissimilar. Using florescent labeling and immunostaining, we show that the cortex is highly variable in cell type as well as total neurons labeled, remaining consistent with the in vivo model. Furthermore, we demonstrate the characteristics of neurons that are retrogradely labeled within each device. The majority of the retrogradely labeled neurons are GAD 67 negative (non-inhibitory) and are typically located closer to the microgrooves, thus able to extend their axons into the other compartment. Because the hippocampus contains a higher percentage of excitatory neurons than the cortex, the choice to use the hippocampus in these studies for retrograde labeling is supported.

Impact of Nodal Perturbation on the Distribution of Nuclear Transport Proteins in Sea Urchin Neurogenesis.

Shams R, Byrum CA
Department of Biology and Program in Neuroscience, College of Charleston

The sea urchin embryo (Lytechinus variegatus) is a valuable model for studying the molecular basis of embryogenesis, because it is easy to culture, is genetically and physiologically simple, and, as a deuterostome, has molecular pathways similar to those in vertebrates. During embryogenesis, three germ layers arise: ectoderm, mesoderm, and endoderm. Our lab is particularly interested in the formation of neural ectoderm and how

karyopherins may influence the ability of the transcription factor Nodal to influence formation and spatial distribution of ectodermal structures. Nodal and other transcriptions factors are transported to and from the nucleus by nuclear transport proteins called karyopherins. Our lab is examining how expression of karyopherins impacts embryogenesis and which neurodegenerative processes are implicated with the disruption of target karyopherins. One of the karyopherins considered in this study is KPNA2/7, a karyopherin that may help maintain embryo pluripotency before neural differentiation. Using the chemical inhibitor NiCl2 to induce Nodal overexpression, we have compared karyopherin expression in NiCl2-treated and untreated embryos, examining the spatial distributions of karyopherins using wholemount in situ hybridization. In untreated embryos, KPNA2/7 is typically expressed around the mouth, in the gut, and in clusters of cells associated with the ciliary band. Our preliminary studies show that this pattern is perturbed in NiCl2-treated embryos, where KPNA2/7 is found primarily in the archenteron and vegetal regions. To better quantify relative changes in karyopherin levels, our lab is performing reverse transcriptase polymerase chain reactions (RT-PCR) and also plans to examine roles of additional karyopherins expressed in neural tissues. Results of this study will improve understanding of how nuclear transport processes impact neurogenesis and may further implicate karyopherins in neurodegeneration at a systems-based level.

The Accessory Stimulus Effect is enhanced by a prepulse preceding an intense startle-eliciting acoustic stimulus .

Sivakumar AP, Snipes LB, Blumenthal TD
Wake Forest University

The Accessory Stimulus Effect (ASE) involves a speeding of reaction time to a target stimulus when that target is accompanied by an intense stimulus in another modality. An intense acoustic stimulus can elicit a startle response, and this intense stimulus speeds responding to a concurrent visual target. A weak acoustic pulse (a prepulse) presented shortly before the startle stimulus inhibits the startle response, referred to as Prepulse Inhibition (PPI) of startle. In the current study, startle eyeblink responses were measured while participants completed a simple two-color Stroop task. A startle stimulus resulted in speeded reaction time on the visual Stroop task, and this ASE was enhanced by the presentation of a prepulse 120 ms before startle stimulus onset. This suggests that the prepulse inhibited the response to the startle stimulus, which enhanced the processing of the startle stimulus, strengthening the ASE. This finding has implications for processing task-relevant stimuli in the presence of cross-modality distracting and facilitating stimuli.

Impacts of Glucagon-Like Peptide-1 (9-36) on de novo protein synthesis-associated signaling pathways in a mouse model of Down syndrome.

Stern JE, Wang X, Day SM, Ma T
Department of Neuroscience, Wake Forest University

Down syndrome (DS) is linked with impaired learning and memory. Glucagon-Like Peptide-1 (9-36) amide (GLP-1 (9-36)) is a cleavage product from GLP-1 (7-36), an incretin hormone released in response to nutrient ingestion. We have recently demonstrated that GLP-1 (9-36) improves memory in Ts65DN mouse model of DS, but its exact molecular mechanism of action is unclear. The purpose of this study was to determine if GLP-1 (9-36) alters the phosphorylation levels of several signaling proteins involved in de novo protein synthesis which are known to be required for long-term memory and synaptic plasticity. Using Western

blot assays, we have examined activity and phosphorylation status of eIF2α, eEF2, mTOR, and AMPKα. Oxidative stress is higher in Ts65Dn mice and is linked to dysregulation of de novo protein synthesis. We consequently hypothesized that GLP-1 (9-36)-treated Ts65Dn mice would have restored phosphorylation levels of the aforementioned signaling molecules (indicating the activity) compared to the saline-treated control mice. The preliminary results do not demonstrate statistical difference between treatment groups for eIF2α, eEF2, mTOR, or AMPKα phosphorylation levels.

Design and implementation of a custom-made device to measure two-bottle choice of fluid consumption in mice.

Tanas JK, Schmidt KT, McElligott ZA
Bowles Center for Alcohol Studies, UNC Chapel Hill

Our lab is interested in probing specific neural pathways underlying consummatory behaviors. In order to compare the relative reinforcing efficacy of various liquids (e.g. alcohol, water, or sucrose), two bottle choice procedures allow the subject to decide which liquid to consume. There are currently two predominant approaches to measure and record fluid consumption in mice: periodically observing the fluid volume change of two bottles or using a capacitive sensor to measure the electrical disturbance when the spout is licked. Each of these approaches has limitations regarding the temporal sensitivity and accuracy to gauge liquid consumption. To improve on current methods used in our lab, a custom 3D printed, Arduino-based, two-bottle choice apparatus was designed and tested to report lick count, duration and precise timing of licks with mice (https://hackaday.io/project/158279-automated-mouse-homecage-two-bottle-choice-test#menu-description). The design operates by two photo-interrupter sensors which were placed beneath each bottle. When the lixit is licked, the infrared beam from the sensor would break causing the signal to detect a lick. Once construction of the lickometer device had been completed, various tests were conducted on two mice placed in a single cage. For both water vs water and water vs sucrose tests, the lickometer was left recording overnight for a week duration. Consumption behavior was observed with both water and sucrose tests. The sucrose tests suggested that the mice could identify and follow the preferred fluid bottles when the bottles were switched. The future direction of this research would be to apply these devices to study the effects of alcohol on consumption and sleep behavior.

Blunting Drug Related Gene Expression Changes Using a CRISPR/dCas9 Strategy.

Thukral S, Savell KE, Day JJ
Department of Neurobiology

Drugs of abuse increase dopamine concentration in the nucleus accumbens, a key reward structure that integrates contextual and cue-related information and regulates motivated behavior. This surge of dopamine triggers cell signaling cascades that converge in the nucleus to cause changes in gene expression. These changes are thought to lead to the observed functional and structural alterations in the reward circuit after exposure to drugs of abuse. One alteration is the chronic induction of Fosb, a Fos transcription factor family protein that is induced in the nucleus accumbens and dorsal striatum as a response to exposure to drugs of abuse and signals downstream changes in gene expression. To attempt to blunt the initial elevation of Fosb mRNA following dopamine receptor activation, we employed a neuron-specific CRISPR-dCas9 repressor system in cell cultures to achieve decreases in gene expression. Different parts of the Fosb promoter were targeted, including the promoter

region, transcription factor binding sites, and the first exon of the gene to block transcription. Primary embryonic rat striatal cultures were transduced with the CRISPR-based repressor system, and cells were treated with a D1 dopamine receptor agonist to mimic the signaling cascades found in vivo after exposure to drugs of abuse. Preliminary results suggest that targeting of the exon region in the Fosb promoter can block Fosb mRNA induction. Future directions involve infusion of these constructs into the adult nucleus accumbens in a rodent model system to see if the blunting of Fosb can affect drug-related reward learning.

Meeting young neuroscientists at their individual starting points.

Tucker H, LaFratta B, Franssen RA, Hennings M, Franssen CL
Longwood University

With the rapid increase in undergraduate neuroscience programming, many students encounter neuroscience in one form or another during their collegiate journey. At our small southeastern university, both Psychology majors and Neuroscience Studies Minors of other majors are required to take a course in Introduction to Biopsychology, which serves as an entrance into the realm of neuroscience (or not, for those who choose not to continue in that specific direction). In Fall 2018 we taught our first course of Introduction to Neuroscience, a general education course to meet scientific objectives for primarily non- STEM majors which we hoped would draw more neuro minors. The course turned out to be fraught with challenges- ultimately calling to question the appropriate concepts and methods for the introductory level of neuroscience. Here, we examine the introduction to neuroscience delivered in our two courses. We describe the coursework and methodology for each, and consider how these courses for within a context of being goals of scientific literacy, scientific reasoning skills, and -more specifically- goals of introducing neuroscience content. We further contemplate the content and approach of these introductory neuroscience courses at our university as they compare to other similar universities, and as they fit e with a broader neuroscience curriculum. Finally, we use different evaluations and reflections to create a clear plan for introductory neuroscience at university for the coming year.

Comparative study of flicker fusion frequency in three cockroach species.

Vaughan DT, Triblehorn JD
Department of Biology and Program in Neuroscience, College of Charleston

The ability to process visual information rapidly is an important factor in determining how quickly animals may move through their environment. Fast moving animals need to process visual stimuli at a higher rate than those that move slowly because objects approach faster moving animals at higher velocities. Visual processing speed of an animal can be determined by measuring the Flicker Fusion Frequency (FFF), also called the critical fusion frequency. The FFF is the frequency at which the animal perceives repeating light pulses as a continuous light stimulus (perceptual definition) or individual light pulses no longer elicit neural responses to individual pulses, but evokes the same response as a continuous light stimulus (neural definition). In vertebrates, flying animals have higher FFFs (i.e. 148 Hz in pigeons) while non-flying vertebrates have lower FFFs (i.e. 30 Hz in mice). Slow flying insects (wing-beat frequencies <100 beats/minute) have lower FFFs than flying insects (i.e. 90 Hz in locusts) while fast flying insects (wing-beat frequencies > 100 beats/minute) have some of the fastest FFFs (i.e. 240 Hz honey bees). In this study, we determine whether visual processing speed varies with flight ability in closely related species by comparing the FFFs in three cockroach species: Periplaneta americana (American cockroach), Blaberus craniifer (Death's head

cockroach), and Gromphadorhina portentosa (Hissing cockroach). Both P. americana and B. craniifer possess the ability to fly while G. portentosa lacks wings. We measured FFFs of summed photoreceptor responses in the compound eye using electroretinogram (ERG) recordings under dark adapted conditions. After 10 minutes of dark adaptation, an LED controlled by an isolated pulse stimulator generated one second trains of flickering light stimuli using two ms pulses and frequencies from 5 - 100 Hz. Prior to presenting flickering light stimuli, a one second continuous light stimulus was presented to provide a template for a fused neural response. Preliminary data show the two flying species (P. americana and B. craniifer) have higher FFFs (64±11.4 Hz and 48+5.67 Hz, respectively; mean+SD) than the non-flying species (G. portentosa: 37±4.33 Hz). We are currently investigating how FFFs vary with stimulus intensity in these three species.

Stress and College Students: The Impact of Nature and Tree Climbing.

Willihnganz SB
Department of Psychology, Warren Wilson College

Shinrin-yoku, the concept of spending time in a wooded area or green space, or 'forest bathing', promotes overall well-being and decreased stress (Park et al., 2010). Driven by an interest in mitigating college students' stress due to its negative impact academic success and physical and mental health, this study investigated whether recreational tree climbing, as modified forest bathing, could similarly reduce stress in a college demographic. Twenty participants engaged in two climbing conditions for prescribed periods of time, with recreational tree climbing as the intervention and wooden tower climbing as the control. Participants' stress was measured pre- and post-climbing for both conditions by completion of a self-reported survey to measure perceived acute stress, and physiologically via measuring heart rate, electrodermal activity, salivary cortisol concentration, and alpha brainwave activity levels. Results were significant for reductions in stress levels of college students post recreational tree climbing as measured by perceived acute stress, heart rate, and electrodermal activity. Additionally, alpha brainwave activity, a measure of stress absence, was significantly lower in the tree climbing group compared to the tower climbing group. Further study will provide additional insight into the potential of recreational tree climbing to provide a low-cost, accessible way for college students to manage stress.

The Effect of Valence Priming Labels on Ratings and Neural Processing of Ambiguous Sounds.

Willingham K, Zeitlen D, Barrera M, Neelon M
Department of Psychology, University of North Carolina Asheville

This study seeks to find if there is a difference in neural processing when ambiguous sounds are positively or negatively labeled. Twenty-two sounds were taken from the International Affective Digitized Sounds Database and then edited down to ~1s to enhance ambiguity. Fourteen sounds were labeled positively or negatively depending on the condition, and the remaining eight were labeled neutrally. These labels were taken from the Affective Norms for English Words. In a pilot study conducted by our lab, a group of around 40 participants were presented with the labeled sounds, and they rated them based on valence and arousal levels. Ratings were consistent with the label presented, but this may be due to demand characteristics as opposed to the actual reaction of the participants. The present study also used these labeled sounds, and added the element of electroencephalography (EEG). On each trial, subjects were presented with a label, a fixation cross, then a sound, after which they

rated their responses to the sounds in terms of valence and arousal. Subjects were randomly assigned to see either negative or positive labels for 7 of the valenced sound clips and then opposing valence labels for 7 different sound clips. All subjects saw the same labels for the neutral sounds. Data will be analyzed to determine if priming a sound via a positive or negative label affects the resulting auditory ERP and hence the actual perception of an ambiguous sound.

Reducing scrimmages may significantly reduce concussion rate in high school football.

Zimmerman B, Kelley M, Contillo N, Flood W, Urban J, Stitzel J.
Wake Forest School of Medicine, Department of Biomedical Engineering

Concussions in American football have acute and long-term health consequences and are known to occur more frequently in games than in practices. Scrimmages are a common drill conducted in practice, are intended to simulate games, and may therefore be a modifiable risk factor for concussions. This study seeks to evaluate the relationship between a football team's practice structure and the frequency of concussions that occurred during a single season. On-field head impact data were collected from athletes participating on one high school football team using the Head Impact Telemetry (HIT) System during all practices and games. Video was recorded for all sessions, and post-season video analysis was performed to eliminate impacts that occurred when athletes were not helmeted. Video was also used to assign each head impact occurring in practices to a drill type. Drill types were characterized as either tackling, skill, or scrimmage. Tackling drills included: Oklahoma, tackling circuit, small group tackling, and one-on-one. Skill drills included: position-specific drills and special teams. The duration of each drill was determined by one-minute interval checks by a video rater. The date and session type were recorded for all clinically diagnosed concussions. Mixed effects models were used to assess differences in head impact exposure and time spent between session (i.e. practice and game) and drill type on weeks concussions did and did not occur. 7,004 impacts were evaluated over 12 games and 28 practices from 23 football players. Weeks with concussions correlated with significantly more time spent on scrimmaging and less on skill practice drills, with a parallel increase in the frequency of high magnitude impacts during games. Reducing head impact exposure during games is a noble goal, practices are an environment amenable to change; therefore, limiting time spent on scrimmaging while maximizing skill development may reduce concussion rates in high school football.