Symposium for Young Neuroscientists and Professors of the SouthEast

Saturday March 28, 2020
SYNAPSE STUDENT AWARD WINNERS (MORNING)

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College of Charleston Honors College
Dr. Jennifer Wilhelm

The role of estrogen signaling on synaptic plasticity around axotomized spinal motoneurons. After peripheral nerve transection, changes in spinal cord circuitry occur including the withdrawal of synaptic inputs from the somata and proximal dendrites of axotomized spinal motoneurons. Moderate daily treadmill exercise after transection injuries has been shown to mitigate the reduction in synaptic coverage; however, different exercise protocols are required in male and female animals. The mechanisms that underlie this sex-dependent effect of exercise are not well understood. Androgen receptor signaling has been shown to be an important factor. In this study we tested the hypothesis that estrogen receptor (ER) signaling also is a part of the mechanism by which treadmill exercise mediated its effects on synaptic inputs. Gonadally intact male and female C57BL/6 mice underwent a unilateral sciatic nerve transection and lateral gastrocnemius motoneuron pools in the spinal cord were retrogradely labeled. Mice then were treated with various combinations of estradiol (E) treatment, ER antagonist treatment, and treadmill exercise for the two weeks after nerve transection. The average synaptic coverage of glutamatergic inputs onto labeled motoneurons was assessed fourteen days after injury. We found no significant reduction in coverage in mice treated with E alone. Additionally, we found that blocking ER signaling during treadmill exercise prevented the sustaining effects of the exercise and resulted in an increased loss of inputs compared to mice not receiving the ER antagonist. Based on these results we suggest that ER signaling is an important part of the machinery required for the sex-dependent exercise-mediated effects on synaptic inputs onto axotomized motoneurons after sciatic nerve transection.

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Romanova EV, Doncheck EM, Grant RI, Vollmer KM, Hohmeister MR, Winston K, Holman H, Otis JM
Department of Biology, Program of Neuroscience, College of Charleston; Neurosciences Department, Medical University of South Carolina, Charleston, SC
Dr. Elizabeth Meyer-Bernstein

Prefrontal cortical activity dynamics during cue-induced natural- versus drug-reward seeking

Presentation of environmental cues associated with rewards, such as food or drugs of abuse, can trigger cravings and initiate reward-seeking behaviors. This is especially problematic in substance use disorder, as drug-associated cues can trigger relapse despite prolonged periods of abstinence. While the mechanisms underlying cue-induced reward seeking are not fully understood, the prelimbic-prefrontal cortex (PrL-PFC) is particularly important for cue-induced cravings. Not only does PrL-PFC integrate the information necessary for cue-reward recall, but it is composed of richly heterogeneous classes of neurons whose activation is necessary for reward-seeking behavior. Despite this, responses of unique PrL-PFC cell ensembles during cue-driven reward seeking have not been characterized. Here, we paired head-fixed reward-seeking behaviors with simultaneous in vivo PrL-PFC multiphoton microscopy. Mice received intra-PrL calcium indicator microinfusions and GRIN lens implantation to allow for activity pattern visualization of thousands of PrL-PFC neurons during either Pavlovian sucrose conditioning or intravenous heroin self-administration. During sucrose conditioning, animals were trained to associate an audio cue with sucrose availability and licking responses following both cue and sucrose presentation were recorded. During heroin self-administration, active lever presses triggered an audio cue which co-terminated with intravenous heroin infusions. In either paradigm, post-training presentation of audio cues alone induced reward-seeking behavior. Preliminary analyses reveal at least 8 different PrL-PFC cell clusters displaying unique activity patterns during cue-induced reward-seeking behavior. Ongoing investigations of specific PrL-PFC cell ensembles and their activity...
during cue-driven reward seeking aim to determine their function and potential overlap in their contribution to natural- versus drug-reward seeking behavior.
SYNAPSE STUDENT AWARD WINNERS (AFTERNOON)

Razavi S, Gehle N, Harris N, Mitrano DA
Christopher Newport University
Darlene Mitrano

Norepinephrine and Dopamine in the Dorsal Hippocampus and Locus Coeruleus

In previous studies, the ventral tegmental area (VTA) was presumed to be the main source of dopamine (DA) innervating the dorsal hippocampus in rodents. However, recent data has challenged this by proposing the locus coeruleus (LC) as the main center for DA and norepinephrine (NE) release in the dorsal hippocampus. The dorsal hippocampus is a brain area of interest because it is engaged in arousal, spatial memory, and learning tasks. This study explores the possibility of both DA and NE being released from the same axon terminal arising in the LC and projecting to the dorsal hippocampus. In order to test this, this study uses markers (antibodies) for the norepinephrine reuptake transporter (NET), dopamine β-hydroxylase, the enzyme that converts dopamine to norepinephrine (DBH), and the dopamine reuptake transporter (DAT) at the electron microscopic level to determine if the neurotransmitters are released from the same axon terminal. Rodent tissue is currently being single- and double-labeled with these antibodies to establish an anatomical basis for the possible co-release of DA and NE. Furthermore, to gain a better understanding of how the LC is activated and to observe if there are noradrenergic receptors on LC neurons that may be regulating its activity, rodent brain tissue is being labeled for the alpha1-adrenergic receptor and will then be examined at the electron microscopic level. Overall this study hopes to provide a neuroanatomical basis for the actions of DA and NE in this brain circuit.

Kahle AC, Cleland CL
James Madison University
Corey L. Cleland

Identification of muscle synergies in rat extrinsic tail muscles by multi-channel, selective electromyography

Movement planning by the central nervous system (CNS) is complex, partly resulting from an abundance of muscles that have similar mechanical actions, known as muscle redundancy. There is evidence that the CNS simplifies its control of movement by activating groups of muscles together, termed muscle synergies, rather than activating each muscle individually. Muscle synergies are commonly identified through recording electromyograms (EMGs) during movement. Rat tails are an ideal, under-utilized model system because they are hyper-redundant, with 300 muscles acting on the tail. However, selectively recording from tail muscles has not previously been successful due to their small size and close proximity. Thus, our goal was to develop a selective EMG recording technique to record extrinsic tail muscle activity in order to deduce muscle synergies. Eight bipolar EMG recording electrodes were implanted into the SDL, an extrinsic tail muscle in rats (n=8). To identify the muscle being recorded, muscles were electrically stimulated through the electrodes, and the resulting tail movements were video recorded. The tail was heated with a 980nm laser to evoke an isometric nociceptive withdrawal response, and the resulting EMGs were recorded. We successfully developed selective EMG recording by using closely spaced (<1mm), fine wire bipolar electrodes that were percutaneously inserted into the muscle. Using this technique, we unexpectedly observed activity in both left and right extrinsic muscles, demonstrating a co-contraction synergy that would be expected to stiffen the tail during movement. These findings suggest that the CNS uses a novel strategy to simplify the control of tail muscles.
FEATURED STUDENTS: PROJECT BLITZ PRESENTATIONS (MORNING)

Robinson LE, Cronic TL
Furman University and Prisma Health Neuroscience Associates
Dr. Victoria Turgeon (Furman); Dr. John Absher (Prisma Health PI)

RAP Study: Repository Analysis of Parkinson's disease

There is a need in the scientific community to understand the differences between subgroups (Tremor Dominant [TD] and Postural Instability and Gait Disorder [PIGD]) of Parkinson's Disease (PD). Estimating the degree of structural and clinical variability between existing PD patients offers a better way to differentiate and diagnose the disorders. Currently, clinicians lack a reliable and objective subgroup diagnostic tool which can lead to incorrect, inconsistent diagnoses. Patients with PIGD can be diagnosed because they feature greater postural and gait instability compared to those with TD. The Supplementary Motor Area (SMA) is the brain region that controls bilateral balance and higher order movement planning, and if damaged, may demonstrate bimanual deficits of gait. Using existing retrospective repository data obtained from the Parkinson’s Progression Markers Initiative (PPMI), this study attempts to classify TD and PIGD subjects based on clinical data such as the Unified Parkinson Disease Rating Scale part III (UPDRS-III) postural stability scores, and examine anatomical differences in the SMA between these PD subgroups and healthy controls (HC). We hypothesize that the PIGD subjects will have lower SMA volume compared to TD subjects, after normalizing for whole brain volume and correcting for confounds. The study has the potential to identify important neuroimaging and clinical biomarkers specific to Parkinson's subtypes. New diagnostic biomarkers may prove useful to better understand other variations of PD, such as Atypical Parkinson's Disease, and contribute to the redirection of the movement disorder research community toward personalized medicine.

Lostoski EL, Gendle MH
Elon University
Dr. Mat Gendle

Effects of Oral 5-HTP Administration on Wisconsin Card Sorting Task Performance

5-hydroxytryptophan (5-HTP), the precursor to the neurotransmitter serotonin, is both naturally occurring and sold over the counter as a dietary supplement. Historically, it has most often been utilized to treat mood disorders (Turner et al., 2006; Birdsall, 1998). Theoretically, 5-HTP supplementation should not alter endogenous levels of released serotonin because neuronal autoreceptors should prevent the release of any additional synthesized serotonin. However, there is evidence to suggest that oral 5-HTP may paradoxically reduce cortical dopamine under some circumstances (Gendle and Golding, 2010; Gendle et al. 2013). This double-blind, placebo-controlled study intended to further explore the hypothesis that oral 5-HTP administration produces a reduction in dopamine activity that is detectable by certain dopamine-dependent neurocognitive tasks. Eighty-four university undergraduates were recruited from an institutional participant pool at a mid-size university in the southeastern U.S. and were randomly assigned to receive capsules containing either a total oral dose of 150 mg 5-HTP or a matched placebo. Participants were instructed to take the capsules following a specific schedule prior to arriving at the lab for neurocognitive assessment. All participants completed the Wisconsin Card Sorting Task (WCST), a measure of attentional set shifting that is sensitive to reductions in dopamine, but not to increases in serotonin. The groups did not significantly differ on any WCST outcome (all p's >= 0.23). This result indicates that oral 5-HTP did not cause a dopamine deficiency as was hypothesized in prior published research, or that participants’ levels of compliance in consuming the 5-HTP/placebo before the testing session was low.
Are Body image attitudes affected by media exposure among Latinx young adults?

Positive body perceptions are salient to the healthy development of adolescents. Media and TV exposure has been shown to play a central role in body dissatisfaction among adolescents and are associated with the eating concerns and disorders, as well as a negative self-perception. Research studies have shown youth generally report body dissatisfaction after viewing thin media images. These results have been especially significant among White youth. The present study seeks to examine how body image attitudes are affected by media exposure among Latinx young adults. 198 Latinx college students (70% female; 43% first generation college students) completed questionnaires to assess television influence and body image attitudes. Among female young adults, bivariate correlations revealed that perceived realism of television and active viewing of television were positively associated with eating disorder symptoms (r=.210 & .204, p<.05). No associations were found among media measures and young adult male’s body image perceptions. The findings suggest that Latinx young adult’s perceptions of media may have different effects for males and females.

Sex Differences in Sucrose Sensitivity in Drosophila melanogaster

Physiological states affect animal behavior, emotion, and perception of external stimuli. Hunger changes behavioral responses in a variety of species, including Drosophila melanogaster, the common fruit fly. Despite known anatomical differences between male and female flies, many studies exclusively focus on one sex, which may overgeneralize findings to the entire species. We sought to explore sex differences in feeding behavior using an assay known as the Proboscis Extension Reflex (PER). PER is a feeding-related behavior that occurs when a fly senses an appetitive stimulus, such as sucrose. Under fed conditions, a greater fraction of female flies exhibit PER at lower sucrose concentrations than male flies. This finding suggests a sex difference in sucrose sensitivity. We also measured PER under starved conditions, which is known to increase sucrose sensitivity. Our results indicate that when both male and female flies are starved, they exhibit increased sucrose sensitivity compared to fed flies. We did not see significant differences between the sucrose sensitivity of starved male and female flies. These findings imply that studies exploring behavioral responses of fruit flies should include sex as a biological variable. Additionally, they support the need for investigation of the mechanisms underlying these sex differences. Pilot experiments are underway to investigate how L-DOPA affects sucrose sensitivity in male and female fruit flies. Previous studies have demonstrated that L-DOPA treatment increases sucrose sensitivity in female flies, and this treatment would allow us to determine if differences in dopamine neuromodulation are involved in the sex differences in sucrose sensitivity observed.
Abbie Preston, Summer Chenault, M. Beth Barnes, Natalie Dean, Dr. Ann Cralidis  
Longwood University  
Dr. Ann Cralidis  

The relationship between verbal fluency performance and an online grocery shopping task in young participants with no-brain-damage  

The purpose of this pilot investigation is to explore the relationship between phonemic and semantic verbal fluency performance, as indicated by the total number of correct words produced, and performance on an online grocery shopping task in young adult participants with no-brain-damage. There are no recent investigations that have explored the potential relationship between performance on tasks of phonemic and semantic verbal fluency and grocery shopping efficacy in younger participants without brain injuries.

Tolley TN, Cleland CL  
James Madison University  
Corey Cleland  

Identification of Muscle Synergies based on Mathematical Reconstruction of the Tail Nociceptive Withdrawal Response in Rats  

Computational complexity in movement planning by the central nervous system (CNS) arises in part from muscular redundancy, in which there are more muscles than necessary to produce a movement. The CNS may simplify the control of movement by controlling muscles in groups, known as synergies, rather than individually. In order to experimentally identify synergies it is necessary to determine the patterns of activity of muscle, which has typically been done by electromyography (EMG). However, it is difficult to selectively record EMG in the small, closely packed muscles that control the rat tail. The aim of this study is to develop a new approach by deconstructing the nociceptive withdrawal response (NWR) movement into linear combinations of tendon primitives. The NWR of the rat tail was evoked by heat stimuli (980 nm laser) and recorded with high-speed video. The following day, surgery was performed under anesthesia, during which 30-40 tendons arising from the extrinsic tail muscles (SDL, SDM, and ACD) were individually pulled; the resulting movement of the tail, or tendon primitive, was video recorded. To determine which tendons contribute to the NWR, various combinations of tendon primitives were added to identify the combination that provided the best-fit to the NWR. Our results suggest that small movements of the tail can be attributed, surprisingly, to a single tendon. Larger movements, however, require the recruitment of multiple tendon primitives acting as a synergy. Our findings suggest that rats may simplify the control of movement by using multiple strategies, including synergies.
Brooke N. Jones, Adenike Iry-Shabazz, Antoniette M. Maldonado-Devinci, Ph.D., and Jian Han, Ph.D.
North Carolina Agricultural and Technical State University

Jian Han

High fat diet impairs sensorimotor integration in male, but not female, C57BL/6J mice

Sensorimotor integration is defined as the central nervous system’s capability to integrate different stimuli, as well as to transform such inputs into motor actions. Using the beam traversal test to measure balance and motor coordination can serve as a proxy for measuring sensorimotor integration. In this experiment, male and female C57BL/6J mice were received at 3 weeks of age and placed on regular rodent chow for one week. Following this acclimation period, mice were placed on chronic high fat diet (HFD) or control diet and monitored for sensorimotor integration using the beam traverse test. The beam traverse test measures sensorimotor integration by placing mice on a cylindrical beam that is suspended 50 cm above the ground. Mice are introduced to the beam on a brightly lit side and travel to the dark goal box. Mice are trained for three trials to cross the beam, with at least 10 minutes between trials. The fourth trial is the test trial and is video-recorded for offline analysis. The male HFD-exposed mice took longer to cross the beam compared to male control mice beginning after three months of HFD exposure. This effect was absent in females. Together, these data demonstrate sex differences in HFD-induced impaired sensorimotor integration following chronic exposure to high fat. Currently this study is ongoing and we plan to determine neurobiological markers in the brain that may mediate these changes in key brain regions involved in motor function and sensory perception.

Tatlock Lauten, G. Jean Harry
NIEHs, National Toxicology Program

Jean Harry

Glial Response to ASC Specks Released by NLRP3 Inflammasome Activation

Inflammasomes are multiprotein complexes that form in the cytosol of immune cells in response to extracellular signals and serve as a key determinant of chronic inflammation. One such inflammasome, the nucleotide-binding oligomerization domain – (NOD-) like receptors (NLRs) family, pyrin domain-containing receptors (NLRPs) recognizes a range of signals. Upon activation by a primary signal, the cell forms a protein aggregate comprised of NLRP, apoptosis- associated speck-like protein (ASC), and caspase 1. This process promotes cleavage of pro-caspase-1 to mature caspase-1 and the release of mature interleukin 1 beta (IL-1β) and interleukin 1 alpha (IL-1α), and with pyroptotic cell death, a release of the “ASC speck”. While the release of IL-1β and IL-1α can have detrimental effects on cells in proximity, recent data suggests that the physical ASC speck can also present as an adverse factor within the tissue. This includes the swelling of microglia cells through the predicted uptake of ASC specks and prevention of breakdown of the ASC speck by the microglia. The question as to whether this is due to an ineffective ability of the lysosome of the microglia or by the direct effect of ASC specks is being investigated. To address this question, ASC specks were generated to examine their biological impact on microglia cells and levels of pro-inflammatory cytokine release. Previous work demonstrated a differential pro-inflammatory response with a microglial cell line and microglia/astrocyte co-culture, when exposed to ASC specks, showed an increase in pro-inflammatory cytokines significant for microglia and astrocyte cells. These data suggest a neurotoxic effect of ASC specks on microglia cells and the role of microglia-astrocyte cell response. These, in addition to the changes induced by ASC specks on microglia function will be the focus of this study.
FEATURED STUDENTS: PROJECT BLITZ PRESENTATIONS (AFTERNOON)

Meghan Babington, Zoe Anderson, Daniela Gil, Samantha Lambeth, Katherine Vaughn, Nicole Rivero Ballon, Jennifer Rainville, Georgia Hodes

Virginia Tech
Georgia Hodes

Sex Differences in the Immune Cell Response to the Long-Term Variable Stress Model for Depression

Depression is the leading cause of disability, impacting around 300 million people worldwide. Depression in humans is associated with inflammation meaning they have a higher white blood cell count compared to the average person. In our lab my focus was on the effects of stress on wild-type mice both males and females to measure the changes in their white blood cell count. Being able to model this helps connect the immune system and potential biological markers for depression. The 28-day variable stress model involves three major stressors, one test given each day for 28 days followed by a submandibular blood draw, and five different behavior tests for the following week immediately after stress. These behavior tests help us identify mice that are stress susceptible or stress resilient. After the blood draw, we performed flow cytometry and achieved the following results from targeting specific populations of cells within the adaptive and innate immune systems. In the innate immune system, we screened for neutrophils and monocytes while in the adaptive immune system we looked for B cells, T-helper cells, and Cytotoxic T cells all involved in protecting the body. Based on our results, we found no evidence for lasting changes to immune cell populations in circulation 24 hours following the last stress of a 28-day variable stress paradigm. However, we observed a sex difference in the number of cytotoxic T cells. Cytotoxic T cells’ role are to induce apoptosis in virally infected cells, although their role in response to stress is unknown.

Washington University in St. Louis
Dr. Tien-Phat Huynh

Lack of hepatic apoE does not influence early Aβ deposition: observations from a new APOE knock-in model

The apolipoprotein E (APOE) gene has been identified as the greatest genetic risk factor for late-onset Alzheimer’s disease (AD). The three most common isoforms of APOE– APOE-E2, APOE-E3, and APOE-E4– modulate risk of developing AD. One copy of E4 increases the risk of developing AD 3.7-fold, and two copies of E4 increase risk 12-fold compared to the E3/E3 genotype. Furthermore, amyloid-beta (Abeta) plaques are one of the foremost biological hallmarks of AD, with fewer plaques possibly indicating less harmful pathology.

Previous studies have shown that mice carrying two copies of the APOE-E4 gene had significantly greater amyloid plaque burden compared to E3/E3 and E2/E2 mice. Yet, we have not looked at the cell-specific effect of APOE expression on amyloid pathology.

In the brain, the apoE protein is produced by both astrocytes and microglia. In the periphery, apoE is produced by hepatocytes. Thus, we aimed to study the effect of cell-specific APOE expression on amyloid pathology using a new APOE KI mouse model.

We found that the majority of apoE surrounding amyloid plaques was found in astrocytes. As seen in earlier studies, E4/E4 mice contained a higher density of plaques compared to E3/E3 mice. Finally, we observed the effect of cell-specific APOE expression on cerebral amyloid deposition. Depleted hepatocyte APOE expression did not lower brain apoE levels but did lead to a significant decrease in plasma APOE levels. Yet, despite the marked decrease in plasma apoE levels when hepatic APOE expression was mitigated, cerebral Abeta plaque deposition was unaltered.
Eberhard JM, Matthews LJ, Vaden Jr. KI, Dubno JR, Eckert MA
Department of Psychology and Program in Neuroscience, College of Charleston; Department of Otolaryngology-Head and Neck Surgery, Medical University of South Carolina
Dr. Beth Meyer-Bernstein
Lower general cognitive function is uniquely related to elevated low-frequency thresholds and poorer dichotic listening in older adults
A decline in general cognitive function is frequently reported in older adults with identified hearing loss. It has been postulated that this relationship may be due to age-related speech recognition difficulties that occur with declines in the peripheral and central auditory system. The present study examined the extent to which: 1) general cognitive function is significantly correlated with deficits in audition in a relatively large cross-sectional sample; and 2) whether the association could be explained by speech recognition difficulties. Regression analyses were used to examine the association between total Mini-Mental Status Exam (MMSE) score and auditory performance. Across participants, lower MMSE performance was observed when low- and high-frequency hearing thresholds (250 Hz to 8000 Hz) were elevated. Potential sex effects were also seen as women displayed greater low-frequency loss associated with lower MMSE scores, while men showed significant high-frequency loss. Speech recognition errors were more pronounced with lower MMSE performance for females (p<0.001) and males (p<0.005), after accounting for the low-frequency threshold and demographic variables. Hearing thresholds only accounted for, at most, ~5% of the variance in MMSE performance. Although the selective attention and attention switching demands of speech recognition tasks may explain the association with MMSE performance, the reason for modest associations between low-frequency thresholds and MMSE performance remain unclear. We are currently exploring the effects of estrogen-based hormone replacement therapies as a contributing mechanism to low-frequency loss in older women.

Xueqin Wang, Zan Xu, Fangli Zhao, Kuanhung J. Lin, Joshua B. Foster, Tianqi Xiao, Nydia Kung, Candice C. Askwith, John P. Bruno, Valentina Valentini, Kevin J. Hodgetts, Chien-liang Glenn Lin
Department of Neuroscience, College of Medicine, The Ohio State University, Columbus, OH, USA; Department of Psychology, College of Arts and Sciences, The Ohio State University, Columbus, OH, USA; Department of Neurology, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA, USA
Dr. Chien-liang Glenn Lin
Restoring Tripartite Glutamatergic Synapses: a Potential Therapy for Mood and Cognitive Deficits in Gulf War Illness
As a chronic and multi-symptomatic disorder, Gulf War Illness (GWI) is associated with a combination of war-related traumatic stress and exposure to toxic substances such as pyridostigmine bromide (given to soldiers as an anti-nerve agent pretreatment), sarin nerve agent, pesticides, and smoke from burning oil wells. Currently, there are no effective treatments, and the exact pathophysiology remains elusive. Neurological problems are among the most commonly reported GWI symptoms. In this study, we investigated the glutamatergic system in the hippocampi of mice exposed to GW-related agents and stress. These mice developed GWI-like symptoms, including cognitive impairments, mood deficits, and fatigue. They also exhibited the following pathological changes in hippocampi: elevated extracellular glutamate levels, impaired glutamatergic synapses, astrocyte atrophy, loss of interneurons, and decreased neurogenesis. LDN/OSU-215111, a small-molecule which has been developed and studied extensively in our laboratory, has shown to strengthen the structure and function of tripartite glutamatergic synapses, which are composed of astrocytic processes and synaptic boutons. We found that LDN/OSU-215111 effectively prevented the development of cognitive and mood deficits in mice when treatment was implemented immediately following exposure to GW-agents and stress. Moreover, when symptoms were already present, LDN/OSU-215111 still significantly ameliorated these deficits and impressively, benefits were sustained one month after treatment cessation. LDN/OSU-215111 effectively normalized pathological changes. These results provide strong evidence that restoration of
tripartite glutamatergic synapses by LDN/OSU-215111 is a potential therapy for GWI, and suggests the potential for treatment of other glutamatergic neurodegenerative diseases.

Barry GB, Martinez D, Wood D, Hughes B, Taniguchi M, Penrod RD, Cowan CW. Department of Biology and Program in Neuroscience, College of Charleston; Department of Neurosciences, Medical University of South Carolina

Dr. Meyer-Bernstein

Understanding the functional role of the activity-regulated cytoskeleton-associated protein (Arc/Arg3.1) in the nucleus accumbens using shArc and cre-dependent shArc as knockdown strategies

Drugs of abuse create lasting alterations in brain function that can contribute to the persistence of addiction. Previous research has shown that glutamatergic plasticity in medium spiny neurons (MSNs) of the nucleus accumbens (NAc) is a locus for drug-induced changes in the brain. Understanding the molecular pathways underlying these changes is important for treating drug addiction. We have focused our attention on the activity-regulated cytoskeleton-associated protein (Arc) which is an important regulator of glutamate receptor expression and is upregulated by cocaine exposure. To understand Arc’s function in addiction, we knocked down Arc mRNA and protein expression in the NAc using a constitutive shRNA and cre-dependent shRNA target system and examined effects on addictive behaviors. Viral expression and region-specific Arc knockdown were validated using western blot, qPCR techniques, and immunocytochemistry. Animals with NAc-selective knockdown of Arc demonstrated reduced anxiety, sensitivity to novelty, and sensitivity to drugs. Interestingly, some behavioral effects were sex-specific. Current work uses cre-dependent shRNA virus in mice expressing cre recombinase under control of cell type-specific promoters to assess the contribution of different MSN sub-types to the previously observed effects in the constitutive shRNA experiments. Ongoing work is focused on validating cell type-specific Arc knockdown using IHC and RNAscope methods. The goal of current work is to manipulate Arc in a region (NAc) and cell type-specific manner and examine behavioral effects. Understanding the mechanism’s underlying Arc’s function will provide a better understanding when developing future therapies.
Role of dopamine receptor type-2 expressing neurons in the nucleus accumbens and ventral pallidum in the suppression of reward seeking under punishment

Addiction is associated with compulsivity and resistance to the suppressive effect of punishment on reward seeking. This resistance to punishment could be a risk factor, but the brain circuits mediating learning from punishment are not well defined. Dopamine neurons from the ventral tegmental area project to downstream targets, such as the nucleus accumbens (NAc) and ventral pallidum (VP), and activity of dopamine-responsive neurons in these regions is associated with risk-preference. For example, NAc neurons expressing dopamine receptor type-2 (D2) are involved in suppression of reward-seeking. Accordingly, we hypothesize that inhibiting D2-expressing neurons will lead to resistance to punishment in reward-seeking. To test this, transgenic D2 cre+ rats underwent stereotaxic surgery for injection of cre-dependent inhibitory Gi DREADD in the NAc or VP to inhibit D2-expressing neurons. Rats were tested for the suppressive effect of footshock on seeking of food reward. Specifically, rats were trained to lever press on one of two levers that yielded a large reward (3 food-pellets) that was immediately followed by aversive footshock, or a small reward (1 pellet) not followed by punishment. We found that inhibition of D2-expressing neurons in the NAc or VP caused a moderate, but not statistically significant, increase in the amount of footshock rats were willing to endure to receive the large food-reward. While these results are encouraging, small sample sizes limit our ability to draw strong conclusions. Future research will be critical to support initial findings but results suggest that activity in these regions is important in suppressing reward-seeking under punishment.
Watson HG, Ashchi AT, Riley CN, Caron AL, Saunders CJ, Silver WL
Wake Forest University
Wayne Silver

The Development of Epidermal Sensory Organs in the Earthworm Eisenia hortensis

Although the earthworm’s chemosensory capacity has been studied for centuries, little is known about the cells they use to sense stimuli in their environment. We are interested in the development of sensory structures which purportedly detect environmental chemical stimuli in Eisenia hortensis. The earthworm literature speculates that receptor cells responsible for detecting chemical stimuli are clustered in structures known as epidermal sensory organs (ESOs). Our investigation used scanning electron microscopy (SEM) and florescent confocal microscopy (FCM) to determine if ESOs are present at various stages of earthworm development. ESOs consist of clusters of ciliated cells, which were visualized for FCM using an antibody against acetylated tubulin. Samples of the prostomium (an organ analogous to the tongue) were taken from newly hatched, adolescent, and adult earthworms for analysis. ESOs were observed at each of the three age points, both with SEM and FCM. Additionally, there was a significant correlation between segment size and mass, which could be used to estimate earthworm age. Based on our results, ESO diameter significantly changes from hatching to adulthood (1-way ANOVA, f(2,99)=15.556, p<.001). Analysis using Tukey’s HSD showed a significant difference between the number of ESOs at each age point (Adolescent-Hatchling=0.05, Adult-Hatchling=1.3e-6, Adult-Adolescent=0.0069). We are comparing the estimated age using linear regression to the number and density of ESOs. We are also conducting behavioral assays to determine whether newly hatched and adolescent worms respond to chemicals (such as ally isothiocyanate, menthol and NaCl) in a manner similar to those we have shown previously for adult earthworms.

Seidling K, Garcia-Keller C, Penaloza T, Galuska C, Neuhofer D, Kalivas P
College of Charleton, Medical University of South Carolina
Beth Meyer-Bernstein

Does THC modulate heroin seeking? A behavioral and neuroimaging study in rats.

The overprescription of opioids for pain management has led to a health crisis in the United States. As a result, less addictive alternatives are being explored as clinical analgesics in an effort to decrease the addiction rates and consequent overdoses that accompany opioid use. The recent legalization of cannabis in several states has led to an increase in its popularity as a potential analgesic. Cannabinoids such as tetrahydrocannabinol (THC) warrant further study to determine if repeated use of THC affects the development of opioid use disorder. The present study examines whether THC pre-exposure increases heroin self-administration and/or increases the risk of relapse to heroin after withdrawal. Adults rats were trained to self-administer THC using a standard behavioral protocol. Following an extinction period, rats were subsequently trained to self-administer heroin or a sucrose control. Active lever presses were recorded under each condition and quantitative comparisons were made to saline and sucrose control groups. Preliminary results indicate that THC self-administration is associated with subsequent decreases in heroin self-administration (F(2,27)= 3.614, p<0.05). Morphological differences in medium spiny neurons containing dopamine D1 and D2 receptors in the nucleus accumbens are being analyzed to determine whether THC use is associated with structural changes seen with heroin use. Prior research has indicated a drop in dendritic spine density associated with THC use that matches those seen with opioid use. Ultimately this research will lend insight into the possible modulating role of THC on the addictive qualities of opioids.
**POSTER PRESENTATIONS (MORNING)**

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<td><strong>Longwood University</strong></td>
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<td><strong>Dr. JoEllen Pederson</strong></td>
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<td><strong>The Relationship between Volunteering and Mental Health: A mixed-methods sociological approach</strong></td>
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The objective of this research is to examine the health implications of volunteering at a rural therapeutic riding program. A brief review of the literature on the health benefits of volunteering and discussion the use of cortisol, DHEA-S, and alpha-amylase in sociological research illustrates the importance of a methodological approach joining these two fields of study. Therefore, a mixed-methods approach was used to understand the physical, mental, and social health implications of volunteering. First, in-depth, semi-structured interviews were conducted with volunteers. Then, saliva samples were collected from volunteers. The saliva analysis indicates the distinction between the initial collection, prior to volunteering, compared to the subsequent collection, after participants completed their volunteering experience. Findings suggest there is a health benefit to volunteer that could have significant implications for rural health initiatives.

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<th>Jacob Dunn</th>
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<td><strong>High Point University</strong></td>
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We investigated the ability of CBD to protect against a hypoxic insult in a pure neuronal population. Concurrent with a 24-hour injury, cells are exposed to varying concentrations of CBD, measuring viability using an MTT assay. We found that CBD did not protect against the injury and may be neurotoxic itself.
Microglial CD11b Expression During a Critical Period of Circuit Assembly in the Multimodal Midbrain

The lateral cortex of the inferior colliculus (LCIC) is a multimodal shell nucleus of the auditory midbrain which receives segregated somatosensory and auditory afferents. Its characteristic modular-extramodular organization develops during an early critical period (postnatal day, P0 to P12), with somatosensory inputs targeting glutamic acid decarboxylase (GAD)-specific modules and auditory inputs surrounding calretinin (CR)-positive extramodular zones. Microglial cells (MGCs) have been implicated in the development and refinement of analogous discretely organized maps in a variety of unimodal systems. One pathway important for neuronal-glial interactions whereby underutilized synapses are selectively tagged for elimination is the classical complement cascade. The present study examines complement signaling in the developing LCIC, focusing on MGC-specific expression of the complement receptor, CR3. Immunostaining for CD11b, a subunit of CR3, was performed in GAD67-GFP and CX3CR1-GFP mice during the defined critical period for LCIC development. GAD67+/GFP mice enable visualization of emerging LCIC modular fields, while CX3CR1+/GFP mice highlight microglia expressing the fractalkine receptor (a known MGC marker). CR staining was also employed as a marker for LCIC extramodular domains. CD11b expression appears homogeneous throughout the LCIC at birth and up to P4. By P8 and P12, as LCIC compartments sharpen and multimodal afferent streams segregate, CD11b expression becomes concentrated to modular fields. In CX3CR1+/GFP mice, CD11b labeling does not colocalize with GFP-labeled MGCs, suggesting that unique subsets of microglia exist in the nascent LCIC with different molecular signatures and responsibilities. Changing CR3/CD11b patterns in the developing LCIC suggest complement signaling involvement in refining early LCIC multimodal connections.

Toxicity of vape juice on cultured neuronal cell lines.

Electronic cigarettes have been advertised as a healthy alternative to traditional cigarettes. Although being marketed as a safer alternative, there is a paucity of research on the effects of inhaling e-cigarette vapor. Exposure to vape products in animal models leads to increased microglia activation, altered neurotrophin expression, and decreased hippocampal cell viability. Electronic cigarettes contain nicotine like traditional cigarettes, but are delivered in a “vape juice” containing ingredients such as propylene glycol, vegetable glycerol, and flavoring. In this study, we are using multiple neuronal cell lines to test effect of culturing the cells with varying concentrations of vape juice solution, with and without the presence of nicotine, on neuronal cell viability.
Hesterman R, Hyde D, Ackerman K  
Highpoint University, University of Notre Dame  
Kristin Ackerman  

**Taurine-Induced Photoreceptor Differentiation in the Developing Zebrafish Retina**

World-wide, approximately 39 million individuals are blind. The leading cause of visual impairments involves permanent photoreceptor cell death. Efforts in regenerative medicine are focused on either 1) implanting exogenous cells that must integrate/replace the lost photoreceptors or 2) the induction of remaining healthy cells to produce additional new cells. It is well documented in vitro using iPS cells, embryonic stem cells, and/or developing retinal explants that taurine is critical for the differentiation of progenitor cells into rod/rod-like photoreceptors. In the vertebrate retina, taurine concentration and retino-genesis are highly correlated. As the rat, chick, and cat retina differentiates to become more mature, taurine levels and taurine transporter (TauT) activity increases. Additionally, taurine depletion results in retinal degeneration and eventual blindness. To date, limited studies address the mechanisms by which taurine induces rod photoreceptor differentiation. Thus, we set out to determine if the zebrafish retina could be the first in vivo model system to examine taurine-induced photoreceptor differentiation. Currently, we have utilized both a genetic and pharmacological approach to examine the role of taurine on photoreceptor differentiation (using the Xops:GFP transgenic line to visualize rod photoreceptors). We confirmed that taurine exposure significantly increased the number of rod photoreceptors within the developing retina and that the number of ventral rod photoreceptor significantly decreased after TauT-knockdown. However, exogenous taurine application in the TauT-knockdown did not rescue the phenotype, indicating that taurine was not signaling via the TauT to induce differentiation. Preliminary studies indicate that taurine is signaling through a glycine receptor to alter differentiation.

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Balch LE  
Longwood University  
Dr. Franssen  

**Stress Management**

Our project was an outreach project that we presented at Fuqua high school. We gave a presentation to high school seniors and sophomores to empower them to have successful tools to manage their stress. We not only gave them the tools to manage their stress, but we also educated them on what stress is and what stress can do to effect their body in the short term as well as the long term. Our hope was that these students could learn effective strategies now so that they could use them as they progressed more in life. Based on the data we collected from the note cards and a Kahoot, we found that students had a lot of different ways to deal with their stress and most of them felt stress of some kind. We also found that most students had a relatively good idea of what stress was.
Trzcinski LO, Black AK, Knudsen KA, Mangone GN, Watts EC, Brown JW
James Madison University
Justin W Brown PhD
The Role of Brain Stem 5HT1A and GABA-A Receptors in the Thermoregulatory Response to Hypoxic Stress
Alterations in brainstem Serotonin (5HT) and GABA neuronal development have been linked to the etiology of Sudden Infant Death Syndrome (SIDS). Specifically, alterations in 5HT1A and/or GABA-A receptors at the brainstem Nucleus Raphe Pallidus (NRP) are known to alter normal cardiovascular stress responses. Here, the protective thermoregulatory responses to hypoxia are explored. Using aseptic techniques, Sprague-Dawley rats were instrumented with radiotelemetry probes to non-invasively measure core temperature (Tc), and a steel cannula was inserted into the brainstem which allowed microinjection at the NRP. After recovery, rats were housed in a thermal gradient which quantified their select ambient temperature (STa) and thereby allowed for behavioral thermoregulation. Then, 30mM of either a 5HT1A agonist (8OH-DPAT; N=4), antagonist (WAY; N=6), a GABA-A agonist (Muscimol; N=3), antagonist (Bicuculine; N=3), or ACSF (control; N=5) was then microinjected into the NRP immediately before exposure to 6% O2 for 60 minutes. DPAT injection resulted in a greater drop in Tc compared to ACSF, while the other drugs had minimal sustained effects on STa or Tc. These data suggest activation of the 5HT1A receptor exacerbates the hypothermic response to hypoxic stress while alteration of the inhibitory GABA-A receptor had minimal effects. The STa changes in ACSF and DPAT groups help facilitate Tc changes suggesting coordination between behavioral and autonomic mechanisms in the protective response to hypoxia. This suggests the 5HT1A receptor is at least involved in mediation of protective thermoregulatory responses to hypoxic stress and, if poorly developed in an infant, may contribute to the etiology of SIDS.

Passarelli G, Huezo K, Ackerman K, Ahrens H
High Point University
Heather Ahrens
Distribution of Nicotinic Cholinergic Receptor Subtypes in the Adult Zebrafish
Adult zebrafish serve as an exceptional model for the central nervous system, with the dopamine, noradrenaline, serotonin, and histamine neurotransmitter systems previously described. The goal of this project was to examine and describe the distribution of the cholinergic/cholinocceptive system in the adult zebrafish. Specifically, we mapped the expression of nicotinic cholinergic receptor subtypes (nAChRs α4 and α6) to determine where acetylcholinesterase (AChE) is found in the central nervous system. The fish heads were sliced at 16 μm thickness, and a binding assay was used to map the α4 (Epibatidin) and α6 (Conotoxin) nAChRs. The α4 subtype is dense in the retina, olfactory bulb, optic tectum, superior reticular formation, medial longitudinal fascicle, and hypothalamic inferior lobe. Additional areas of weak α4 presentation include the optic chiasma, ventral habenular nucleus, and central nucleus of torus semicircularis. The α6 nAChR was found very prominently in the retina, as well as in the olfactory bulb, optic chiasma, ventral habenular nucleus, and optic tectum. Farther caudally, the locus coeruleus, superior reticular nucleus, the nucleus isthmi, the vagal motor nucleus, and the caudal octavolateralis nucleus displayed weak expression of α6. It is important to note the significant, high concentrations of both α4 and α6 within the visual areas of the central nervous system. The similarity between the adult zebrafish and human central nervous system can lead to more specified clinical developments related to the role of nicotine in the central nervous system.
Jacob Dunn  
High Point University  
Dr. Michael Grider  
**Cannabidiol Does Not Protect Against an in vitro Model of Stroke.**

Although almost 700,000 patients in the United States suffer from an ischemic stroke each year, 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 a neonatal hypoxia model. Following adult injury, CBD decreases the reactive gliosis process following CNS damage, although the neuronal viability was not changed. We suggest that CBD-mediated neuroprotection in animal models may be mediated by CBD metabolites or through interactions of CBD with the vasculature, immune system, or glial cells. Therefore, we examined CBD's effect on neuronal cell populations in vitro in an attempt to identify specific mechanisms that may be altered in neurons. In the current experiments, we investigate the ability of CBD to protect against a hypoxic, glucose withdrawal insult in a pure neuronal population utilizing the neuronal cell lines RN33B, PC12, and chick forebrain neurons. The initial injury was not sufficiently robust, and cell were potentially using alternative energy sources (not glucose) for energy metabolism. As a result, the injury was modified to exclude the potential alternative energy sources including pyruvic acid. Concurrent with a 48-hour injury, cells are exposed to varying concentrations of CBD or a vehicle control. Neuronal viability is measured using an MTT assay. We found that CBD did not protect against the injury and, at higher concentrations, may be neurotoxic as evidenced by decreases in cell viability.

Zanghi NP, Mathias WJ, Grider MH  
High Point University  
Dr. Michael Grider  
**Cannabidiol potentiates oxidative stress in cultured peripheral neurons.**

Oxidative stress-related injury in nerve cells is considered to be a principal result of many neurodegenerative disorders and injuries. However, the vulnerability of neurons to oxidative stress varies greatly across different neuronal sub-populations. Peripheral nerves, due to their lack of a vascular barrier and lymph drainage, are particularly sensitive to injuries resulting from oxidative stress. Cannabidiol (CBD) is the primary non-psychoactive component of marijuana (Cannabis sativa). Previous publications indicate that CBD, depending on the model implemented, can have either pro- or anti-oxidant effects. To further elucidate these diverging results, we investigate the potential neuroprotective effects of CBD on an oxidative injury (H2O2) in a cultured peripheral neuron cell line. Following injury, cell viability was quantified using MTT analysis. We find that neurons treated with CBD had lower viability following an injury, as compared to those only treated with H2O2. This data supports the findings that CBD potentiates oxidative stress in peripheral neurons. We are further investigating the interaction of CBD and oxidative stress by using a neuronal cell line from the central nervous system, and by varying the concentration of CBD to test dose dependency.
Lopuch AJ, Swinehart BD, Widener EL, Holley ZL, Bland KM, Vidal GS  
James Madison University  
George Vidal  
A role for integrin beta 3 in cerebral cortex-mediated behaviors in mice  
There is overwhelming evidence of a positive association between mutations in integrin beta 3 (Itgb3) and intellectual disabilities, including autism spectrum disorder. Global knockout of Itgb3 in mice leads to autistic-like behaviors, in which various social, anxiety, and grooming behaviors are altered. No current studies have revealed any specific cortical functions of Itgb3 that are relevant to autistic-like behaviors. Thus, the specificity of altered social behavior in conditional KO of Itgb3 in the forebrain is currently unknown. We hypothesize that Itgb3 is required in the cerebral cortex of mice for normal social behaviors. To test this hypothesis, we observed adult Itgb3 conditional KO and control mice in the 3-chamber sociability and social preference tests and performed control and non-social behavioral experiments. Our data suggest that cortical Itgb3 KO only have altered social behavior. By isolating a specific set of behaviors modified by conditional KO of Itgb3, we have uncovered a putative link between Itgb3 function, excitatory cortical circuitry, and social behavior.

Lacanin SC, Reed AT, Cisse FN, Birgbauer E  
Winthrop University  
Eric Birgbauer  
Investigating the Responsiveness of Embryonic Chick Retinal Ganglion Cells to Semaphorin-3A  
During development, neuronal axons are guided by a terminal structure, the growth cone, which respond to cues in the environment to grow over long distances to find their synaptic targets. When a growth cone encounters a repulsive cue, it collapses and grows in a different direction. One such axon guidance cue is Semaphorin-3A (Sema-3A). Luo et al. (1993) showed that Sema-3A causes growth cone collapse from embryonic chick dorsal root ganglion cells (DRGs) but not from chick retinal ganglion cells (RGCs). However, we have found that Sema-3A can cause growth cone collapse of chick RGCs. There are several possible hypotheses for why these 2 experiments differed. There could be a dose-dependent or age-dependent difference. There could also be a time-dependent difference, since the previous assay was for 60-minutes while we used a 15-minute assay. We have tested these hypotheses and found a dose-dependent growth cone collapse from chick RGCs at different developmental ages, although we still need to compare it with DRGs. Furthermore, the time response of RGCs to Sema-3A was investigated and found to peak after 15-minutes, and that they started regrowing at 20-minutes. This may explain the reason why Luo et al. had a different result at 60 minutes. We also found expression of Sema-3A receptors in the chick retina by RT-PCR. In conclusion, we have found evidence that Sema-3A induces a dose-dependent and developmentally relevant growth cone collapse in embryonic chick RGCs. Furthermore, embryonic chick RGC growth cones rapidly desensitize to Sema-3A.
Effects of Taurine on Zebrafish Development

Despite improvements in IVF, implantation rates remain disappointingly low with only ~21.3% of transferred embryos implanting. After successful IVF fertilization, half of the cultured embryos arrest during pre-implantation development. Thus, efforts have been focused on enhancing the culture environment to support increased developmental outcomes. Taurine, a non-essential amino acid, supports development of 2–4-cell stage human embryos to the blastocyst stage, but the mechanisms by which taurine augments early development is unknown. Zebrafish (Danio rerio) are a valuable model system for studying early vertebrate development. Fertilization is external; thus, zygotes can be collected and exposed to environmental agents, chemicals, or pharmaceuticals beginning at the 1-cell stage. Mitotic divisions of the blastomeres, morula compaction, and initiation of gastrulation can easily be viewed via light microscopy in the first six hours after fertilization. As our laboratory is interested in determining the mechanisms by which taurine may be protective in guiding blastomeric development, we first aim to verify that taurine supplementation is not toxic during the first couple of days of zebrafish development. Supported by literature in various animals, we hypothesize that exogenous taurine exposure will not be harmful to teleost development. Thus, zebrafish embryos were exposed to 100 mM, 50 mM, 30 mM, 10 mM, and 1 mM taurine at the 1-cell stage and analyzed at 6 hpf, 24 hpf, 48 hpf, and 72 hpf for alterations in gastrulation, mortality rates, coiling (spontaneous movement), teratogenicity, hatching from the chorionic egg sac, heart rate, escape responses, eye size, and overall body length.
Bhalerao, J 1; Kent, M 2; Bowen, G 1; Jackson, E 1; Meisel, E 1; Ploppert, E 1; Jacob, J 1; Olives-Navaarrete, R 3; Puetzer, J 3; Lambert, K 1

1 University of Richmond, Richmond VA USA; 2 Virginia Military Institute, Lexington VA USA; 3 Virginia Commonwealth University, Richmond VA USA

Dr. Kelly Lambert

The pervasive effects of early-life stress in male and female Long-Evans rats: Unpacking influences of postpartum restricted resources on social, emotional, neural, immunological, and developmental/physical outcomes

Early life stress associated with disrupted maternal care alters maternal responsiveness, resulting in negative impacts on offspring. The current study investigated the effects of probiotics in male and female rats exposed to early life stress (ELS; i.e., restricted nesting and bedding materials during the postpartum period) or standard postpartum conditions on various anxiety and immune functions (n=6/group; N=48). Following weaning, pups were given either probiotics (Lactobacillus; daily dose of 10^9 CFU in treated cereal squares) or vehicle control. In subsequent assessments to examine various behavioral responses, results indicated differential effects of probiotics such as an anxiolytic effect in standard housed rats that was absent in restricted animals. For social play, increased play bouts were observed in low resource animals. For immune assessment, wound healing was observed; a significant 3-way interaction indicated that, in the low resource animals, probiotic treated males had the slowest rate of healing whereas no effects of sex or probiotic treatment were observed in the standard resource group. Histological analyses are ongoing to determine the effects of early-life resources, sex, and probiotic treatment on microglia immunoreactivity and peripheral levels of the cytokine, IL-6. In a second cohort of animals with no probiotic exposure, physical parameters including tail length and collagen/bone architecture of the tail, foot, and femur were assessed. In accordance with past findings, the restricted resource animals exhibited compromised bone and cartilage tissue. Thus far, results suggest that probiotic intervention differentially affects males and females exposed to early life stress characterized by restricted resources.

Musa R, Matada H, Huber E

UNC- Chapel Hill

Monica M Gaudier-Diaz

Influence of Acute Social Stress on Emotional and Inflammatory Responses

The field of psychoneuroimmunology seeks to evaluate the bidirectional interaction between the nervous system and the immune system. In particular, stress has been shown to impact both psychological and physiological processes. Thus, the present study investigates the influence of acute social stress on emotional and inflammatory responses. Specifically, emotions and functional immunity were evaluated before and after exposure to the Trier Social Stress Test (TSST). Emotions were assessed with the PANAS Circumplex scale, which divides emotions into four discrete categories based on varying levels of positive and negative valence and arousal. Preliminary results suggest that positive valence/negative arousal emotions and negative valence/positive arousal emotions are influenced by acute social stress. Specifically, positive valence/negative arousal emotions decreased (p<0.01), whereas negative valence/positive arousal emotions increased (p<0.01). Preliminary analysis of functional immunity, which was assessed with a histamine scratch, suggests little-to-no effect of acute stress on immune reactivity (p-value>0.05). From these results, we conclude that acute stress can impact emotions, which supports the connection between social and psychological experiences. However, the effect of the TSST on the histamine scratch remains inconclusive.
Sahil Dhawan, Fredi Mino, Michelle Angeles, Kristen Willingham, Daniel Zeitlen, Michael Neelon
University of North Carolina at Asheville

Michael Neelon
The Effect of Valenced Priming Labels on Neural Processing of Ambiguous Sounds

This study seeks to find if there is a difference in neural processing when ambiguous sounds are positively or negatively labeled. Twenty-three sounds were taken from the International Affective Digitized Sounds database, then edited down to ~1s to enhance ambiguity. Labels for sounds were taken from the Affective Norms for English Words database. Fourteen sounds were labelled positively or negatively depending on the condition, and the remaining nine were labelled neutrally. On each trial, subjects were either presented with a positively-valenced, negatively-valenced or no label, then a fixation cross, immediately followed by an ambiguously-valenced or neutral sound. After the sound was presented, the subjects were asked to provide valence and arousal ratings. Subjects were randomly assigned to one of 3 groups: 1) positive labels for seven of the valenced sound clips and negative labels for the remaining valenced sound clips; 2) negative labels for the original seven sounds and positive labels for the remaining valenced sounds; or, 3) a control condition in which no labels were presented. All label-condition subjects also saw the same neutral labels for neutral sounds. Electroencephalography (EEG) data was recorded while subjects listened to and rated the sounds on valence and arousal. ERP analysis shows that labels of either valence create an enhancement of the N1 peak, suggesting an attention-related priming effect; however, there do not appear to be any other ERP differences due to positive or negative labels. Further analyses will look into whether valenced labels influences difference in spectral content or global field power.

Rebecca Reid, Madison Cox, Eric Henderson, Jesse Sargent
Francis Marion University

Dr. Jesse Sargent
Symmetry and the Visual Buffer

Spatial span (how many sequentially presented locations can be recalled in order) is greater if the locations together form a symmetrical pattern. Does this benefit manifest in the visual or the spatial component of visuo-spatial working memory? Participants viewed a 5x5 grid of squares on a computer screen. Three to seven of the squares lit sequentially for 1 s. each, then there was a 10 s. delay after which participants clicked on the remembered locations in order. The delay was filled with either visual (dynamic visual noise) or spatial (tapping) interference tasks. The memory advantage for symmetrical patterns was smaller after visual interference. Apparently, the relative imageability of symmetrical patterns manifests in the visual buffer and thus was disturbed more by visual than spatial interference. Within the visual buffer, integration over time of sequentially presented information may operate by grouping (e.g., Gestalt) principles similar to those acting in visual perception.
Dobbins ME, Cunnane K, Romero-Sandoval EA  
Wake Forest University School of Medicine  
E. Alfonso Romero-Sandoval  
Association of THC Content and Price in Herbal Cannabis Products by Dispensaries in California  

Background: 80% of medicinal cannabis users are pain patients. Tetrahydrocannabinol (THC), a main component of cannabis, reduces neuropathic pain at low potency (<15%) with limited side effects. However, we discovered high potency products (>15% THC) dominate the cannabis market, medical and recreational. We hypothesize that there is an association between THC content and price. We tested this hypothesis in CA since this was the first state to legalize medical cannabis in 1996. Methods: We randomly selected dispensaries across California and screened for a web presence, a product menu, and THC content for herbal products, price, type of product (flower or preroll), and “strain” (Sativa, Indica or Hybrid). Results: We observed that more potent products (>15% THC) were more prevalent and more expensive than low potency (<15% THC) products. This potency and price association remained in flower products but not in prerolls. Similarly, this association was found in Sativa, and at some extent in Indica, but not in hybrid products. Flower products have higher THC concentration than preroll products, however preroll were more expensive than flower products. We did not find differences in price or THC content among Sativa, Indica, and Hybrid products. Conclusions: High THC content is associated with higher prices in most herbal products, but some factors influence this association (i.e. preroll or Hybrid). Our data indicate that economic motives may influence, at least in part, the composition of medical cannabis market. This study offers evidence to influence policy and help ensure a safe marketplace for patients.

Williams J, Grider M  
High Point University  
Michael Grider  
Modeling Diabetic Neuropathy in Vitro and Investigating the Neuroprotective Role of CBD  

Diabetic neuropathy is a common complication in patients that are unable to control their blood glucose levels. Chronic elevated glucose in the bloodstream limits the oxygen supply to peripheral sensory neurons, leading to nerve damage that decreases sensation and may increase pain. Although results of clinical trials demonstrate the ability for CBD oil significantly decrease the inflammatory and neuropathic pain in patients, there is little evidence for mechanism of CBD-mediated changes directly on the sensory neurons. Here, we describe the effects of CBD on cell viability when we apply a high-glucose injury model to a cultured peripheral neuron cell line (PC12). Additionally, we aim to dissect the specific signaling contributions on diabetic neuropathy by subsequently introducing an injury associated with decreased oxygen levels.
Christina Abram, Dr. Shayna Wrighten  
Francis Marion University  
Dr. Shayna Wrighten  
Interpersonal Interactions : Examining Factors that Contribute to Implicit Racial Bias Among College Students  
Implicit bias, subconscious actions, thoughts or feelings that one has concerning a specific concept, can lead to discriminatory behaviors. Implicit racial bias can be derived from deep-rooted cultural cues and group associations with certain negative and positive behaviors. Despite the illegalization of overt racism, microaggressions and implicit racial bias against African-Americans has made little progress over the years; this can be seen in widespread preference for light skin over dark skin and white over black. Factors that may contribute to implicit racial bias are a combination of social constructs and institutionalized racism within the prefixes of society; these constructs have been the standard for hundreds of years. Most Americans, regardless of race, display a pro-White/anti-Black bias on the Implicit Association Test (IAT). Finding a way to mediate and ultimately eliminate these implicit racial biases is vital in the progression of equality in society. The goal of the current study was to examine implicit racial bias among college students at a diverse university. We found that contrary to the general population overwhelmingly showing bias against African Americans, most students in our sample showed no preference for White or Black individuals or preference for Black individuals. We will now take these findings and begin to tease apart which factors contribute to the decrease in racial bias in our sample.

Valeria Burchard  
Florida State University  
Julia Sheffler  
Exploring the Interaction between Early Life Adversity and Support Groups on Adult Cognitive Functioning  
Background: Early life adversity (ELA) has been associated with cognitive decline in old age and may accelerate aging processes (Lesuis et.al., 2018). There is growing literature regarding factors that are able to moderate and/or mediate the relationship between ELA and cognitive functioning. This project examines how the association between ELA and cognitive status may be buffered by attending a support group in adulthood. Methods: This study used the data from the first two waves of the National Survey of Midlife Development in the United States (MIDUS) which is a longitudinal study of health and well-being. Data from MIDUS I and MIDUS II, Project 3 (Cognitive Project), which included the Brief Test of Adult Cognition by Telephone (BTACT) composite score, was obtained from phone interviews and self-administered questionnaires. For all analyses, we controlled for age, income, and gender. Results: ELA was significantly associated with worse cognitive functioning. Attending a support group was associated with higher cognitive functioning. The interaction between ELA and attending a support group was not significantly associated with cognitive status. Conclusions: Attending a support group was associated with higher cognitive functioning, which has important implications suggesting that social interaction/assistance can protect against cognitive decline at a later age. Further research is needed to investigate the interaction because not everyone in our sample attended a support group.
Carolina S, Malek S, Michael J
George Mason University
Carolina Salvador-Morales

Engineering Atrazine Loaded Poly (lactic-co-glycolic Acid) Nanoparticles to Ameliorate Environmental Challenges

The use of herbicides plays a vital role in controlling weeds and conserving crops; however, its usage generates both environmental and economic problems. For example, herbicides pose a financial issue as farmers must apply large quantities to protect crops due to absorption rates of less than 0.1%. Therefore, there is a great need for the development of new methods to mitigate these issues. Here, we report for the first time the synthesis of poly(lactic-co-glycolic-acid) (PLGA) nanoherbicides loaded with atrazine as an active ingredient. We used potato plants as a biological model to assess the herbicidal activity of the engineered PLGA nanoherbicides. Our method produced nanoherbicides with an average size of 110 ± 10 nm prior to lyophilization. Fifty percent of the loaded atrazine in the PLGA matrix is released in 72 h. Furthermore, we performed Monte Carlo simulations to determine the chemical interaction among atrazine, PLGA, and the solvent system. One of the most significant outcomes of these simulations was to find the formation of a hydrogen bond of 1.9 Å between PLGA and atrazine, which makes this interaction very stable. Our in vitro findings showed that as atrazine concentration is increased in PLGA nanoparticles, potato plants undergo a significant decrease in stem length, root length, fresh weight, dry weight, and the number of leaves, with root length being the most affected. These experimental results suggest the herbicidal effectiveness of atrazine-loaded PLGA nanoherbicides in inhibiting the growth of the potato plant. Hence, we present the proof-of-concept for using PLGA nanoherbicides as an alternative method for inhibiting weed growth. Future studies will involve a deep understanding of the mechanism of plant–nanoherbicide interaction as well as the role of PLGA as a growth potentiator.
Fractalkine-Dependent Recruitment of Microglia to Emerging Multimodal Midbrain Maps

Microglial cells (MGCs) are highly dynamic and have been implicated in shaping discrete neural maps in several systems. MGCs respond to numerous cues in their microenvironment, including a neuronally-expressed chemokine, CX3CL1 (fractalkine). The present study examines microglial and CX3CL1 patterns with regard to the emerging modular-extramodular framework within the lateral cortex of the inferior colliculus (LCIC). The LCIC is a shell region of the auditory midbrain where discrete compartments receive modality-specific connections, whereby somatosensory inputs terminate within modules and auditory inputs target surrounding extramodular zones. A reliable modular marker, glutamic acid decarboxylase (GAD), enables visualization of emerging modular domains in the nascent mouse LCIC. Developing multimodal connectivity patterns interface with its neurochemically-defined patch-matrix-like organization. A developmental series of postnatal (P0, P4, P8, P12, P16) GAD67-GFP and CX3CR1-GFP mice were utilized to explore the potential involvement of MGCs and role of fractalkine signaling in establishing LCIC functional compartments. MGCs occupy the neonatal LCIC, with spatial heterogeneities and densities that change with age and respect to the modular-extramodular framework and its segregating multimodal afferent patterns. MGCs conspicuously border modular-extramodular boundaries prior to invading modular confines by P12. CX3CL1 labeling is clearly modular at P12, in keeping with the notion of fractalkine-mediated recruitment of microglia to modular centers. CX3CR1-GFP/GFP results suggest microglial movement into modules is delayed due to compromised fractalkine signaling. Ongoing experiments aim to further elucidate the role that MGCs and fractalkine signaling play in refining multisensory LCIC compartments during an early critical period.

Most fish cannot be out of water briefly, yet it is essential for other fishes to breathe air and maneuver on land. Kryptolebias marmoratus is a small, amphibious fish that moves on land by means of a tail-flip jump, which is a modified C-start for fish on land. C-start behaviors are driven by a motor pattern produced by a network of reticulospinal neurons. The axial muscle contracts on the side of the body opposite the threatening stimulus, bending the fish into the characteristic C shape, followed by a traveling wave of muscle contraction on the contralateral side that moves the fish away from the threat. The tail-flip on land observed in K. marmoratus is slower than the aquatic c-start response, because the muscle contraction duration is elongated. We are testing air acclimation on jumping performance. Physiological changes occur on land within 14 days, such as the formation of new blood vessels for gas exchange, so these changes likely confer some performance benefit. Forty fish were separated into four groups and were treated with 14 days in water, 28 days in water, 14 days on land to see if time spent in water changes their c-start behavior, and then the fourth group was placed 14 days on land followed by 14 days in water to see if the results were reversible. The experimental hypothesis is fish who have been acclimated to air will jump further and more often than fish that have been taken from the water and placed from land.
Nico Nettemeyer, Matthew Knick, Madison Ward, Wesley Yankey, Katie Matsen, and Dr. Susan Halsell

James Madison University
Dr. Susan Halsell

Cold Nociception in Drosophila melanogaster: Do Innexin Function in Peripheral Nociceptors?

Nociception refers to an organism’s reflexive response to harmful stimuli to minimize injury. This study investigates whether any of the eight Drosophila Innexin gap junction protein family members function in noxious cold peripheral nociceptors.

RNAi knockdown of innexin gene expression in peripheral dendritic arborization sensory neurons (da neurons) assesses their possible function in cold nociception. When available, loss-of-function innexin mutations are also examined. A cold-behavioral assay reveals the effect of the knocked-down expression of each innexin gene.

Wild type third instar larvae exhibit a characteristic “cringe” response when exposed to noxious cold. Inhibition of the cringe response indicates a defect in cold nociception. By comparing the change in percent cringe between the innexin knockdown larvae and appropriate experimental controls, the cold nociception function of each innexin gene can be inferred. At least 100 control and 100 experimental larvae are videotaped after being subjected to noxious cold. Image J video analysis allows quantification of the larval cringe responses. The Two-Tailed Fisher Exact test determines the statistical significance of any changes in cringing.

To date, all eight innexin genes have been knocked down in class III da neurons. It appears that four of the eight innexins may function in cold nociceptors; these include innexins 2 and 3, ogre, and surprisingly, zero population growth. An ogre loss of function mutation exhibits an even more significant inhibition of cringing.
Pferdmenges L, Hemerson M, Muhr J, Jiudice S, Rexha A, Ackerman K
High Point University

Kristin Ackerman
Dark-Adaptation Is Not Necessary to Induce Retinal Regeneration in the Adult Zebrafish
An estimated 12.4 million Americans are afflicted with neurodegenerative visual disorders, defined by a loss of retinal neurons. Danio rerio (zebrafish) don't demonstrate these retinal pathologies due to their innate capacity to regenerate neurons. Specifically, neuronal death initiates Müller glia (a resident stem cell in the retina) to dedifferentiate and divide, yielding neuronal progenitors that migrate to the site of damage to regenerate new neurons. While avian and mammalian Müller glia exhibit proliferation, they cannot regenerate significant numbers of neurons to restore vision. Therefore, investigating the mechanisms which drive robust regeneration within zebrafish is integral to understanding/developing potential therapeutics for visual diseases.

Multiple retinal damage models exist to induce regeneration (genetic manipulation, physical nerve crush, pharmacological exixto-toxicity, and light-damage). Light-damage is a model utilized to specifically damage rod and cone photoreceptors while maintaining the integrity of all other retinal cells. Traditionally light-damage paradigms from goldfish and zebrafish utilize a two week dark adaption prior to exposing the fish to constant intense light. It is thought that the switch from chronic dark to intense light initiates photoreceptor cell death which turns on the regenerative machinery. Our laboratory has hypothesized that dark adaptation is not necessary for initiating the regenerative response in the adult zebrafish retina. Taking an immunohistochemical-based approach in both dark adapted and non-dark adapted fish, we have quantitatively monitored retinal health/cell death (DAPI), changes in rod and cone photoreceptors (transgenic lines and DAPI), and the proliferation of Müller glia-derived neuronal progenitors to replace damage/dying cells (PCNA).

James Madison University

Jeff Dyche
Rodent Psychomotor Vigilance Task Performance Following Chronic Sleep Restriction: Possible Strain Differences?
Chronic sleep restriction has been correlated with various cognitive impairments including detriments to sustained attention measured via the psychomotor vigilance test (PVT). Individual differences in response to sleep restriction have been observed in human subjects, including individual differences in the effects of sleep loss on PVT performance. Possible differences in response to chronic sleep restriction between different rat strains have not been reported. In recent years a rodent model of the PVT was developed called the rPVT. The purpose of this study was to compare sustained attention and the effects of chronic sleep restriction in Wistar Han and Sprague Dawley rats, which are two commonly used strains in behavioral and sleep research. After meeting baseline rPVT criterion, rats were subjected to 6hr/day sleep deprivation using slow moving forced exercise wheels for one week. Results found Wistar Han rats show more prominent impairments in sustained attention following sleep restriction compared to Sprague Dawley rats. Wistar Han rats show an increase in reaction time and a decrease in accuracy. Sprague Dawley rats show little to no change in performance on the rPVT following sleep restriction. Sprague Dawley rats may be more resilient to impairments of sustained attention due to sleep restriction compared to Wistar Han rats. The differences in performance found between the strains may have implications on choice of strain in behavioral research and tasks following sleep restriction, as well as the potential genetic differences underlying sleep loss resiliency.
Ruggeri M, Simpson B, Cleland, CL
James Madison University
Corey Cleland

Influence of Stimulus Location on the Nociceptive Withdrawal Response in the Rat

Nociceptors are responsible for signaling that tissue is at risk for damage. The nociceptive withdrawal response (NWR) is a complex movement used to escape the noxious stimulus. Previous studies support the idea that the location of stimuli affects the withdrawal response in spinalized or anesthetized mammals. Nevertheless, it is unknown if and how the NWR depends on stimulus location in an intact, unanesthetized non-human mammal. The goal of our experiment was to determine how stimulating different locations on the plantar surface and leg of the intact, unanesthetized rat affects the direction and trajectory of the NWR. The caudal left quadrant of male Sprague-Dawley rats were shaved and marked with 3mm diameter circles on the middle toe, fifth metatarsal, lateral malleolus, knee, greater trochanter, and iliac crest to track the changes in joint angle. On the bottom of the foot, five 2mm diameter circles were drawn to target laser heat stimulation. A 980 nm laser diode was used as a source of heat stimulation. The change in position of the left foot was tracked and analyzed. Preliminary findings demonstrate three separate components of the withdrawal response: an initial upward lift of the body, a rapid flexion withdrawal of the foot, and a rapid foot replacement on the surface. Unexpectedly, the rapid flexion withdrawal did not depend on stimulus location; however, replacement of the foot did depend on the initial replacement of the foot. These results suggest that the NWR in intact, unanesthetized rats differs from spinalized rats.

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Corey Cleland

Dependence of the Nociceptive Withdrawal Response on the Location of Electrical Stimulation in the Intact, Unanesthetized Rat

Nociceptors are activated by noxious stimuli that have the potential to cause tissue damage. The response evoked by noxious stimuli, known as the nociceptive withdrawal response (NWR), is evolutionarily advantageous because it allows animals to avoid harmful conditions. Our lab previously studied the NWR in intact rats using heat stimulation, which revealed a 3-phase movement beginning with an initial push up of the leg followed by flexion and extension. However, the effect of electrical stimulus on the NWR has not been demonstrated in an intact non-human mammal model. Evoking a response using electrical stimulation in non-human mammals provides beneficial insight because this mode is commonly used clinically in humans. Our research goal was to determine if non-invasive electrical stimuli administered to the plantar aspect of the paw in an unanesthetized, spinaly intact rat influences the trajectory of leg withdrawal. Unanesthetized rats were placed on a sheet of wire mesh and an electrode was applied directly to the plantar aspect of the rat hind paw in five different stimulus locations. The stimuli were administered at 2x threshold in a brief pulse train of 5 pulses. The NWR was video recorded at 500 fps and tracked using software. Our results, in contrast to heat stimuli, show a lack of initial slow extension preceding the rapid flexion withdrawal. Similar, however, was that the rapid flexion did not depend on stimulus location but the final replacement of the foot did depend on initial posture. Our results provide insight into the spatial organization of NWR.
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Onarae Rice
The Effects of Simultaneous Chronic Methylphenidate and Fluoxetine Usage on Bone Health
Methylphenidate (MP) is the most commonly prescribed pharmacotherapy for adolescents with Attention Deficit Hyperactivity Disorder (ADHD). Likewise, fluoxetine (FLX) is the most commonly prescribed Major Depressive Disorder (MDD) medication. Both drugs have been shown independently to cause significant bone mineral density (BMD) loss leading to height growth reduction in adolescents. Despite MP and FLX’s high co-prescription rate, no study has been conducted to examine BMD loss induced by the MP-FLX interaction. The purpose of the present study is to measure the changes in BMD and osteoclast activity caused by MP and FLX consumption in rats. Adolescent male rats were treated orally with either MP, FLX, or a MP + FLX combination and control rats were given water. Behavioral tests were run at the beginning and end of the treatment window to measure changes in depressive- and anxiety-like symptoms. At the end of the 28-day treatment, the animals were sacrificed and their femora and tibiae were extracted and analyzed. Behavioral results showed that the MP + FLX combination synergistically reduced both anxiety and depressive behaviors in rats. Additionally, the average end body weight of the combined group was markedly lower than all other groups. Bone quality data was inconclusive. These findings support the hypothesis that MP and FLX interact to synergistically reduce growth, potentially caused by BMD loss. Bone quality and osteoclast activity will be analyzed to confirm this mechanism.

Irisyunuel Lopez Hernandes
National Institute of Environmental Health Sciences
Jean Harry, PhD.
Exposure to Emamactin Benzoate and Ozoxystrobin induce inflammasome activation in microglia
There is a growing recognition of transcriptional changes in the brain associated with autism spectrum disorders (ASD) including biological processes such as synaptic transmission and immune function. Environmental exposure may contribute to the risk for ASD. Using analysis of brain transcriptional signatures, prior studies identified a cluster of 22 environmental chemicals out of 297 generated a transcriptional signature similar to ASD and altered immune cell function in isolated rodent cortical neurons Pearson (2016). Given the representation of immune cell function in the published transcriptional signature and the paucity of such cells in the neuronal cultures examined for environmental exposures. Microglia are the resident immune cells of the central nervous system (CNS) serving a critical role in both CNS homeostasis and disease. The current study examined the ability of this library of 12 environmental chemicals from the Pearson (2016) study to affect microglia immune cell functions at the dose levels employed in previous studies. The murine BV-2 microglia NLRP3 reporter cell line was examined for effects of these chemicals on function as a secondary stimuli for NLRP3 inflammasome activation. Two out of the twelve compounds Emamactin Benzoate and Ozoxystrobin induced inflammasome activation. mRNA expression for pro-inflammatory cytokines was also measured. Data from this suggest the ability for environmental exposure to alter microglia cell immune response in BV2 cells.
Holley ZL, Bland KM, Swinehart BD, Lopuch AJ, Widener EL, Song MI, Casey ZO, Handwerk CJ, Vidal GS
James Madison University
George Vidal
Specific roles for integrin beta 3 in dendritic development in layer II/III neurons of cerebral cortex
Dysregulation of dendritic branching and dendritic spine type or number in excitatory neurons of the cerebral cortex may lead to neurological disorders such as intellectual disability and autism spectrum disorder. Integrin subunits have been implicated in axonal and dendritic outgrowth, as well as dendritic spine plasticity. In particular, a strong 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, we use an approach that targets layer II/III cortical pyramidal neurons to either label them (control) or to label them and cause Itgb3 loss of function (mutants). When compared to controls, dendritic length is increased in Itgb3 mutant neurons surrounded by WT neurons. Itgb3 mutant cortical pyramidal neurons have increased dendritic length while dendritic spine density is unaltered, suggesting that Itgb3 KO neurons have more total excitatory synapses and dendritic length compared to controls. This altered level of connectivity could change excitatory-inhibitory coordination in cortex, leading to intellectual disability.

Leftwich T, Chambers S, Rice J, Walker MT Ph.D.
James Madison University
Marquis Walker, PhD.
UNCOVERING NOVEL PATHWAYS IN LIGHT-DEPENDENT SIGNALING FOR PUPIL CONSTRICION IN MAMMALS
In mammals, all light detection occurs in the photosensitive retinal tissue of the eye. Photoreceptor cells within the retina provide the visual signaling input necessary for image forming and non-image forming behaviors. Pupillary light reflex (PLR) is an involuntary non-image forming behavior in which iris muscle in the eye constricts in response to increasing ambient light intensity. All retinal photoreceptor classes contribute light-activated signals to drive the PLR behavior, but these signals are exclusively carried from the retina to the olivary pretectal nucleus (OPN) of the brain by intrinsically photosensitive retinal ganglion cells (ipRGCs). Neurons in the OPN control the signaling input that initiates constriction of the iris muscle. Recently it has been demonstrated that the iris muscle can respond directly to light and will constrict independent of neurological signals from the OPN. Light signaling in the iris sphincter muscle requires the photopigment melanopsin. Our laboratory has been able to isolate iris tissue in culture and maintain light dependent constriction in the tissue. What these responses suggest is that there is a novel photosensitive pathway independent of signaling from the OPN that is also able to activate iris constriction. It remains unclear if light signaling for this novel pathway is initiated in the retina or in the iris muscle. Our preliminary results suggest that light responses driving intrinsic constriction in the iris result from photosensitive efferents which originate in the retina.
### Retinoic Acid Receptor Function in hindbrain and spinal cord specification

Tissue alignment during embryogenesis is controlled by cell communication signals. Retinoic acid (RA) from the mesoderm is an essential signal to align the hindbrain and spinal cord to occipital and cervical somites. While RA represses the spinal cord specification genes (Cdx) in the hindbrain, RA does not do this in the prospective spinal cord territory. A survey of the literature has revealed differential transcription of RA receptors (RAR) in the neural tube, raising the possibility that different RARs mediate the different functions of RA in the hindbrain and spinal cord. To elucidate the function of each receptor in cell specification, we used pharmacological reagents to inhibit RAR-alpha exclusively. First, we characterized the effect that different RAR-alpha-specific inhibitors have on hindbrain and spinal cord cell populations. We then showed that different RAR-alpha inhibitors cause similar defects, suggesting that the inhibitors are RAR-alpha specific. Finally, we showed that inhibition of RARα cause hindbrain and spinal cord defects that are a subset of the defects caused using a pan-RA inhibitor. Together, our results suggest that RAR-alpha has specific functions in preventing spinal motor neuron development in the caudal hindbrain. Future work involves comparing these phenotypes with morphological effects caused by CRISPR-induced RAR-alpha deletion mutants and understanding the role of RAR-gamma in spinal cord and hindbrain development. Funding is supported by the University of Richmond College of Arts and Sciences and the National Science Foundation (NSF-1051755386).

### Perceived Stress and Sense of Belongingness in First-Generation College Students

Generation in college associates with academic performance. First-generation college students tend to obtain lower grades relative to their continuing-generation counterparts, but the underlying causes of this remain poorly understood. To examine perceived stress and sense of belongingness as potential causes, we are having college students complete a survey including demographic measures and the perceived stress, social assurance, and social connectedness scales. Preliminary analysis suggests that none of the psychosocial factors evaluated vary by generation in college. However, it is worth noting that the perceived stress mean score is higher among first-generation college students, a trend that might become significant once we reach our target sample size.

Previous studies demonstrate that first-generation college students are more vulnerable to stress and more likely to feel like they do not belong in the college environment. Moreover, it is well-established that increased stress and a lack of sense of belonging are both predictive of poor academic performance. Thus, it is essential to further investigate the relationship between generation in college and these psychosocial factors. With the large population of first-generation students entering higher education, it is becoming increasingly important to ease their transition and acclimation to the college setting.
Catalan V, Joseph S, Russell E  
UNC- Chapel Hill  
Monica M Gaudier-Diaz  

Effects of Exam Stress on Working Memory  

Stress can negatively impact academic performance. To further explore this association, the present study examines the influence of exam stress on working memory. For this, we are having participants visit our laboratory for a baseline session on a week in which they do not have any exams and for a stress session on a day in which they have an exam for their hardest class. During each session, participants complete the digits-backwards and Stroop tasks, cognitive tasks designed to assess working memory. Preliminary analysis suggests that exam stress does not alter working memory, as we found no differences when comparing performance between the baseline and stress for either of the tasks. As this is an ongoing study, our null findings could be due to a small sample size (n=14). Therefore, we anticipate that a greater sample size will potentiate the relationship between working memory and academic stress at the conclusion of the study. If stress negatively impacts cognition, on the day of a stressful exam students will perform worst on the tasks. In addition to examining the effects of exam stress on working memory, we are investigating psychological and physiological factors that might mediate the interaction between stress and cognition, in the hopes of identifying targets of intervention to attenuate the stress effects on academic performance.

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Medical College of South Carolina  
Dr. Jim Otis  

Designing a Behavioral Paradigm That Allows Large-Scale Recordings of Cellular Activity During Drug Seeking Behavior  

Opioid use disorder (OUD) is a major epidemic within the United States. Patients suffering from OUD are more likely to relapse to compulsive opioid seeking and taking when presented with drug-associated environmental cues, despite the risk of facing significant negative consequences. Despite knowing this, how opioid-associated cues engage brain reward circuits for the control of drug-seeking behaviors is unclear. To study this phenomenon, it is necessary to develop novel neurotechnologies which allow us to visualize neural activity during drug seeking behaviors. We designed a state-of-the-art head-fixed heroin self-administration paradigm, which allows in vivo two-photon imaging during heroin-seeking behaviors. Within this model, mice are placed within a head-fixation apparatus and presented with two levers; upon pressing the ‘active lever’ mice will receive an infusion of heroin and an auditory cue, whereas pressing the ‘inactive lever’ will have no effect. We have found that, despite being head-fixed, mice will acquire lever pressing for heroin and will associate the active lever with a heroin infusion and heroin-associated cue. Additionally, we have discovered that mice will extinguish heroin-taking behaviors when pressing either lever (active or inactive) does not have an effect. Lastly, we have demonstrated that mice will reestablish their heroin-seeking behaviors when presented with heroin-associated cues. Overall, using this novel head-fixed heroin self-administration paradigm will allow us to view neuronal activity during heroin-seeking behaviors. Having a better understanding of how neuronal circuits are modulated by continued heroin use and exposure to heroin-associated cues may give insight into potential therapeutics for the treatment of OUD.
Garris R, Henken H  
University of North Carolina Asheville  
Angel Kaur  
Effects of ALS-associated mutation in Fused in Sarcoma on cell viability, mRNA and protein expression in the HEK-293 cell line.

Amyotrophic Lateral Sclerosis (ALS), a progressive neurodegenerative disease primarily affecting motor neurons, is characterized by an 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. Cytoplasmic aggregates of FUS appear to cause cell toxicity, however, the mechanism by which this occurs remain largely unknown. Mutations in FUS, a nuclear RNA/DNA binding protein, are specifically associated with familial ALS, with mutations in the non-classical nuclear localization signal of the C-terminus associated with aggressive, juvenile-onset variants of the disease. Overexpression of wild-type FUS has been shown to result in aggregation and cell death. To avoid toxicity by overexpression, Promega’s Flexi Vector System, a two plasmid system that allows for tuning of protein expression via varying concentrations of the antibiotic coumycin, was utilized. Wild-type FUS and the P525L mutation were transfected into the HEK-293 cell line for analysis of structure and viability. Furthermore, the lysate of these cells were collected for analysis of changes in protein and RNA expression. This two vector system has not previously been used to study the effects of FUS mutations on cell structure, function, or viability, and the findings of this experiment provides insight not only into the effectiveness of this system but also expands the current understanding of the molecular mechanisms behind FUS mutation driven neurodegeneration in ALS.

Leonard L, Lavach H, Waters RP  
University of Mary Washington  
Parrish Waters  
Chronic exercise and social enrichment influence stress related physiology and behavior

While both physical exercise and social enrichment acutely increase levels of stress ligands, chronic application of these stimuli decrease stress levels and improve cognition and mood in mice. In this experiment we directly measured the influence of these stressors on mouse behavior and determined changes in neural systems that are related to these behavioral effects. We measured short-term and long-term spatial memory using the Barnes Maze and Novel Object Recognition test, and anxiety using the Elevated Plus Maze. We measured central levels of BDNF via qRT-PCR and ELISA assays and focused on the hippocampus and pre-frontal cortex, brain regions that are involved in these behavioral traits. We will also measure levels of corticosterone, a primary stress hormone, using in samples of trunk blood from all mice.
Alvarado RA
Longwood University
Dr. Catherine Franssen

Community-Based Social Marketing, the Environment, and Neuroscience

Single use plastics, such as balloons and straws, are dangerous to sea life if not properly discarded. A partnership with Clean Virginia Waterways, a non-profit organization focused on litter prevention, has suggested that social marketing may offer a solution to the difficult task of preventing littering behavior. An active Community-Based Social Marketing Campaign (CBSMC) targets a single, specific behavior that is directly correlated to environmental harm, like the release of balloons or the general overuse of single-use-straws, and works to test messages, programs, and trends that function as social influences for shaping an individual's specific behavior away from one that is environmentally harmful. The aim of this project is to explore how the application of behavioral and social neuroscience topics, such as decision making and social behavior, can support a sustainable behavior change focused on a key environmental issue and assist CBSMC’s in achieving their goals.

Kellar, H., Kruse, A., Henderson, E., Sargent, J.
Francis Marion University
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NEURAL RESPONSE TO SYMMETRY DOES NOT TRACK MEMORY ADVANTAGE

Visuo-Spatial Working Memory (VSWM) is the cognitive system humans use to maintain and manipulate location and visual information over short periods of time. Visual stimuli that are symmetrical are easier to remember (symmetry effect). Symmetrical stimuli are associated with a specific neural response (sustained posterior negativity: SPN) observed using electroencephalography (EEG). The goal of this study is to establish a relationship between the memory benefit for symmetrical patterns (symmetry effect) and the neural response associated with perceiving symmetrical patterns (SPN). Twenty one undergraduate participants performed a VSWM task on a PC while EEG activity was recorded. On each trial, six yellow squares chosen from an invisible 6 x 6 grid lit up simultaneously for 3 s, after which the grid appeared and participants used a mouse to click on the squares that had been lit. On half (60) trials the lit squares were selected randomly and on the other half the lit squares made a pattern that was symmetrical about the vertical midline. Both the symmetry effect and the SPN were observed but the size of the symmetry effect did not correlate with the size of the SPN across participants. Whatever cognitive process is involved in the memory benefit for symmetrical patterns, it does not appear to be related to the automatic neural response to symmetry indexed by the SPN.

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