Faculty for Undergraduate Neuroscience
Agenda and Abstract Book

Sunday, November 4, 6:45-8:45 pm
Marriott Marquis Grand Ballroom
Welcome to the 2018 FUN Poster Session and Social – an event that embodies the mission of FUN to facilitate and celebrate undergraduate neuroscience research and education.

This year we have 168 posters being presented from over 95 institutions. We are tremendously thankful for the support of the Society for Neuroscience. In addition, we thank those institutions, organizations, and companies that have sponsored student travel awards. Their commitment to undergraduate neuroscience research and education is greatly appreciated!

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Agenda for the FUN Social and Poster Session
6:45-8:00 pm: Poster presentations
8:15-8:45 pm: Awards ceremony
Opening remarks, Leah Chase (Past-President)
Announcement of new officers, Ronald Bayline (President-Elect)
Travel Award winners recognized, Hewlet McFarlane (President)
Brain Awareness award winner recognized, Hewlet McFarlane (President)
FUN Faculty Awards, Leah Chase
  Mentor Award
  Service Award
Recognition of the Out-Going President, Leah Chase
Closing Remarks, Ronald Bayline

FUN Faculty Awards Committee:
Leah Chase (Hope College), Past-President
Hewlet McFarlane, Chair (Kenyon College), President
Shelly Dickinson (St. Olaf College), Councilor

FUN Social Organizing Committee:
Leah A. Chase (Hope College), Past-President
Shelly Dickinson (St. Olaf College), Councilor
Alexis Martin (Society for Neuroscience), Meeting Programs Assistant

FUN Student Travel Awards Committee:
CHAIR: President-Elect: Ronald Bayline (Washington and Jefferson), Arseny Khakhalin (Bard College), Gary Muir (St. Olaf College), Jennifer Honeycutt (Northeastern University), Anthony Kline (University of Pittsburgh School of Medicine), Christina McKittrick (Drew University), Lora Becker (University of Evansville), Leah Chase (Hope College), Carolyn Pytte (Queens College), Jacqueline Morris (Baldwin Wallace University), Anthony Rauhut (Dickinson College), Sarah Holstein (Lycoming College), Tyisha Williams (Wilkes University)
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PHYSICAL AND FUNCTIONAL INTERACTION BETWEEN GAMMA-PROTOCADHERINS AND NEUROLIGIN-2 IN THE DEVELOPMENT OF INHIBITORY SYNAPSES

Steffen, D.; Marcucci, C.; Molumby, M.; Weiner, J.
Department of Biology and Iowa Neuroscience Institute, University of Iowa

The mammalian Pcdha, Pcdhb, and Pcdhg gene clusters encode a diverse group of cadherin superfamily adhesion molecules, the α-, β-, and γ-Protocadherins, respectively. The 22 γ-Protocadherins (γ-Pcdhs) are combinatorially expressed in the brain, and play critical roles in synaptogenesis, dendrite arborization and patterning, and the survival of subsets of neurons in vivo. The γ-Pcdhs can interact promiscuously with each other, and with other clustered Pcdhs, in cis, but interact strictly homophilically in trans. Mice lacking the γ-Pcdhs in the cerebral cortex in vivo exhibit severely reduced dendrite arborization (Garrett, et al., Neuron, 2012). Recently, we further demonstrated that these cortical mutants exhibit a significant increase in the density of dendritic spines and excitatory synapses, and found that the γ-Pcdhs physically interact in cis with neuroligin-1, a postsynaptic adhesion molecule implicated in autism and schizophrenia that is important for the maturation of excitatory synapses. In an “artificial synapse assay” in vitro, γ-Pcdhs could inhibit the ability of neuroligin-1 to induce presynaptic differentiation (Molumby et al., Cell Reports, 2017). While this study identified a new mechanism through which γ-Pcdhs regulate excitatory synapses, their effect on inhibitory synapse development has not yet been examined. Here, we provide evidence that the γ-Pcdhs can also negatively regulate inhibitory synapse development in forebrain neurons. Using co-immunoprecipitation assays, we find that γ-Pcdhs interact both in vitro and in vivo with neuroligin-2, which is found at inhibitory postsynaptic sites and promotes inhibitory synapse maturation. Utilizing the artificial synapse assay, we find that multiple γ-Pcdhs can, when co-expressed in COS cells, strongly inhibit the ability of neuroligin-2 to promote presynaptic clustering of synaptic vesicle proteins synapsin and VGAT in contacting axons. To ask whether the γ-Pcdhs negatively regulate inhibitory synapse density in vivo, we analyzed mice in which a conditional Pcdhg allele has been excised in excitatory cortical neurons (the postsynaptic sites of many inhibitory synapses) using Emx1-Cre. Using immunostaining for synaptic markers, we find that inhibitory synapse density is, indeed, significantly increased in the absence of γ-Pcdhs. Together, these data suggest that γ-Pcdhs interact with neuroligin-2 in cis at inhibitory postsynaptic sites to negatively regulate the formation and/or maturation of inhibitory synapses.

Theme: Development
The size of hippocampal place fields progressively increases from the dorsal to ventral region of the hippocampus (Jung et al., 1994; Maurer et al., 2005; Kjelstrup et al., 2008), leading to the hypothesis that there is a spatial gradient along the long axis of the hippocampus. Small place fields in the dorsal hippocampus are associated with spatial representation while broader fields in the ventral hippocampus facilitate emotional and homeostatic processing (Bannerman et al., 2004). Although the dorsal-ventral axis of transcription is differentiated at birth (O’Reilly et al., 2015), place cells and spatial selectivity do not appear for approximately 2 more weeks. Therefore, to understand the developmental trajectory of the spatial selectivity along the long-axis of the hippocampus, we characterized the expression of the activity-regulated immediate early gene, Arc, at ages P14 to P21. Pups underwent a two-epoch object exploration task, in which the position of two out of the four objects are swapped (Ramsaran, Westbrook, & Stanton, 2016). This design provides the capability to assess spatial selectivity as well as any potential correlates to object-place selectivity. Our preliminary data demonstrates that at P14, there is no significant difference in Arc activity between the dorsal and ventral regions of CA1, however by P21 a gradient is evident. In addition, this gradient is a result of increased expression of Arc in the dorsal region, as the activity in the ventral region remains relatively stable. Ongoing experimentation will seek to focus on the development of the spatial gradient in the CA3 region in addition to the previously studied CA1.

Theme: Development
CHARACTERIZATION OF A NEW MOUSE MODEL OF SUPRATENTORIAL EPENDYMOMA BRAIN TUMOR

Randazzo, Ericka, Dunrack, Jesse, Fang, Justin, & LoTurco, Joseph
Department of Physiology and Neurobiology, University of Connecticut

Ependymomas are a class of brain tumor characterized by neoplasms containing a mixture of abnormal glial and endothelial cells. The third most common brain tumor in children, ependymomas can be divided into several subclassifications based upon their location, and molecular genetic features. An important subclass of these tumors are supratentorial ependymomas (ST-EPN), which form in either the third or lateral ventricles and have been found to be significantly correlated with a novel somatic genetic fusion between the C11orf95 and RELA genes (FUS1). Here, we present evidence that cellular transgenesis of FUS1 combined with somatic mutagenesis of TP53 by CRISPR/Cas9 in fetal forebrain neural progenitors at the surface of the fetal ventricular zone is sufficient to cause lethal tumors in mice that resemble C11orf95-RELA+ ST-EPN in humans. RNA-seq analysis of tumors in mice showed an upregulation of genes in the NF-κB pathway, consistent with increased RELA function, but also in genes not in the NF-κB pathway and associated with cell adhesion, growth factor, and NOTCH signaling pathways. We will conduct CUT & RUN ChIP-seq experiments to test the hypothesis that the FUS1 protein binds to the promoter regions of regulated genes in these tumors. In addition, by culturing cells isolated from induced tumors in mice we were able to establish an immortalized novel murine ependymoma cell line which should prove useful in screens for agents that might decrease ependymomal cell proliferation. We tested three anti-tumor drugs on the growth of these cells in culture and found that the BET bromodomain inhibitor JQ-1 is sufficient to decrease cell proliferation in a dose-dependent manner. Together, these data are consistent with FUS1 as a major oncogenic driver in ST-EPN, and establish novel in vivo and cell culture systems to investigate the mechanisms by which FUS1 transforms neural progenitors into ST-EPN tumor stem cells.

Theme: Development
A NOVEL ROLE FOR NOTCH IN MECHANOSENSORY NEURON CONNECTIVITY IN C. ELEGANS

Richard Guerrero, Quin Brown, and Rachid El Bejjani
Biology department, Davidson College, Davidson, NC

Notch receptors are conserved transmembrane proteins that regulate key developmental processes and promote stem cell proliferation and renewal. Notch signaling remains active in the nervous system from birth to adulthood. Notch pathway genes are highly conserved across many species, including humans, and extensive work to decipher Notch signaling was initially done in Caenorhabditis elegans. While these animals’ mechanosensory neurons have been well characterized behaviorally, little is known about the late stage development of the connections between them. We show that animals lacking the Notch metalloprotease sup-17/ADAM10 have significantly higher rates of the ALM-AVM nerve ring not forming compared to wild type animals. Significantly higher rates of improper development were also found in Notch receptor and gamma-secretase complex mutant animals, confirming that the Notch pathway is likely involved in ALM-AVM connection at the nerve ring. Notch mutants did not exhibit different opening rates across life stages. This suggests that the defect is likely to be developmental rather than degenerative or the result of a developmental delay. To ask if Notch functions cell-autonomously, we are currently performing tissue specific rescue experiments for both the mechanosensory neurons, the surrounding glia, and muscle arms. We are also working on a sup-17 rescue using CRISPR to determine if its reinsertion will result in normal nerve ring development.

Theme: Development
EXPLORING THE RELATIONSHIP BETWEEN THE INNATE IMMUNE SYSTEM AND ADULT NEUROGENESIS IN THE BRAINS OF PROCAMBARID CRAYFISH

Dugar A, Kelley V, Beltz BS
Neuroscience Program, Wellesley College, Wellesley MA 02481

Adult neurogenesis, the production of new neurons in the adult brain, is a highly conserved phenomenon that occurs in both vertebrates and invertebrates. We choose to study this process in the crayfish *Procambarus clarkii* because they are a relatively simple model organism with quantifiable neuronal numbers and a clearly defined lineage of neural precursor cells. It is often thought that mammalian neuronal stem cells are self-renewing, however studies in crayfish have suggested that neural precursors lack this ability and are instead replenished by an extrinsic source. Recent *in vitro* and *in vivo* studies in crayfish suggest that blood cells originating in the immune system—specifically semi-granular hemocytes—replenish neural precursor cells in the neurogenic niche and can become functional neurons that innervate olfactory and higher order processes. In order to investigate the intimate relationship between the immune system and adult neurogenesis, this project aims to (1) test the relationships among the size of the neurogenic niche size, total hemocyte counts, and crayfish body size, (2) identify the specific immune tissue from which neural precursor cells are released and isolate the factors that enhance the attraction of immune cells to the niche, and (3) elucidate the molecular mechanisms underlying differentiation of adult-born neurons in crayfish. Insight into the molecular mechanisms involved in adult neurogenesis and may lead to better treatments for a variety of neural diseases including stroke, depression, and Alzheimer’s disease.

Theme: Development
IDENTIFICATION OF PSD-95 AS AN INTRACELLULAR BINDING PARTNER OF THE SYNAPTIC ADHESION PROTEIN SLITRK2

Ursinus College Biology Department and Neuroscience Program

Slitrks are a family of leucine-rich repeat containing transmembrane proteins that promote synaptogenesis in the developing nervous system. Slitrks localize to the postsynaptic density, where they induce synapse formation via trans-synaptic interactions with the LAR family of receptor protein tyrosine phosphatases. While trans-synaptic binding partners of Slitrks have been reported, little is known about the intracellular proteins that interact with Slitrk’s cytoplasmic domain. Here we report an interaction between Slitrk2 and the synaptic scaffold PSD-95, as shown by co-immunoprecipitation from postnatal mouse brain. Mapping analysis in yeast demonstrates that the PDZ and SH3 domains of PSD-95 are required for the interaction. We also show that PSD-95 and Slitrk2 co-localize at neuronal synapses, and PSD-95 induces robust clustering of Slitrk2 in 293T cells. Future studies will examine the functional significance of interaction between PSD-95 and Slitrk2. PSD-95 may mediate localization of Slitrks to synaptic sites and/or recruit additional intracellular signaling molecules involved in postsynaptic differentiation.

Theme: Development
Caffeine and taurine are common ingredients listed on the nutritional label of energy drinks. However, in many drinks, these compounds are marketed as a “specialized blends”. The consumption of energy drinks has increased in recent years, yet there is little research on neural morphology and the dosages of ingredients in these “specialized blends”. The present study investigated the relationship of varying dosages of caffeine and taurine on primary rat cortical neurons by measuring the neuronal outgrowth of primary, secondary, and tertiary neurites within each treatment and concentration condition. This evaluation included seven treatments (excluding the control group) of both caffeine (100 μM -1mM), and taurine (50 μM -2mM), cultured over two weeks. Neurons were imaged using phase-contrast microscopy and analyzed using NeuronJ. A One-way ANOVA and Factorial ANOVA were employed. Data collected was used to establish a dose-response curve for each agent. Caffeine displayed a biphasic curve inferring two mechanisms working on neurite regulation, and taurine displayed a U-shaped curve. An interaction between neurite type and concentration on neurite outgrowth was apparent [F(12, 5901) = 3.34, p = .005, η2 = .007], suggesting concentration impacts the frequency of the types of neurons, which ultimately contributes to overall neuron length. Further, main effects for treatment (p <0.001), concentration (p <0.001), and neurite type (p <0.001) on neurite outgrowth were revealed and will be discussed.

Theme: Development
DECODING DYSLEXIA

Gabel, L.1, Johnson, E.2, Truong, D.3, Murray, E.1, Esch, K.1, Voss, K.1, Grigaux, O.1, Paniagua, S.3, Gruen, J.2
1) Lafayette College, Easton, PA 2) Bosie State University, Bosie, ID 3) Yale University, New Haven, CT

Reading disorder (RD, a.k.a Dyslexia) is a specific learning disability that affects reading. If learning disabilities remain untreated, a child may experience long term social and emotional problems which influence future success in all aspects of their lives. Early detection and intervention will help close the gap between typically developing and reading impaired children in acquiring reading skills. The complete explanation of a complex neurodevelopmental disorder requires and understanding across multiple levels, including, but not limited to, cognition, behavior, and genetics. Although our understanding and treatment for dyslexia has greatly increased in the last 20 years, a significant percentage of children with dyslexia are either identified too late or have a specific manifestation of the disorder that is not understood well enough to design and deliver a successful remediation. This outcome is exacerbated when the child is non-native speaker. Research examining the connection among genetic, cognitive, and behavioral aspects of reading disorder offers promise for early identification and intervention to successfully address specific phenotypes of RD. Recently, we demonstrated the animal models of dyslexia (i.e. genetic models based on candidate dyslexia susceptibility genes) and children with specific reading impairment show a common deficit on a virtual Hebb-Williams maze. This deficit is consistent across language orthographies (i.e. transparent and non-transparent languages). In this study we examined the link between maze performance, phonological processing, family history of dyslexia, and genetic risk in a longitudinal study. We examined reading ability and maze performance at 5-6 and 8-9 years of age and determined whether early reading measures, maze performance, or genetic risk is a better predictor of reading ability by the third-grade.

Theme: Development
A theory for how neurons achieve selective muscle connectivity is through specific extracellular proteins lining the membrane of both the presynaptic and post-synaptic membranes that interact for target recognition (Skidmore College Neuroscience Program, 2018). In Drosophila Melanogaster, DIP-α interaction with Dpr10 is believed to be essential for connection to muscle 4 in RP2 motor neurons (Skidmore College Neuroscience Program, 2018). However, the DIP-α/Dpr10 interaction may not tell the whole story. Other proteins or mechanisms may mediate synaptic connection between RP2 motor neurons and muscle 4. To investigate which genes may be interacting partners for DIP-α, we observed the connectivity to muscle 4 in larvae expressing deficiency on potential interacting partner regions. If an important gene is located on one or more of these regions, then making them deficient in flies should affect overall connectivity to muscle 4 from DIP-α containing-neurons. We found that deficiency on regions 7571 for females, 7634 for males, and 7638 for females resulted in a statistically significant difference in connectivity to muscle 4 for these groups of flies compared to control groups of the same sex, implying that important structures for the development and pathfinding mechanisms of Muscle 4 may be located on these chromosomal regions.

Theme: Development
DIFFERENTIAL REARING IN RATS CAUSES CHANGES IN HIGH DOSE ETHANOL METABOLISM BUT DOES NOT IMPACT ESTROUS CYCLE SYNCHRONY

Stearns, R. B., Taylor, S. Wukitsch, T. J., Moser, T., Campbell, R. M., Cain, M. E.
Kansas State University

Rats reared in isolated conditions exhibit more ethanol-seeking behaviors than rats reared in enriched conditions (Deehan, et al, 2011). Additionally, rearing in different environmental conditions could alter levels of exercise and play. Chronic exercise results in faster ethanol clearance in rats (Ardies, et al., 2018). The role of the estrous cycle is also important to ethanol-related behavior. Previous research on operant drinking found female rats with synced estrous cycles lever-pressed more for ethanol when they were in diestrus (Roberts et al., 2006). If a rearing group has higher ethanol metabolism or is in a different stage of the estrous cycle, there may be baseline differences in reward sensitivity when administered identical amounts of ethanol. Therefore, the current study examines differential rearing’s effect on ethanol metabolism and estrous cycle. Experiment 1: Male and female Long-Evans rats arrived in the lab at PND 21 and were randomly assigned to either an isolated (IC), enriched (EC), or standard condition (SC). All rats received ethanol injections at PND 52 (1.5g/kg, i.p.) and again at PND 58 (3.0g/kg, i.p.). Two hours post-injection, blood samples were taken from each rat for blood ethanol content (BEC) analysis. A third injection at PND 72 (1.5g/kg, i.p.) sampled blood 0.5 hours post-injection. While differential rearing did not alter BEC following the lower doses of ethanol, there were effects in the higher dose with ECs having lower BECs than SCs. This indicates that at higher doses ECs metabolize ethanol faster than SCs. Experiment 2: From PNDs 56-67, estrous stage was monitored for synchrony via vaginal cytology throughout Experiment 1. Analysis of vaginal cytology showed no differences in estrous stage between rearing environments across time. Together, these results suggest that differential rearing alters ethanol metabolism at a high dose but does not cause desynchrony of estrous cycle.

Theme: Development
Poster #11

ORGANOPHOSPHATE TREATMENT OF XENOPUS LAEVIS DURING EARLY DEVELOPMENT INDUCES ABNORMALITIES IN THE CYTOARCHITECTURE OF SPINAL NEURONS

Degner, K.; J. Hidalgo Lopez; M. Bryson; A. Ward; F. L. Watson
Department of Biology & Program in Neuroscience, Washington and Lee University, Lexington, VA

Organophosphate pesticides (OPs), a subset of acetylcholinesterase-inhibiting chemicals, are widely used to protect crops, homes, and businesses from insects. Despite a U.S. ban on residential use of organophosphates such as chlorpyrifos (CPF), widespread use of these pesticides continues in agricultural settings. Developing countries have no restrictions and OPs continue to be widely used. Because it is difficult to mitigate exposure to these pesticides, understanding both the short and long-term exposure to OPs is important. We utilized a line of frogs expressing green fluorescent protein (GFP) in their spinal neurons and used immunofluorescence co-localization with a dorsal root ganglion neuron (DRG) marker, gamma synuclein, to establish DRGs as a subpopulation of neurons expressing GFP. We then examined the effects of CPF exposure on the neurons of developing Xenopus laevis over a 21-day period. While ecologically relevant concentration varies between 0.1 - 0.25 μM, to assess acute toxic exposure levels we exposed embryos to CPF concentration between 0 to 10 μM. Using confocal microscopy, our preliminary data show increased defects in neuronal migration and decreased neuronal growth at developmental stages 37 and 47. Specifically, DRGs of stage 47 Xenopus exposed to 10 μM CPF showed significant increases of neuronal migration from the midline. We also observed that DRGs of stage 37 Xenopus exposed to 0.1 μM, 0.5 μM, and 10 μM and stage 47 Xenopus DRGs exposed to 0.1 μM, 5 μM, and 10 μM exhibited a trend towards decreased neuronal growth. Our preliminary results provide evidence that OPs have profound neurological effects in the sensory neurons of developing vertebrates. Our ongoing studies investigating sub-acute levels of CPF exposure will provide a more complete analysis of neuronal defects caused by OP exposure.

Theme: Development
Drug addiction is a multi-factorial disorder, and environmental factors during childhood have a large impact on susceptibility to addiction during adolescence. Rearing rats in enriched (EC), impoverished (IC), or social (SC) conditions post weaning causes neurobiological and behavioral changes that impact future drug taking behavior. In particular, a protective effect develops during rearing as EC rats self-administer less psychostimulant than IC and SC rats at low unit doses. The majority of differential rearing studies have been conducted in male rats, and at the current time no studies have investigated cocainc self-administration dose effects and reinstatement in differentially reared female rats. The current study investigated drug taking behavior using female Sprague Dawley rats, which were reared in either an EC, IC, or SC conditions, and trained to self-administer cocaine or saline utilizing a standard 2-hr self-administration paradigm. Following self-administration and extinction rats underwent a randomized series of cue-induced, cocaine-primed, or yohimbine plus cue reinstatement. Immediately after cue-induced reinstatement sessions, tissue was extracted from the prefrontal cortex, infralimbic cortex, and nucleus accumbens for western blot analysis of GluA1. Results corresponded with differential rearing literature in male rats, revealing that EC rats self-administer less cocaine then IC and SC rats at low doses. IC rats displayed significantly higher drug seeking behavior when undergoing cue-induced, and yohimbine + cue-induced reinstatement.
EXAMINING GENETIC DIFFERENCES IN BINGE DRINKING AT BASELINE AND IN RESPONSE TO STRESS

Dimitratos, E.
Villanova University

Binge drinking exerts a tremendous toll on American society through its numerous direct and indirect effects, including the promotion of increased risk-taking behaviors, aggression, sexual violence, poor academic performance, liver damage, and the development of psychological disorders, such as major depression. Two of the many factors that influence binge drinking are gender and stress. Although stress is typically considered to increase alcohol consumption, there is evidence to suggest that the relationship between stress and alcohol intake is complex and may be moderated by sex. For example, previous research has found that stress is positively associated with binge drinking in male college students but negatively associated with binge drinking in female college students. However, whether this clinically observed sex difference results from sexually dimorphic neurobiological responses to stress (or alcohol) in males vs. females or from societal or other factors has not been established. The current work sought to evaluate whether c57BL6/J mice exhibit sex differences in stress-induced alterations in binge drinking behavior. In addition to examining behavior, we also sought to provide new insight into the mechanisms through which stress might impact binge drinking in males and females. To do this, we used real-time PCR to examine stress and alcohol-induced alterations in gene expression in several brain regions that have been implicated in binge drinking. Given that the endorphin and glucocorticoid systems are both highly stress responsive and have been highly implicated in stress responses to alcohol, we compared the expression of several genes involved in endorphin and glucocorticoid signaling in male and female mice after chronic restraint stress and under control conditions. Although no significant sex differences in binge drinking were observed, stress was shown to significantly increase binge alcohol consumption and to lead to a significant upregulation of several genes in the amygdala, including corticotropin-releasing hormone and proopiomelanocortin. Overall, this study provides new insight into the effects of chronic stress on binge drinking and may help identify novel molecular targets to protect against or reverse stress-induced increases in alcohol consumption.

Theme: Development
The proper development of mammalian nervous system requires that cells and their axons navigate a complex and dynamic environment as the brain grows and matures. Cells respond and adapt to regulatory extracellular cues by exchanging activated surface membrane proteins and targeting damaged or unnecessary surface membrane proteins for lysosomal degradation. The small GTPase Rab7A operates within both autophagic and endocytic pathways as a master regulator of lysosomal degradation and membrane trafficking. We have used a conditional Rab7A knockout in neural progenitor cells and their post mitotic progeny to look at the consequences of improper trafficking events in the endocytic and autophagic pathways. Immunofluorescence of cortical layer markers in the conditional knockout reveals perturbations in layer V development and the subplate. Furthermore, loss of Rab7A resulted in aberrantly projecting axon bundles in the lateral and medio-dorsal cortex, highlighting a potential role for Rab7A in subplate-mediated axon guidance. Ultrastructurally, knockout cortices exhibited intracellular accumulations of vesicular machinery and evidence of failed fusions with LAMP1-positive lysosomal structures. Disrupted trafficking translates to proliferative deficits in ventricular zones of the cortex. This work suggests novel functions for Rab7A during key processes of cortical development.

Theme: Development
PYRETHROID TREATMENT OF XENOPUS LAEVIS DURING EARLY DEVELOPMENT INDUCES MORPHOLOGICAL ABNORMALITIES AND DECREASED SURVIVAL AND LOCOMOTOR ACTIVITY

Agostini, T.; M. Bryson; T. Loughery; A. Fielder; K. Degner; J. Hidalgo-Lopez; A. Ward; A. Love; F.L. Watson; H. I’Anson
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Synthetic pyrethroids, a common class of insecticides synthesized from the pyrethrum extract of the Chrysanthemum flower, are commonly used to control insects in a wide range of settings, including agricultural, domestic, and industrial settings. The frequent and various uses of pyrethroids leave aquatic vertebrates susceptible to the effects of runoff due to a significant amount of aquatic environmental contamination. Here we assess the effects of pyrethroids on aquatic vertebrates by examining the impacts of four pyrethroids from two different classes – permethrin (Class I), and cypermethrin, cyhalothrin, and cyfluthrin (Class II) on the physiology of developing frog embryos, Xenopus laevis. These frog embryos provide a good model to assess the effects of potential teratogens on vertebrates due to their physical transparency during early embryonic stages, rapid growth, and external development. Specifically, we evaluate the toxicity of pyrethroids (LD50) and conduct physiological and behavioral measurements that include heart rate, swimming activity, body length, kyphosis (spine curvature), and cardiac edema. Preliminary data suggest all four pesticides caused significant mortality. We report a positive correlation between spinal length and presence of spinal bends with increasing pyrethroid concentrations for each of the four pesticides. In addition, we observed reductions in swimming activity in response to all four pyrethroids. Interestingly, exposure to permethrin, the least toxic pyrethroid, caused the highest increase in heart rate in 4-day old tadpoles followed by a decrease in heart rate in 7-day old tadpoles. In our on-going studies, we will investigate the sublethal effects of pyrethroid exposure to Xenopus laevis embryos and the underlying biological and neurological mechanisms involved.

Theme: Development
INCREASED NRF2 EXPRESSION MITIGATES THE DECLINE IN SUBVENTRICULAR ZONE NEUROGENESIS DURING A CRITICAL MIDDLE-AGE PERIOD

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Our recent studies have examined the function of rat subventricular zone (SVZ) neural stem and progenitor cells (NSPCs) during aging. This work indicates that although NSPC function continuously declines with advancing age, there is a critical time period during middle-age (13-15 mos) when a prominent reduction in NSPC survival and regeneration occurs. We also find that this specific temporal pattern of NSPC deterioration is mediated via the reduced expression of the redox-sensitive transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Based on these data, we next investigated whether increasing Nrf2 expression could potentially mitigate the decline in NSPC function across the identified critical middle-age period. To test this, we administered recombinant adeno-associated viral (rAAV2/1) vectors carrying Nrf2 (or eGFP vectors as controls) into the SVZs of aging rats, before the critical period at 11 mos of age, and analyzed the animals at the end of the critical period at 15 mos of age. Specifically, we stained sagittal and coronal sections of the rat brain with markers for proliferation (Bromodeoxyuridine (BrdU)) and differentiation (early-neuronal Doublecortin (DCX), late-neuronal (NeuN) and subtype-specific neuronal (Tyrosine hydroxylase (TH)) to visualize changes in adult neurogenesis. In doing so, we found significantly higher levels of BrdU in the SVZ and throughout the rostral migratory stream (RMS) in Nrf2-overexpressing animals compared to controls. Additionally, we found significantly higher levels of DCX in Nrf2 animals indicative of increased migration throughout the RMS. These animals also had a pronounced increase in the number of differentiating cells marked by higher levels of NeuN. In summary, these findings indicate increased levels of neurogenesis and survival of SVZ NSPCs in Nrf2-overexpressing rats compared to controls. To further assess the effects of Nrf2 on the NSPC population, we are currently investigating the level of NSPC integration into the glomerular layer of the olfactory bulb using NeuN, TH, and other subtype-specific markers.

Theme: Development
Poster #17

LOCAL NETWORK SYNCHRONIZATION IN THE RAT DORSAL AND VENTRAL HIPPOCAMPUS THROUGHOUT DEVELOPMENT

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Medial temporal lobe epilepsy (mTLE) is a prevalent form of focal epilepsy defined by spontaneous recurrent seizures generated within the medial temporal lobe—seizure foci that originate within the hippocampus are especially common. Interestingly, the probability that the local hippocampal network will synchronize and precipitate a seizure varies with location along longitudinal hippocampal axis, such that the anterior hippocampus (rather than the posterior hippocampus) is most often associated with seizure generation and hippocampal sclerosis. Moreover, the seizures associated with mTLE tend to begin during adolescence, and continue into adulthood. Given the age, and location dependence of seizure generation in the hippocampus, we sought to explore the mechanisms of local network synchronization across the longitudinal hippocampal axis throughout development using Sprague-Dawley rats ranging from two weeks to six months old. Here, network synchronization was accomplished by applying 0 mM Mg$^{2+}$ artificial cerebrospinal fluid to acute hippocampal slices at near physiological temperature (31–33°C), while network activity was monitored using an extracellular electrode placed in the CA1 cell body layer. Interestingly, the dorsal hippocampus (DHC; the rodent homolog of the human posterior hippocampus) transitioned from hyperexcitable to hypoexcitable (determined by steady-state event frequency) throughout development, whereas the activity within the ventral hippocampus (VHC; the rodent homolog of the human anterior hippocampus) remained stable throughout this developmental timeframe. This result suggests significant remodeling of the local circuits within the DHC throughout development, which presumably protect this region from inappropriate network synchronization, and, by extension, could shed light onto the mechanisms of the seizure generation in adolescents with mTLE.

Theme: Development
Poster #18

STEM CELL DYNAMICS DURING REGENERATION: CHARACTERIZATION OF THE NEOBLAST IN LUMBRICULUS VARIEGATUS

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Univ. of the Incarnate Word

*Lumbriculus variegatus* is one of a select few model organisms that is capable of regenerating lost body segments following injury and recover full function. Our lab has developed Lumbriculus as a model to investigate changes within the nervous system as small worm fragments regenerate. While we have previously investigated the molecular components of head regeneration, our current project extends our proteomics work to the identification and characterization of stem cell populations found in Lumbriculus. We are most interested in characterizing proteins which are important contributors to stem cell development. Using an antibody raised against proteins found in wound blastema (1D9-E11), we detected 2 protein bands that measure 214 kDa and 137 kDa on immunoblots. These 2 bands are present in both anterior and posterior regenerating and non-regenerating worm fragments. Therefore, it is predicted that these proteins may already be present in segmental tissue prior to an injury event and are increased in expression during regeneration. Immunohistochemical analysis demonstrates 1D9-E11 immunoreactive puncta are present within the neuropile of the ventral nerve cord and within the body cavity more closely associated with the nervous system. Total worm lysates will be further purified using immunoaffinity columns with 1D9 antibody attached to sephadex beads. 1D9 column purified proteins will then be sent for mass spectrometry analysis using the MALDI-TOF method. This work will ultimately determine the protein identity of the 1D9 immunoreactive protein epitopes which are involved in regeneration in Lumbriculus. Finally, co-localization of the 1D9 positive cells with bromo-deoxy-uridine (BrDU) will provide further evidence that 1D9-E11 is positively identifying a subpopulation of stem cells in Lumbriculus. Overall, understanding the mechanisms of regeneration utilized by Lumbriculus will further our understanding of these complex processes and perhaps the knowledge gained will be applicable in higher order organisms.

Theme: Development
Chloride (Cl-) is a negatively charged ion that plays a major role in regulating the electrical excitability of neurons throughout the nervous system. GABA binding to ionotropic GABA-A receptors opens high conductance chloride channels, but the effect on neuron excitability depends on the basal concentration of Cl- inside neurons (Cl-[i]) relative to extracellular levels. With high basal Cl- [i], GABA-A receptor activation drives an outward Cl- current, which is depolarizing and excitatory. With low Cl-[i], GABA drives an inward Cl- current, which is hyperpolarizing and inhibitory. During development, most vertebrate neurons undergo a shift from high to low Cl-[i] concentration, which reverses the ionic driving force of GABA and shifts its effects from excitatory to inhibitory. Disruption of this switch to GABA inhibition has been proposed to contribute to Epilepsy and Autism Spectrum Disorders, inspiring the development of methods to modify this developmental process with drugs. Methods: The current study investigated the GABA switch in zebrafish larvae, a model system offering several advantages for investigating modulators of neuronal excitability. We used optical imaging of a genetically-encoded fluorescent calcium indicator (elavl3:GCaMP6s), to record activity throughout the brain before and after application of the selective GABA-A receptor agonist, muscimol. Neuronal activity was evoked with a visual ‘darkflash’ stimulus (sudden darkening of ambient light) and was measured at 6 ages (each day from days 4-9 post-fertilization). To detect potential regional effects of muscimol, 4 separate brain regions were analyzed: telencephalon, diencephalon, mesencephalon, and rhombencephalon. The statistical model therefore analyzed neural activity as a function of age, muscimol, and brain region. Results: Rostral brain regions (telencephalon, diencephalon) showed consistent activation by the visual stimulus whereas caudal regions did not. Within rostral brain regions, there was a significant effect of age due to the youngest larvae (4 days old) having larger calcium responses than older larvae. There was no main effect of muscimol treatment on neural activity but there was an interaction between muscimol, age, and brain region, caused by muscimol inhibiting the normal youth-related hyperactivity in forebrain regions. Ongoing analyses will measure muscimol effects using finer spatial resolutions as there is clear variability in activity within the brain regions analyzed. Conclusions: These results support that in zebrafish, as in other vertebrates, immature neurons are hyperexcitable. Subsequent studies will examine whether early treatment with modifiers of Cl- [i] homeostasis, including the oxytocin analog, bumetanide, can alter the shift toward lower neuronal excitability as zebrafish age.

Theme: Development
Fetal alcohol spectrum disorder, which is observed in children who have been exposed to alcohol in utero, is a leading cause of neurodevelopmental disability. Human fetuses with a history of prenatal alcohol exposure have shown abnormal cortical vasculature that accompanies microencephaly, where reduced regions of the brain include the basal ganglia, diencephalon, and the cerebellum. Studies in rodents have shown that prenatal ethanol exposure results in decreased blood flow from the umbilical artery to the fetal brain, compromising neuron formation and angiogenesis in the maturing brain. The same phenomena have been observed in oviparous fetuses such as in chick yolk sac membrane model, where ethanol exposure dramatically inhibits angiogenesis in the 9-day-old chicken embryos in a dose-dependent manner. Previous studies on ethanol exposure in zebrafish larvae have shown craniofacial and brain structural defects. Ethanol has shown to disrupt coronary artery formation in the embryonic zebrafish, but the effects of ethanol on the developing cerebral blood vessels and neurogenesis in zebrafish larvae remains poorly understood. We exposed zebrafish larvae to three different concentrations of ethanol (1%, 1.5%, and 2%) at 3 hours post fertilization (hpf) and at 27 hpf for 24 hours, and imaged the brain at 27, 51, and 75 hpf. We used Tg(ETvmat2:GFP) to visualize monoaminergic neurons and Tg(fli1:EGFP) to image cerebral blood vessels under confocal microscopy. The two groups differed in the developmental stage at which the 24-hour ethanol exposure was administered. Our results suggest that ethanol at 2% leads to reduced vessel diameter and number of cranial blood vessel branching, and reduced number of neurons in the locus coeruleus, arch-associated neurons, and raphe nuclei. We show that ethanol at 2% leads to teratogenic effects in the developing zebrafish brain and cranial vessels.

Theme: Development
ZEBRAFISH (DANIO RERIO) PREFER SOCIAL REWARD OVER FOOD REWARD

Christopher Newport University

Zebrafish (Danio rerio) are an increasingly popular subject for psychological and neurological studies. Food rewards are typically used in many studies of instrumental learning and choice behavior in zebrafish. This study sought to establish social reward in the form of a mirror presentation as an alternative to typical food rewards. Subjects were placed in a T-maze and in thrice-daily trials were given the opportunity to choose between food or social rewards. Counterbalanced chamber color indicated the reward type and a small green plastic plant was placed within the T-maze to serve as a form of enrichment. Trials continued until each subject indicated a reward preference. Significantly more fish preferred the mirror reward, and fish which stabilized on mirror preference had significantly fewer trials-to-criterion (TTC) than fish which stabilized on food preference. While there was no main effect of color on TTC, the interaction of color by reward type on TTC was significant, indicating that the color of the discriminative stimuli had mediating effects on reward preference. Addition of enrichment in the form of a plastic plant, showed no change in social reward preference when compared to previous trials. The preference for social reward over food reward indicates that social rewards may be a more effective reward for Zebrafish than the classic food reward.

Theme: Development
IDENTIFICATION OF GENES INVOLVED IN THE DEVELOPMENT OF NOCICEPTIVE NEURONS IN THE MOUSE DORSAL ROOT GANGLION

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The mouse dorsal root ganglia (DRG) are paired structures that lie adjacent to each vertebrae along the spinal cord. The DRG contain the cell bodies of a collection of sensory neurons that include nociceptors; the neurons that sense painful stimuli. In an ongoing attempt to understand how the correct number of neurons are formed in the correct locations during embryonic development, this study attempts to characterize part of the gene regulatory network that is required to generate pain sensing nociceptors in the DRG during embryonic development. One key gene that is involved in this network is the transcription factor neurogenin1 (neurog1).

Neurog1 is expressed by progenitor cells in the DRG and this gene is required for the generation of nociceptors since knockout mice that lack the neurog1 gene do not produce nociceptive neurons. Because neurog1 is a transcription factor, we hypothesize that it is a key node in the gene regulatory network that produces nociceptors and that transcriptional targets of the neurog1 gene are also members of this network. To identify transcriptional targets of the neurog1 gene, we have compared the transcriptome of embryonic day 11.5 (E11.5) cervical dorsal root ganglia from wild-type and neurog1 knockout embryos. Differential gene expression analysis has identified several genes that appear to be downregulated in the knockout and therefore are potential transcriptional targets of the neurog1 gene. To confirm the differential expression of the genes identified in the transcriptome analysis, we are currently examining their mRNA expression pattern in the cervical DRG of wild-type and neurog1 knockout E11.5 embryos by in situ hybridization with complementary RNA probes. The results of this comparative analysis will be presented. Supported by the Keith Sutton and Hugh Stevens Moseley Undergraduate Research Awards, the Hendrix Odyssey Program, and the Arkansas INBRE Program.

Theme: Development
Marijuana, or cannabis, is one of the most widely used drugs worldwide, second only to alcohol. Since its ban in the US and Canada in the early 20th Century, the effects of both chronic and acute cannabis use have been difficult to research due to its Federal Schedule I status. With the recent flood of legalization efforts in both US states and Canada, it is critical that more clinically translatable research is carried out so that we may better understand this drug’s full effect on the brain. The aim of this study was to assess the effects of a single inhaled dose of Δ9-tetrahydrocannabinol (THC) on blood-oxygen level dependent (BOLD) activity in drug-naïve, awake mice, to obtain a THC-specific map of activation in clinically-relevant brain regions and systems. Dried, pulverized cannabis plant matter (9% THC) was vaporized and inhaled by the mice during a functional magnetic resonance scan, resulting in increased positive and negative BOLD signals compared to vehicle, especially in areas rich in the cannabinoid type 1 (CB1) receptor. These results offer unique maps of activity, or, ‘fingerprints’, associated with inhaled THC administration. This specific administration modality is particularly understudied in preclinical research, despite being the most common route of administration in humans. The data collected from this study can be used for further comparisons with additional doses or compounds, or between other THC administration modalities (i.e. inhaled vs. ingested vs. systemic), which ultimately adds to the translatability assessment of THC-induced BOLD activity between humans and animals.

Theme: Development
Poster #24

THE INFLUENCE OF NEONATAL PAIN ON MATERNAL BEHAVIOR

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Premature infants who spend time in the Neonatal Intensive Care Unit (NICU) are at higher risk of developing anxiety-disorders later in life, likely due to exposure to painful/stressful events. Although time in NICU predicts a greater susceptibility to latter disorder, still the majority of NICU patients go on to be healthy. This raises the issue of why some are susceptible and some are not. Mother-infant interactions could potentially affect the infants by making them more resilient to later life stress they might experience. This project was designed to test the hypothesis that maternal behavior may have an effect on how pups respond to later life fear and stress by assessing nurturing and non-nurturing dam-pup interactions. This will help gain insight into the role of maternal behavior in the emotional and cognitive development of pups. The pups were placed into three different conditions: Inflammatory pain, Acute pain, and Control. The inflammatory pups were subjected to left hindpaw plantar injections of either carrageenan or saline on their first and fourth day of life. The acute pain pups were subjected to a needle to their left hind-paw, four times a day for the first week of life. The Control pups were an undisturbed litter that spent their first week of life with their mother and didn’t have any handling. The results show that with both acute and inflammatory pain, dams are spending a similar amounts of time with their pups as the control litters. However, the behaviors differ per condition. The dams of the pups in pain exhibited significantly less time providing pup care than the control dams. They also spend somewhat more time nursing pups in pain. As other literature shows that changes in pup licking can alter HPA-axis function and stress responding later in life, these results suggest that changes maternal care following pain could be a contributing factor underlying the long-term effects of neonatal trauma.

Theme: Development
ANALYZING SHOALING BEHAVIOR OF RELN-/- ZEBRAFISH

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The Reelin (RELN) glycoprotein plays a large role in prenatal development and post-birth synaptic transmission, with many studies showing a linkage between decreased RELN expression and neuropsychiatric disorders. While mice have been the established animal model for studying RELN insufficiency, it is unknown whether RELN-/- zebrafish exhibit behavioural impairments resembling those of reeler mice. We present preliminary findings of abnormal social behaviour in RELN-/- zebrafish when placed in shoaling tests and report various methods of visualizing and quantifying trajectory data. By working to identify robust behavioural characteristics, further studies may help validate zebrafish as an appropriate tool for modeling neurodevelopmental disorders.

Theme: Development
Impact of gestational exposure to a ketogenic diet on behavioral and neuroendocrine function in CD-1 mice.

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The development and expression of social and affective behavioral responses are highly influenced by the prenatal environment. It is not clear, however, how the availability of different metabolic fuels influences these processes. Ketogenic diets (KDs) are high fat, low carbohydrate formulations that have recently been shown to increase sociability when administered to juvenile and adult individuals in preclinical models of autism. Given that the brain oxytocin system (1) is strongly implicated in normative and disordered social responses, and (2) initially develops during the late prenatal period, we predicted that gestational exposure of male and female CD-1 mice to a KD would enhance sociability via enhancing oxytocin signaling in these animals. In this study, breeder mice were assigned to either a KD or a control diet (CD) and then mated with an individual of the opposite sex from the same diet treatment. The young adult offspring were then assessed for sociability and depression-like behaviors, and their brains were processed immunohistochemically for oxytocin expression. Although gestational exposure to a KD did not impact sociability, treated mice did show improved positive affect as evidenced by increased active coping responses in the forced swim test. Work is currently underway to determine if changes in oxytocin expression correlate with the behavioral impact of gestational exposure to a KD.

Theme: Development
SYNDIG1 EXPRESSION IN PURKINJE CELLS AND ITS ROLE IN EXCITATORY SYNAPTIC MATURATION

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During development of the nervous system, synaptic connections between pre- and postsynaptic neurons are formed and subsequently sculpted by experience to give rise to mature neural networks. The regulated enrichment of weak or functionally silent synapses with AMPA receptors is a key postsynaptic mechanism underlying experience-dependent maturation of synaptic communication. However, this mechanism is not well understood. SynDIG1 is a transmembrane protein that interacts with AMPA receptors and regulates excitatory synapse development. Previous studies have shown that disruption of SynDIG1 results in a decrease in the number of mature synapses in the hippocampus and impairs synaptic plasticity. In the cerebellum, SynDIG1 mRNA is selectively expressed in Purkinje cells (PCs; the principle output neurons of the cerebellar cortex) and is upregulated during postnatal development. However, the role of SynDIG1 in the cerebellum has not been studied. The purpose of the present study is to investigate the role of SynDIG1 in the maturation of excitatory synapses onto Purkinje cells. Using immunohistochemistry, we determined the cellular and subcellular distribution of SynDIG1 in cerebellar slices collected from postnatal day 12 (P12) mice. First, we validated anti-SynDIG1 antibodies in tissue from animals lacking SynDIG1. Preliminary experiments with the validated antibodies showed that SynDIG1 is uniformly expressed across lobules of the cerebellum. We are currently following up on initial observations of differential distribution between proximal vs. distal dendrites, as well as age differences in expression of SynDIG1 in PCs. PCs receive excitatory inputs from parallel fibers and climbing fibers. Using electrophysiological recordings, we determined the effects of SynDIG1 disruption on evoked glutamatergic transmission from parallel fibers and climbing fibers to PCs in cerebellar slices collected from P8-P12 mice. Whereas parallel fiber-PC connectivity remains unaffected in SynDIG1-deficient mice compared to wildtype littermates, normal elimination of poly-climbing fiber innervation of PCs is impaired. In agreement with the electrophysiological result, immunohistochemical studies showed increased vesicular glutamate transporter (VGLUT2; a marker for climbing fiber synaptic boutons) signal in SynDIG1-deficient mice. Based on these findings, we propose that SynDIG1 plays a key role in regulating normal climbing fiber elimination and synaptic maturity in the cerebellum, potentially through interacting with AMPA receptors and influencing glutamatergic signaling.

Theme: Development
Depression and anxiety are common outcomes for those who experience bullying and other social stressors. Social defeat is one animal model that mimics this pattern. The extent to which one experiences these maladaptive outcomes is dependent on the resiliency or susceptibility of an individual to stress. Deficits in the paternal care an offspring receives is one potential stressor, as there is growing evidence that has shown not only how paternal care affects behavioral changes in the offspring but also how deprivation of this care can have an impact. While the social defeat and paternal deprivation paradigms’ influences have been tested heavily in adulthood, producing sex-specific results, the effect of deprivation or defeat occurring during adolescence has received little attention. The present study investigated how paternal deprivation prior to the experience of social defeat in adolescence influenced behavioral outcomes in both sexes. California mice (*Peromyscus Californicus*) were either raised with two parents, or had the father removed on post-natal day (PND) 3, and then experienced either social defeat or a control condition from PND 42-44. Outcomes were operationalized through use of a Sucrose Preference test and Social Interaction Test, respectively. The hypothesis was that paternal deprivation prior to social defeat would lead to increased depressive- and anxiety-like behaviors in females, while males would show resiliency to these effects. However, results showed that paternal deprivation and social defeat impacted the development of anhedonia and anxiety-like behaviors differently in males and females. On PND 90, females who were socially defeated consumed a smaller percentage of sucrose, $F(3, 38) = 11.12, p = 0.002$, while males who were paternally deprived consumed a higher percentage of sucrose solution, $F(3, 26) = 9.62, p = 0.005$. There failed to be significant differences in traditional measures of social interaction, although there were effects seen in ambulation across different phases of the task. Specifically, social defeat lead to increased ambulation in males during the interaction phase, when the focal animal was exposed to a stimulus mouse, and an interaction of both deprivation and social defeat lead to decreased ambulation in females during the acclimation phase of the task. Thus, our results suggest that females that are exposed to stress in the form of defeat are susceptible to developing anhedonic symptoms, whereas a combination of the stressors is necessary for anxiety-like behavior. Conversely, males demonstrated resilience to anhedonia when paternally deprived, but social defeat lead to resilience in developing anxiety-like symptoms.

Theme: Development
Poster #29

THE ANXIOTROPIC AND COGNITIVE EFFECTS OF CHRONIC ADOLESCENT NICOTINE EXPOSURE IN ADULT MALES AND FEMALES

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Nicotine has been known to affect the developing brain during adolescence (Slotkin, 2002, Yuan et al. 2015). The aim of the current research was to better understand the nature of potentially long-lasting anxiotropic and cognitive effects of chronic adolescent nicotine exposure in early adulthood. This was achieved by exposing male and female Long Evans rats to a chronic nicotine exposure regimen (1.0 mg/kg/day) throughout adolescence from postnatal day (P) 25-59. In early adulthood after this exposure, beginning on P65, an array of behavioral tests were conducted to measure levels of anxiety and investigate cognitive ability. Specifically, the current research included measures of anxiety utilizing the Marble Burying Task (MBT) and the Open Field (OF). The MBT has been historically used as a rodent model of associated anxiety features characteristic of Obsessive-Compulsive Disorder and autism spectrum disorders. Simply, a higher report of digging behavior (i.e. more marbles buried) in the MBT, is indicative of greater anxiety (Angoa-Pérez et al., 2013). The OF is a widely used assessment of anxiety in rodent models. Related to nicotine exposure, OF performance is known to be impaired in adult animals following chronic nicotine exposure that occurred during adulthood (Iwamoto, 1984), but the nature of any adult impairment following chronic adolescent exposure has not been well established. Finally, a cognitive assessment of explicit, non-spatial memory was also conducted using the Novel Object Recognition (NOR) task. Despite being a well-documented assessment of explicit memory, it is unclear what the impact of chronic adolescent nicotine exposure would likely have on NOR in male and female adult rats, as findings on this question have not been published. Collectively, these behavioral tests provide a better understanding of the role adolescent nicotine exposure plays in adult measures of anxiety and cognitive function in males and females.

Theme: Development
In utero stress is a known predictor of anxiety and Autism Spectrum Disorder. The current study looks to further our understanding of the neurodevelopmental effects of in utero stress in the Caenorhabditis elegans animal model by implementing a multi-leveled model of mild stress. First generation worms were subjected to one of three stress conditions (control, liquid suspension or liquid suspension in motion) in utero, followed by analysis of spontaneous locomotor behavior and confocal imaging of NMR-1 and GLR-1 glutamate receptors expressed in the locomotor command interneurons. Behavior data were suggestive of a transgenerational effect of in utero stress, specifically in increased paused behavior in the parent generation and notable decreases in backward locomotion (i.e., avoidance) in F1 generations of stressed animals. Confocal imaging revealed decreased expression of NMR-1 glutamate receptor subunit expression in F1 adult worms; however, only for the liquid suspension stressor group. Initial findings with GLR-1 receptor expression indicates an overall decrease in the number of GLR-1 expressing puncta across stress groups. Together, these data begin to piece together how in utero stress can differentially affect locomotor subcircuits (forward approach vs backward avoidance locomotion) and provide a foundation to investigate neuronal effects of in utero stress in C. elegans.

Theme: Development
Sensation Seeking is a personality trait that is associated with the engagement in risky behaviors. Prior research has shown a gender difference in Sensation Seeking, with males reported being higher in Sensation Seeking than females. Other studies in our lab at California State University, Sacramento have shown a difference between females using hormonal birth control and females who are naturally cycling, with females using hormonal birth control having similar levels of disinhibition and overall Sensation Seeking to that of males. Research also suggests that Sensation Seeking is associated with low levels of dopamine, while Schizophrenia is associated with high levels of dopamine. Additionally, males are typically diagnosed with Schizophrenia at higher rates than females. The Wisconsin Card Sorting Test is commonly used on patients with Schizophrenia and they typically perform poorly on the test. The present study aims to examine the relationship between Sensation Seeking, as measured by the SSS-V and the ZKPQ, performance on the Wisconsin Card Sorting Test, and self-reported hormone use. We are looking to explore various factors relating to sensation seeking, including sex differences, the influence of hormonal birth control, and performance on the Wisconsin Card Sorting Test. We hypothesized that 1) females using hormonal birth control and males will score higher on the Sensation Seeking Scale than naturally cycling females; 2) those who are high in sensation seeking will make less perseverance errors on the Wisconsin Card Sorting Test than those who are not high in sensation seeking; and therefore, 3) naturally-cycling females will make more errors on the Wisconsin Card Sorting Test than either males or females using hormonal birth control. If females who are using hormonal birth control exhibit the same levels of Sensation Seeking as males when compared to natural cycling females, they should also perform similarly in tasks correlated with dopamine levels, impulsivity and risk-taking. Preliminary results support our hypotheses; though additional results will be presented at the poster session. This is important to study in order to better understand risky behaviors. Additionally, future research studies examining these constructs should take into account female hormonal levels.

Theme: Development
PRENATAL ALCOHOL DOES NOT AFFECT E12.5-BORN ISLET1- OR BRDU-LABELED CELL COUNT IN MEDIAL GANGLIONIC EMINENCE

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Fetal Alcohol Spectrum Disorders (FASD) is an umbrella term used to describe the range of deficits and disorders an individual may encounter when prenatally exposed to alcohol. FASD alters sensory and motor processes, leading to deficiencies in attention, memory, language and learning. Thalamocortical axons (TCAs) convey sensory and motor information from the thalamus to different cortical regions. Axons require complex, specific pathways for proper guidance. Corridor cells, found in the medial ganglionic eminence (MGE), build a necessary passageway for TCAs through the nonpermissive MGE. Because sensory processing deficits are observed with FASD, we predicted a decrease in the number of corridor cells in the MGE of E14.5, alcohol-exposed mouse embryos, leading to misguided TCAs. We injected pregnant dams with 15µL/kg of ethanol daily between embryonic day (E) 7.5 to E14.5 to model moderate drinking during pregnancy. Controls received daily saline injections. We injected bromodeoxyuridine (BrdU) at E12.5 to label progeny of dividing cells. We removed embryos via C-section at E14.5. Immunostaining for Islet1 and BrdU labeled corridor and progeny cells, respectively, in the MGE, which we photographed through a microscope and counted using the Cell Counter plugin on ImageJ. To compare between groups, we calculated cell count and area ratio within the MGE. We found no significant difference in the number of Islet1 or BrdU-labeled cells in the MGE at E14.5 in this preliminary study. Future directions include focusing on earlier stages of development, before corridor formation, and analyzing the distribution of cells in the MGE rather than cell count.

Theme: Development
ACUTE CORTICOSTERONE TREATMENT HAS NO EFFECT ON ANXIETY BEHAVIOR IN EARLY- AND MID-ADOLESCENT FEMALE RATS

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Adolescence is a period of dramatic hormonal changes and increased exposure to stressors, the combination of which often results in heightened stress reactivity. Early stress affects development of critical brain regions involved in moderating the fear response to stressful stimuli (Hanson et al., 2010). We previously found that mid-adolescent male rats treated with corticosterone (CORT) showed a significant increase in anxiety-type behavior as well as increased dendritic elongation and arborization in the orbitofrontal cortex (OFC), as compared to early-adolescent CORT-treated rats who showed no effects. The goal of the present study was to determine if CORT has similar behavioral and neural effects on female adolescent rats. Female adolescent Sprague-Dawley rats were given CORT (10 mg/kg) or saline vehicle injections in early adolescence (28 days old) or in mid-adolescence (36 days old). Anxiety behavior was assessed 12 days after injections using the elevated plus maze and open field test. There was no change in anxiety-related behavior in rats that received a CORT injection as compared to controls in either age group on the elevated plus maze or on the open field task. Rats in the early adolescent group and those injected with CORT displayed higher general activity levels in the elevated plus maze. While no effect of CORT injection was found on the anxiety measures, future work will focus on particular effects on dendritic morphology of OFC neurons. Our initial findings suggest that female rats respond differently to stress than males with respect to their outward anxiety behavior, but may show similar effects of stress on dendritic morphology to males. This research was funded by Grinnell College.

Theme: Development
DIFFERENTIAL EFFECTS OF PRENATAL AND EARLY POSTNATAL STRESS ON THE BEHAVIOR OF ADULT C57BL6/J MICE

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Exposure to stress has been demonstrated to affect a variety of physiological and psychological systems, increase susceptibility to illness and inflammation, and contribute to the development of pathological conditions such as depression, anxiety and dementia. However, it is currently unclear if exposure to prenatal or postnatal stress yields differential effects on behavior in adulthood. To test this question, the current project exposed C57BL6/J mice to predictable prenatal, postnatal, or no-stress paradigms and evaluated its behavior in adulthood. To simulate prenatal stress, pregnant dams were exposed to consecutive cold-water immersion and removal of bedding for 21 hours for eight days prior to birth. To simulate postnatal stress, nursing females were separated from pups for 1 hour a day for eight days immediately after birth. Control females were left undisturbed throughout the pregnancy period and after birth. Five weeks after the procedure was completed, seven weeks old female offspring were tested using animal models of human activity, anxiety and despair/depression. Results demonstrated a differential effect on behavior in experimental mice compared to control mice, specifically for minute five of the forced swim test. Further exploration of the effect on prenatal and postnatal stress on behavior will need to be conducted to allow the interpretation of these findings.

Theme: Development
Poster #35

TRAVEL AWARD WINNER
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ROBUST EXPRESSION OF 5HT2A AND 5HT2B IN GLIA CELLS: A COMPARATIVE IMMUNOHISTOCHEMICAL STUDY OF NON-PRINCIPAL CELLS

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Serotonin action on principal excitatory cells is implicated in mood regulation and thought to be mechanistic for part of the dysfunction in many psychiatric disorders. While pharmacological treatments targeting serotonin signaling can be highly effective their exact mechanism is not clearly understood. Released serotonin may bind to any of seven 5HT receptor subtypes, with the 5HT2 family having a critical role in mood disorder pathology. Recent findings in cultured cells and expression systems have demonstrated serotonin receptor expression and function in non-principal cells, yet a comprehensive and comparative localization of these receptors in intact tissue has yet to be completed. In the present study, we examined 5HT2A and 5HT2B receptor expression in parvalbumin-positive inhibitory interneurons, GFAP-positive astrocytes, and Iba1-positive microglia in the mouse cortex and hippocampal CA1 region. Using immunohistochemistry and confocal microscopy, we characterize differential 5HT2A and 5HT2B receptor expression that varies both by cell type and brain region. We detected robust expression levels of 5HT2A in microglia cells, which are not conventionally thought to participate in serotonin signaling. These findings elucidate the potential contributions of specific 5HT2 receptor subtypes to normal brain function via non-principal cells and may have implications for the mechanisms of action of drugs that target these receptors.

Theme: Neural Excitability, Synapses and Glia
CHARACTERISTICS OF ATP-INDUCED EXTRACELLULAR ACIDIFICATION FROM RETINAL MÜLLER (GLIAL) CELLS

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Within the retina, acidification of synaptic environments has been shown to significantly attenuate synaptic transmission. This study characterizes a novel non-neuronal pathway in the retina that evokes an extracellular acidification. In this pathway, Müller glia, upon activation by the signaling molecule ATP, acidify the extracellular environment. Measurements of proton fluxes from the apical (photoreceptor) end of isolated salamander Müller cells were performed using H⁺ sensitive self-referencing microelectrodes, an effective method for measuring relative ion fluxes from cells (see Kreitzer et al., 2007). Calcium imaging experiments using Oregon Green implicate that ATP binds to a P2Y₁ receptor, and in combination with self-referencing experiments point toward the ATP-dependent acidification requiring Ca²⁺ release through an IP₃ pathway. We report here a dependency of the acidification on the presence of extracellular Na⁺. Removal of Na⁺ was shown to significantly attenuate the ATP-induced extracellular acidification. Attenuation of the acidification by the sodium transport blocker amiloride and the sodium-hydrogen exchanger blocker cariporide were also observed. Surprisingly, the ATP-induced extracellular acidification was sensitive to extracellular K⁺. Reintroduction of K⁺ from a nominally 0 K⁺ solution significantly potentiated the ATP-induced acidification. However, this K⁺ sensitivity was dependent upon the presence of Na⁺. We hypothesize that Müller glia are potent acidifiers of the extracellular space. This extracellular acidification induced by ATP is mediated by a P2Y₁ receptor calcium-dependent pathway and is highly sensitive to the presence of Na⁺ and K⁺ outside of the cell. Our findings also point to a potential role for Na⁺/H⁺ exchange in driving this acidification. We hypothesize glial cell based extracellular acidification may be an important regulator of signaling throughout the nervous system.

Theme: Neural Excitability, Synapses and Glia
AUTORECEPTOR INHIBITION OF DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS IS ALTERED BY D2 ANTAGONISM AND DOPAMINE TRANSPORTER INHIBITION

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The mesolimbic dopamine pathway has an established role in disorders such as addiction, ADHD, and schizophrenia. This pathway consists of dopamine cell bodies in the ventral tegmental area (VTA) that project to the nucleus accumbens (NAc), which is classically divided into 2 compartments, core and shell. NAc dopamine transmission is continually being regulated via dopamine autoreceptors (DARs) and dopamine transporters (DATs). D2 dopamine receptors have a well-established role as DARs. D3 receptors have also been suspected to function as DARs, although this is debated in the literature. Less is understood about the interaction of DARs and DATs. The present study aimed to distinguish the regulatory role of D2 and D3 receptors on VTA stimulation-evoked dopamine release in the NAc core and shell using in vivo fixed potential amperometry in anesthetized mice. Stimulation parameters specifically designed to assess DAR functioning were applied during dopamine recordings before and after local infusions of D2 or D3 receptor antagonists. To assess the impact of DAT functioning on DAR-mediated inhibition, DAR tests were also employed before and after a systemic injection of the DAT inhibitor nomifensine (10 mg/kg, i.p.). In both the NAc core and shell, infusing a D2 receptor antagonist significantly decreased DAR functioning compared to vehicle infusions, whereas infusions of the D3 receptor antagonist made no differences in DAR-mediated inhibition relative to vehicle infusions. Furthermore, DAR-mediated inhibition was increased following DAT blockade by nomifensine in both the NAc core and shell. Overall, the results indicate no differences between the NAc core and shell in the measured properties of DAR functioning. Overall, findings indicate D2 receptors but not D3 receptors function as DARs, and DAR functioning is heightened without functional DATs suggesting a potential compensatory relationship. Determining the specific receptor types that serve as DARs and the interplay between DARs and DATs is crucial for understanding disorders related to dopamine dysfunction, especially given that DARs may serve as a therapeutic target for such disorders.

Theme: Neural Excitability, Synapses and Glia
TRUE PROPHYLACTIC TREATMENT EFFECT IN A RAT PTSD MODEL ON PLASTICITY IN VENTRAL HIPPOCAMPAL, LATERAL AMYGDALA, AND MEDIAL PREFRONTAL CORTEX AND MOLECULAR TARGETS

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Post-traumatic stress disorder (PTSD) is a complex anxiety/depression disorder that affects about 1 out of 4 individuals after a stressful/traumatic experience. Two commonly used models that we employed were single prolonged stress (SPS) and social defeat (SD) (both used with 2 weeks of chronic light). First, more naturally anxious rats were selected based on results of an open field test where cat fur and fox urine were placed in one quadrant. Rats were classified as anxious if they avoided that quadrant, froze for long periods of time, did not rear, and frequently urinated or defecated. The naturally anxious rats were used in either the SPS or SD protocols. Next, the elevated plus maze (EPM) and light-dark transition (LDT) tests were used to detect anxious behavior at the conclusion of SPS or SD protocols. We noted that both protocols were able to cause significant anxious behavior when compared to controls, with SD being more significant. Next, we performed field electrophysiology experiments in rat brain slices. The data illustrated that the SD protocol caused significant changes in ventral hippocampus plasticity while SPS did not. Therefore, we used the SD model to look at changes in other brain regions known to have altered plasticity in PTSD. SD caused a significant increase in long-term potentiation (LTP) in the ventral hippocampus (VH), lateral amygdala (LA), and medial prefrontal cortex (mPFC). To determine whether a prophylactic treatment could prevent the physiological changes of PTSD, we simultaneously administered propranolol and mifepristone at 10 mg/kg doses by intraperitoneal injection one week prior and during the entire duration of SD. These drugs significantly decreased LTP in the VH, LA, and mPFC of SD rats that received drug injections when compared to SD rats with no drug injections and controls. However, the SD drug treated rats did not show significant reductions in anxious behavior when tested on the EPM and LDT when compared to the SD rats with no drug injections and still exhibited significantly more anxious behavior than control rats. We then examined potential gene targets involved in plasticity and stress that could be altering the LTP in stressed and drug-treated rats. Significant alterations in the mRNA expression levels of glucocorticoid, mineralocorticoid, and beta 3 adrenergic receptors; AMPAR subunits, and NMDAR subunits were detected using RT-qPCR between all groups in all three brain regions. Overall, our data suggest that propranolol and mifepristone together may be a viable prophylactic treatment for preventing PTSD.

Theme: Neural Excitability, Synapses and Glia
Neuroinflammation contributes to neurodegeneration. Furthermore, neuroinflammation is proposed to play a role in many neurological disorders, from Alzheimer’s disease to Parkinson’s disease to autism. Microglia and astrocytes are known key players in neuroinflammation; however, they are very dynamic cells that require further investigation in order to understand their morphological and molecular changes during neuroinflammation. Our laboratory is currently investigating sex differences in the neuroinflammatory response due to consumption of a high fat diet. Interestingly, females are generally more resistant to diet-induced obesity and have revealed a lack of obesity-induced microgliosis in the hypothalamus. Very few studies have investigated sex differences in gliosis (astrocytes and microglia), especially in multiple brain regions. We fed male and female C57Bl/6 mice a high fat diet or control diet for 14 weeks and then evaluated microglia and astrocytes in the cortex, hippocampus, and hypothalamus. Our studies reveal a significant effect of diet on astrogliosis in the hippocampus and hypothalamus, but not in the cortex. Females have the greatest increase in astrogliosis on the high fat diet. We are currently evaluating the effects of diet and sex on microgliosis. In related work, we are evaluating neuroinflammation in a mouse model of autism. We are using a well-characterized in utero exposure to valproic acid (VPA) as our mouse model. Maternal exposure to different agents, including infection during pregnancy and treatment with the anti-convulsant VPA during pregnancy, has been implicated in ASD pathophysiology. We are also administering a post-natal treatment with the nutraceutical nicotinamide riboside (NR) to determine if increasing cellular NAD+ levels will reverse or improve ASD-linked phenotypes at behavioral, cellular and molecular levels. We are establishing the in utero VPA model in our laboratory and testing 2-month old offspring on a behavioral battery, including: the open field test, 3-chamber sociability test, marble burying and the elevated plus maze. Next, we will evaluate astrocyte and microglia morphology in male and female ASD mice vs. control with NR or regular drinking water. We hypothesize increased microglial activation and increased astrocyte density in male ASD mice, but a decrease in both inflammatory markers following NR treatment.

Theme: Neural Excitability, Synapses and Glia
Poster #40

SYNAPTIC PLASTICITY IN THE CROSSED TEMPORODENTATE PATHWAY IN FEMALE RATS

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Alzheimer’s disease (AD) is a debilitating neurodegenerative disorder characterized by memory loss and other behavioral problems. One of the principal early targets of AD is the entorhinal cortex (EC), which is a primary cortical input to the hippocampal formation. Interestingly, when the hippocampus is deafferented because of EC degeneration resulting from AD, several remaining afferents to the hippocampus undergo axonal sprouting. An established model to explore this concept in rats involves making a unilateral lesion of the EC, which elicits a sprouting response in fibers from the intact EC to the denervated contralateral dentate gyrus (DG) of the hippocampus, the so-called crossed temporodentate (CTD) pathway. Greater synaptic efficacy of lesion-induced, CTD sprouting has been found to occur as early as 6 days postlesion. To date, this model for plasticity has been used almost exclusively in male rats, so it remains unclear whether the female brain evidences a similar kind of neuroplasticity. The present study explores the nature of synaptic plasticity in the CTD in female rats 12 days postlesion. Male and female Sprague-Dawley rats received either unilateral entorhinal cortex lesions or sham operations, a craniotomy over the EC. A stimulating electrode was placed in the contralateral intact EC 12 days after a lesion or sham operation, and evoked field excitatory postsynaptic potentials (fEPSPs) were recorded in the DG ipsilateral to the lesioned EC. The paired pulse paradigm involved one pulse to the EC, known as the “conditioning pulse,” followed by a second “test” pulse at a range of interpulse intervals (IPIs; 10 to 500 ms). Additionally, estrus cycles of female rats were recorded by lavage procedure on days which they received an operation as well as on days of their physiology recordings. Brain tissue is currently being processed for histology. Stains will help determine the location and extent of EC lesion (cresyl violet acetate) as well as extent of the sprouting response in the septodentate pathway (the acetylcholinesterase Naik stain), a hippocampal afferent that also projects to the denervated DG. Analyses of electrophysiological and histological data are currently being completed.

Theme: Neural Excitability, Synapses and Glia
Nociception, or the ability to detect noxious stimuli, is essential to survival and wellbeing (Basbaum, et al., 2009). However, dysfunctions in the nociceptive signaling pathways can cause chronic pain. Cannabinoid-based therapy is one potential treatment for chronic pain, but cannabinoid therapies can have both pro- and anti-nociceptive effects (Christie & Mallet, 2009). One potential explanation for these opposing effects is that there are different sources of endogenous cannabinoid (endocannabinoid) transmitters, such as 2-arachidonoylglycerol (2-AG) and anandamide. Using the well-defined central nervous system of the medicinal leech (*Hirudo verbana*), which possesses the same endocannabinoid transmitters found in mammals, we can study endocannabinoid sources and their potential effects on nociception. Previous experiments using pharmacological methods showed that the L motor neuron, which is suspected to synthesize 2-AG, contributes to the anti-nociceptive effects seen. Our goal is to confirm that this neuron is a source of 2-AG and to identify other neurons that synthesize this transmitter. This was accomplished using Fluorescent *In Situ* Hybridization (FISH) procedures to determine which neurons express diacylglycerol lipase (DAGL) mRNA. DAGL is an enzyme that acts on diacylglycerol (DAG) to synthesize 2-AG. Previously our lab sequenced the *H. verbana* DAGL gene (accession #KU500007), the gene was found to have 34 and 39% amino acid sequence identity when compared to mouse and human DAGL-beta genes, respectively. Analysis of the catalytic domain shows sequence similarities in phosphorylation sites, as well as the presence of the ‘signature motif’ (PLYLPKGIIY) within the regulatory loop. From the completely sequenced version of the *H. verbana* DAGL gene, we developed sense and antisense DAGL mRNA probes using a 510 base pair segment of the gene. Probe validation was first performed with hybridization of human embryonic kidney cells (HEK cells) for both control and DAGL transfected cells. Imaging confirmed that the mRNA probes were selective for detecting *H. verbana* DAGL. The staining for DAGL mRNA was interestingly punctate in the HEK cells, a phenomenon that has been observed in other studies using *in situ* detection of DAGL (Liu, et al. 2008). Hybridization experiments of leech ganglia are currently being conducted and imaged to determine which neurons contain DAGL mRNA. Expression patterns are also being analyzed in a segment-specific manner along the *H. verbana* nerve cord.

Theme: Neural Excitability, Synapses and Glia
COCAINEMEDIATED SIGNALING REQUIRES EXCITATORY D1-LIKE DOPAMINE RECEPTORS TO MODIFY SPECIFIC NEURAL CONNECTIONS ONTO MIDBRAIN DOPAMINE NEURONS

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Dopamine plays an important role in communication between neurons, notably in the neural pathway that is involved in motivation and reward-seeking behavior. This pathway, called the mesolimbic dopaminergic pathway, can be altered by drugs of abuse 24 hours after a single exposure, which can lead to addictive behavior. Our research is attempting to characterize the mechanism of cocaine addiction via the mesolimbic pathway, specifically between the midbrain regions pedunculopontine tegmental nucleus (PPN) and substantia nigra pars compacta (SNc). Prior research has shown that cocaine induces changes in receptor composition at synapses between glutamatergic neurons in PPN and dopaminergic neurons in SNc. The ratio between two glutamate receptors, NMDA and AMPA, is used to assess this synaptic plasticity in response to in vivo cocaine exposure in mice. A virus encoding a fluorescent protein (YFP) and a light-operated cation channel (ChR2) is injected via stereotaxic surgery, allowing us to selectively excite PPN-innervated synapses on SNc dopamine neurons. Using electrophysiological recordings, 24 hours after in vivo cocaine exposure causes a decrease in the AMPA to NMDA receptor ratio. It is unknown, however, whether excitatory D1-like or inhibitory D2-like receptors are involved in this change. Here, a D1-like receptor antagonist (SCH 23390) was injected prior to cocaine treatment, which prevented the cocaine-induced decrease in the AMPA/NMDA ratio. This demonstrates activation of D1 receptors mediates cocaine-induced plasticity of the PPN-SNc synapse.

Theme: Neural Excitability, Synapses and Glia
Alzheimer’s disease (AD) is a form of dementia characterized by devastating loss of memory. The cholinergic hypothesis indicates that this memory loss is a result of mass cholinergic degeneration. The implicated hippocampal circuit includes the septum, the entorhinal cortex (EC), and the dentate gyrus (DG) of the hippocampus. The fiber projection sending information from the septum to the DG, called the septodentate (SD) pathway, is composed of several neurotransmitter systems, including cholinergic, GABAergic, and glutamatergic fibers. Previous studies indicate that a lesion to the SD pathway may have effects that are as detrimental to memory function as a lesion directly to the hippocampus. The entorhinal area projects to the DG in a largely unilateral input called the perforant path (PP). This study used a heterosynaptic model of AD by examining the effect of SD cholinergic neuron loss on the induction of long-term potentiation (LTP; a possible neural substrate of learning and memory) in the PP. Male Sprague-Dawley rats were given an intraseptal injection of either 192 IgG-saporin, a cholinergic neurotoxin, or a saline vehicle. After a 21-day wait period, stimulating electrodes were placed in the medial septum and the EC. Evoked, field excitatory post-synaptic potentials (fEPSPs) were recorded in the DG following stimulation of the EC, both unpaired and paired with stimulation of the septum at a range of inter-pulse intervals (IPIs; from 30 ms to 500 ms). Recordings were collected before and after the induction of LTP through a high-frequency tetany protocol. Shortly after tetanization, an intrahippocampal injection of the muscarinic antagonist scopolamine was made to render any remaining cholinergic inputs inert. The inclusion criteria for this project is determined by two histological procedures. The first stain labels choline acetyltransferase in the medial septum to visualize cholinergic cell bodies. The second stain labels acetylcholinesterase in the DG to measure the density of AChE-containing, cholinergic projections via the SD pathway. Absence of these enzymes in their respective regions indicates the destruction of SD cholinergic projections. We are currently processing the brain tissue and performing analyses of the neurophysiological data. The results of this study will hopefully give the scientific community more insight into the role of septal cholinergic neurons in LTP involving the perforant path and the underlying neurobiology of memory deficits in Alzheimer’s disease.

Theme: Neural Excitability, Synapses and Glia
sAPPα COMPARABLY FACILITATES LTP IN MICE AND RAT HIPPOCAMPAL SLICES REGARDLESS OF STEREOISOMERIC FORM

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Alzheimer’s Disease is a neurodegenerative disorder involving memory deficits due to the oligomerization of amyloid beta (Aβ) and formation of amyloid plaques. Aβ plaques are formed from Amyloid Precursor Protein’s (APP) cleavage by β and γ secretases. However, APP can also be cleaved by α-secretase, which reduces the quantity of Aβ. Secreted APPα (sAPPα) is the end product of this cleavage, and this protein has neuroprotective properties. sAPPα and a peptide within its sequence, arginine-glutamate-arginine (RER) have been shown to improve Long Term Potentiation (LTP) of synaptic transmission in rodent hippocampal slices. Here we compared whether the effects of Ac-RER are comparable to those of Ac-rER, a diastereomeric form that is less prone to degradation by endogenous enzymes. We found that Ac-RER and Ac-rER facilitate LTP to the same extent, approximately 35-45% over baseline, 60 min post-theta burst stimulation, in mouse slices at room temperature. We also investigated whether Ac-rER produced comparable LTP in slices from rats vs mice. Again, we found that Ac-RER and Ac-rER produced LTP, magnitude 30-45%, 60 min post-theta stimulation in rat hippocampi. These results were consistent with those of Jodi Morrissey, a collaborator at the University of Otago, who records at 32°C. Taken together, they demonstrate a remarkable consistency of synaptic effects of this tripeptide across rodent species, regardless of stereoisomeric form or temperature at which experiments are conducted.

Theme: Neural Excitability, Synapses and Glia
CHARACTERIZING THE EFFECTS OF OPTOGENETIC ACTIVATION OF SIFAMIDE NEURONS ON SLEEP IN DROSOPHILA MELANOGASTER

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Although animals spend a huge amount of time sleeping, the mechanisms modulating sleep still remains unclear. Recent studies showed that neuropeptides, a diverse group of proteinaceous molecules involved in neural communication, might play a critical role in modulating sleep. SIFamide (SIFa) is a kind of neuropeptide produced in four specific neurons in the fruit fly Drosophila melanogaster. Previous studies suggested that SIFa plays a critical role in feeding behavior, courtship behavior, and sleep. However, the pathway and the mechanism of SIFa remained unclear. To further characterize the effect and the pathway of SIFa in sleep, we first created transgenic fly lines whose SIFamide neurons would be activated optogenetically by red light. Then, by utilizing the DAM (Drosophila Activity Monitor) system, we recorded and quantified sleeping patterns of experimental Drosophila in response to red light stimulus. Five different sleep experiments with different stimulus durations and stimulus onset were conducted. Then we videotaped experimental animals to quantify their behaviors during and after the red light stimulus, which included grooming, walking, sleeping, and seizing. Our DAM data showed that activity of experimental flies strongly decreased during the stimulus, while that of control flies showed no response to the stimulus. Moreover, DAM data also suggested that female experimental flies had stronger responses than males to activation of SIFa neurons. On the other hand, the acute data revealed that intensity of the stimulus played a significant role in inducing resting response, while the duration of stimulus was not as critical. Our DAM data and acute data supported previous studies, which proposed that SIFa is a sleep promoter, and our DAM data suggested possible sexual dimorphism in response to SIFa.

Theme: Neural Excitability, Synapses and Glia
VALIDATION OF ADENO-ASSOCIATED VIRAL (AAV) KNOCKOUT OF EZH2 IN EMBRYONIC MOUSE HIPPOCAMPAL NEURONS

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Approximately 50 million people worldwide have Temporal Lobe Epilepsy, making it the 4th most common neurological disorder. Epilepsy is a central nervous system disorder marked by a pattern of repeated seizures. Current treatments such as anti-convulsants mitigate single seizure episodes but are unable to modify or stop disease progression. Thus, an understanding of epileptogenesis, the process by which epilepsy develops and progresses, is essential to long-term seizure prevention. Seizures can give rise to enduring changes in gene expression patterns that are mediated by transcriptional or epigenetic factors. Changes in gene expression can dramatically influence the response to repeated seizure episodes and could facilitate the process of epileptogenesis. We have identified the Enhancer of Zeste Homolog 2 (EZH2), the enzymatic subunit of a transcriptional silencing complex called Polycomb Repressive Complex 2 (PRC2) as a principle driver of transcriptional changes after Status Epilepticus (SE). Our lab has shown that levels of EZH2 significantly increase 2-5 days after SE. In addition, brief, systemic administration of the pharmacological EZH2 inhibitor, UNC1999, to mice subjected to SE significantly increases long term seizure burden. This suggests that EZH2 acts protectively rather than pathologically during epileptogenesis. To further test the hypothesis that EZH2 is a protective agent against seizure progression, we will knock out the EZH2 gene using AAV9-CaMKII-H1-eGFP-Cre-WPRE in an EZH2 floxed mouse model. To validate our AAV-Cre-expressing vectors, we have cultured embryonic day 14.5 (E14.5) hippocampal neurons from pregnant EZH2flox/flox mice and used AAV9-CaMKII-H1-eGFP-Cre-WPRE to knock out the EZH2 allele in vitro. We validated the knockdown via genomic PCR, western blot for EZH2 protein, and RTPCR for mRNA. We also validated knockout in neurons using immunofluorescence microscopy. Successful deletion of this allele in vitro will be useful towards future experiments where AAV9-CaMKII-H1-eGFP-Cre-WPRE will be stereotactically injected into mice prior to kainic acid induction. We hypothesize that latency to SE and overall seizure burden will increase after knocking out EZH2. This study will inform whether EZH2 agonists could act as novel anti-epileptogenic drugs. By mimicking the brain’s innate protective mechanism, the clinical approach to treating epilepsy can shift from one of merely managing symptoms to one that targets the root cause of the disease.

Theme: Neural Excitability, Synapses and Glia
Caenorhabditis elegans (C. elegans), an essential research organism in studying molecular aspects of the nervous system, facilitate direct neural cell communication using intercellular electrical channels, called gaps junctions. In C. elegans, these gap junctions are composed of innexins. An essential method of studying these innexins is demonstrated through RNA interference (RNAi), wherein specific RNA sequences bind to mRNA to suppress its production of protein. By using RNAi through feeding, we can suppress innexin gene expression specifically and observe the effects on subsequent behavior. Based on previous research and understanding of the neural circuitry behind head movement, we hypothesize that specific gap junctions localized to the GLR cells, and the innexins that comprise them, contribute to coordinated head movement. Through the use of RNAi-fed C. elegans, we analyzed the effect of Innexin-6 (Inx-6) and Innexin-10 (Inx-10) gene suppression on head movement using head suppression assays. We compare resultant behavior to wild type and control worm behavior to evaluate head movement coordination and look for any effects Inx-6 and Inx-10 might have on gap junction functionality in head movement. We plan to further expand our research by creating transgenic worms using plasmids linking KillerRed, a photosensitizer that can precisely inactivate proteins, to the promoter region of selected innexins. Through both techniques we hope for a more complete understanding of the impact of gap junction function in C. elegans head movement.

Theme: Neural Excitability, Synapses and Glia
Dependence on nicotine-containing products is a major cause of preventable diseases in the U.S. and worldwide. Current smoking cessation therapies with varenicline and bupropion have still a high rate of relapse. Behavioral tests that measure the response to nicotine following pretreatment with a chemical are used in animal models could lead to the discovery and development more effective treatments for smoking cessation therapies. Larval zebrafish represent an excellent model for the screening of chemicals that reduce the neurobehavioral response to nicotine. In addition, comparing gene expression profiles before and after pretreatments and before and after nicotine application could provide new insight into the function of chemicals that reduce the behavioral response to nicotine. When exposed to nicotine in the water, larval zebrafish respond with an increased movement activity. Previously, we have identified chemicals in the serotonin system that reduce the acute response to nicotine. Pretreatment with serotonin receptor agonists for serotonin receptors of type 2 (htr2c1l) and agonists and antagonists for the serotonin receptor type 2a (htr2aa) reduce the acute nicotine response significantly. Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) methods were used to explore gene expression profiles associated with the acute nicotine response including the htr2c and htr2a mediated change of nicotine responses. First experiments confirm a significant increase in cfos gene expression after 30 min of nicotine exposure. In contrast, htr2c1l and htr2aa receptor gene expression appears to remain unchanged. The cfos gene activity returns to background levels within 2 hours after the start of nicotine exposure. No significant change in htr2c1l and htr2aa receptor gene expression seems to occur within 24 hours of nicotine exposure. Higher levels of htr2c1l gene activity have been measured compared to the htr2aa gene. The results indicate that nicotine itself does not appear to change gene expression levels of serotonin receptors htr2c1l and htr2aa within 24 hours of nicotine exposure. In the future, experiments will explore gene expression profiles following pretreatment with serotonin receptor agonists and antagonists that reduce the acute nicotine response.

Theme: Neural Excitability, Synapses and Glia
SYNTHETIC NEUROEXCITATORY VENOM PEPTIDES FOR USE ON ADULT ZEBRAFISH SPINAL NEURONS IN PRIMARY CELL CULTURE

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Venom peptides from a variety of different species have proven themselves as useful reagents for research and pharmacological development. Active neuroexcitatory peptides from the venom of *Conus catus*, a fish hunting cone snail, have been synthesized for use in physiological studies. The linear synthetic peptides were purified and folded in a glutathione buffering system to form disulfides in the active peptides. In order to test the neuroexcitability of these synthetic venom peptides, a primary cell culture of adult zebrafish spinal neurons was developed. The resulting cells exhibit distinct neuronal morphology and behavior, with large cell somas, long extending axons and dendrites, and highly active growth cones on extending neurites. Cells in culture positively label for neuronal and axonal markers using immunofluorescence, supporting neuronal identification. Neurons used for whole cell patch clamp recordings showed strong voltage dependent inward and outward currents, making this preparation ideal for electrophysiological analyses. Such a preparation creates a robust model for studying the neuroexcitability of the synthetically derived venom peptides.

Theme: Neural Excitability, Synapses and Glia
AMANTADINE PLUS ENVIRONMENTAL ENRICHMENT AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY CONFERS AN ADDITIVE EFFECT ON MOTOR AND COGNITIVE IMPROVEMENT

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Traumatic brain injury (TBI) is a significant health care issue with limited treatment options. Amantadine (AMT) is a pharmacotherapy with dopamine receptor agonist activity that has been shown to improve cognition after experimental TBI. Another therapeutic strategy that has consistently been shown to confer cognitive and motor recovery after TBI is environmental enrichment (EE), which consists of a complex living space, novel stimuli, and increased social interaction that is vastly different from standard (STD) housing, and is considered a preclinical model of neurorehabilitation. Hence, the goal of this study was to test the hypothesis that combining AMT and EE would lead to greater motor and cognitive performance after TBI than AMT alone. Anesthetized adult male rats received a controlled cortical impact of moderate severity (2.8 mm tissue deformation at 4 m/s) or sham injury and then were randomly assigned to enriched or STD housing where AMT (20 mg/kg) or vehicle (VEH; 1.0 mL/kg) was administered intraperitoneally 15 min before testing every day for 19 days. Motor function (beam-balance/beam-walk) and spatial learning/memory (Morris water maze; MWM) were assessed on post-operative days 1-5 and 14-19, respectively. Both AMT alone and AMT plus EE performed significantly better than the non-treated STD-housed group on motor and cognition ($p < 0.05$). Moreover, the AMT plus EE group performed better than the AMT alone group ($p < 0.05$), which indicates an additive effect and confirms the hypothesis. The findings provide support for combinational therapies after TBI to optimize outcome.

Theme: Neurodegenerative Disorders and Injury
Methamphetamine (METH) is a widely used psychostimulant drug, and its use in the United States has reached a near-epidemic in the past 15 years, due to the ease with which METH can be manufactured, as well its highly addictive properties. METH use costs the government billions per year through crime, foster care, lost workplace productivity, and other social problems, in addition to causing destructive effects in the lives of users. In humans, METH abuse has been shown to result in long-lasting brain injury as well as significant cognitive impairments. METH interacts with the catecholamine nerve terminals in the brain, inducing non-exocytotic transmitter release, which results in the initial euphoria after taking the drug but then leads to long-lasting brain injury for the user. The neurotoxic effects of the drug are responsible for inducing the cognitive consequences associated with abuse, which include impairments in memory, attention, executive functioning, and decision making skills. The memory impairments caused by METH are seen as the most prominent and persistent cognitive problems, because they interfere with the abuser’s ability to adhere to and benefit from addiction treatment. Therefore, it is of utmost importance to find ways to attenuate these cognitive deficits and thereby improve treatment outcomes for METH users. Exercise is well known for its beneficial physiological effects, and its cognitive enhancing properties. In terms of METH abuse, previous research has demonstrated that in an animal model, post-METH voluntary running can significantly attenuate the dopaminergic neurotoxicity induced by a binge regimen of the drug, potentially due to the upregulation of neuronal growth factors through exercise. Therefore, we have reason to suspect that exercise may also ameliorate the cognitive deficits incurred by METH. The present study examined the effects of post-METH exercise on two well-validated tests of memory in an animal model: object recognition and odor recognition, in hopes of demonstrating an attenuation of METH-induced memory specific cognitive impairments as a result of exercise.

Theme: Neurodegenerative Disorders and Injury
EFFECTS OF PROBIOTICS ON ALZHEIMER’S DISEASE PROGRESSION: ASSESSING COGNITIVE AND CELLULAR MARKERS

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Alzheimer’s disease (AD) is the sixth leading cause of death within the United States and the projected number of affected individuals is on the rise. Currently, no effective treatments have been identified, but the rise in costs and number of affected patients requires identification of easily accessible and affordable strategies to help manage symptoms. Recently, there has been growing interest in the gut-brain axis (GBA), a bidirectional communication system between the brain and the gastrointestinal system, for its potential impact on brain health. The gut microbiota, microorganisms populating the gut, plays an essential role in the host health and microbial imbalances (dysbiosis) have been correlated to several neurodegenerative disorders. We sought to explore probiotics as a potential therapeutic treatment for Alzheimer’s disease in a triple transgenic mouse model (3xTg-AD). In a preliminary study, probiotic treatment decreased cell death and normalized the number of GFAP+ cells present in AD mice. Due to the abnormal behavior of control animals, we repeated the experiment with a new cohort of mice to confirm our original findings. Here, AD mice received a control diet (ADC; n=5) or a diet supplemented daily with probiotic strains *Lactobacillus curvatus* and *Lactobacillus plantarum* (ADP; n=5) and were compared to wild-type mice on control diet (B6129SF2J-1 - WTC; n=5). Behavioral analysis using the Barnes Maze indicated a decreasing trend in escape latency of ADP mice in comparison to ADC mice after 12 weeks. Upon completion of the probiotics treatment, mice were sacrificed and immunohistological analysis was performed on brain tissue. ADC mice showed an overall decrease in total number of DAPI+ cells and a correlated decrease in neurons (NeuN+), while increases in amyloid beta+ and GFAP+-cells were detected. In comparison, AD mice treated with probiotics showed higher cell counts for DAPI+ and NeuN+. Further assessment of the effects of probiotics on all cell populations is being conducted. Overall, our results point toward a beneficial effect of these probiotics on AD pathology progression, possibly driven by a mitigation of the previously reported dysbiosis in the current AD model.

Theme: Neurodegenerative Disorders and Injury
UNDERSTANDING PARKINSON'S DISEASE IN YEAST MODELS: THE NATURE OF A-TOXICITY LINKED WITH A30P, H50Q AND A53E A-SYNUCLEIN MUTANTS

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Parkinson’s disease (PD) is associated with the aggregation and misfolding of alpha-synuclein in midbrain dopaminergic neurons. The gene for alpha-synuclein has six known mutations that cause early-onset familial forms of PD. The pathological determinants of three of these mutants (A30P, E46K, and A53T) are well characterized in diverse model systems and they reveal that each mutant affects cellular toxicity in distinctive ways. The three more recently discovered familial mutants (H50Q, G51D, and A53E) are not extensively studied. We expressed H50Q, G51D, and A53E mutants in budding and fission yeast model systems and hypothesized that each would generate toxicity by altering their membrane association and aggregation properties, and by disrupting cellular pathways including nitrative stress responses and endocytosis, but each would do so in distinctive ways. First, we found that the H50Q and A53E mutants were toxic to yeast, and bound membranes and aggregated within yeast, while G51D was cytoplasmically diffuse and nontoxic. Secondly, we asked whether the loss of the original amino acid or the gain of the new amino acid in each new familial mutant is responsible for disease. We created four substitution mutations for H50Q, G51D, and A53E in both yeasts models corresponding to the four functional classes of amino acids. We found that H50D was cytoplasmically diffuse and nontoxic, G51A bound membranes and aggregated like WT, G51E was cytoplasmically diffuse and nontoxic like G51D, and A53R was cytoplasmically diffuse and nontoxic, suggesting both the loss of the original amino acid and the gain of the new amino acid are key. Thirdly, we found that some of these new familial mutants had increased toxicity in yeast strains altered for nitrative stress (particularly G51D), sumoylation, and endocytosis. Collectively, this work adds insight into the pathogenicity of different familial PD mutants of alpha-synuclein.

Theme:  Neurodegernerative Disorders and Injury
EFFECTS OF DCP-LA ON LEARNING AND MEMORY IN AN IN VIVO ALZHEIMER'S DISEASE MODEL WITH OVARIECTOMIZED RATS

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Alzheimer disease (AD) is the sixth leading cause of death in the United States and affects females disproportionately more than males for reasons unknown. Several promising studies have shown that the PKC-epsilon activator 8-[2-(2-pentyl-cyclopropylmethyl)-cyclopropyl]-octanoic acid (DCP-LA) reverses the effects of AD in in vivo models. Because these studies have been limited to either male transgenic mice or male rats, this study explores the effects of DCP-LA in a pharmacological AD model in female rats. The ferrous-amyloid-buthionine (FAB) model was used to induce AD pathologies. This model infuses a combination of ferrous sulfate, amyloid beta fragments, and buthionine sulfoximine into the lateral ventricle of rats over a four-week period. The FAB model mirrors both behavioral and molecular pathologies of AD and models the role of oxidative stress in AD. In the present study, half of the females also received ovariectomies (OVX) to model postmenopausal conditions, since the female population over 65 is the most susceptible to AD. Osmotic minipumps containing either FAB or saline were implanted into 48 three-month-old female Sprague-Dawley rats with infusion cannulae targeted to the cerebral ventricle; at the same time, animals received either OVX or sham surgery. Four weeks postsurgery, the rats received a single intraperitoneal injection of DCP-LA (1 mg/kg in 5% DMSO) or vehicle 24h prior to exposure to the Morris Water Maze (MWM). Spatial learning was assessed over 5 days of training, and memory was assessed 48 h after the last training session. FAB-treated animals showed significant deficits in both learning and memory as compared to controls. OVX did not affect learning but did lead to memory deficits as compared to controls, with FAB + OVX rats performing worse than all other treatment groups in the memory task. DCP-LA administration effectively restored learning and memory performance back to control levels in the FAB-treated animals but did not fully restore memory deficits in OVX animals, suggesting that FAB and OVX impair memory through different mechanisms. These data suggest that the loss of sex hormones in females may lead to cognitive deficits and these deficits can be exacerbated by oxidative stress conditions similar to those implicated in AD. Furthermore, these results suggest that DCP-LA may have therapeutic potential for treatment of AD, as it has now been shown to reverse the FAB in both male and female rats.

Theme: Neurodegenerative Disorders and Injury
THE EFFECTS OF OLFATORY DEFICITS ON EXECUTIVE FUNCTION AMONG HUMAN SUBJECTS

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Further elucidating a connection between olfaction and cognition has potential implications in maintaining neuronal health among both young and elderly individuals. The hypotheses tested were that olfaction and cognition are correlated and that resolving olfactory deficits may improve cognitive processing. Collaboration with a team of RPI computer scientists allowed for the construction of a testing platform and database to measure subjective and objective olfaction, working memory, attention processing, physical health, psychological profile, along with a number of demographic variables both in person and online. Human subjects have been tested, in the age demographic 18 to 24 to test these hypotheses and validate the novel approach to testing that has been designed. A connection between subjective olfaction and attention processing in the Trail-making Task and spatial processing in the Mental Rotation Task has been determined among young adult participants. Additionally, relationships have been found between subjective and objective olfactory measures in these study participants, validating the in person and online testing approach. Remaining questions being addressed are age differences for these effects and the role of long-term olfactory training. Physicians utilize lost olfaction as a predictor for Alzheimer’s Disease; characteristic neural degeneration is initiated at the olfactory portions of the brain, which may support the claim that amyloid beta (Aβ) contributes to an immune response against invading microbes entering the brain through the Brain-Nasal Cavity interface (B-NC). Aβ plaques may even be the product of aggregating invading pathogens. Investigations into the molecular targets for these effects are ongoing. Research has posited that Cerebrospinal Fluid (CSF), containing the destructive Aβ, clears out of the brain through the lymph vessels protruding from the cribriform plate in the B-NC. Correcting lost olfaction may restore health to this region, thereby, facilitating CSF flow and promoting neuronal health, which is the ultimate goal of this work.

Theme: Neurodegenerative Disorders and Injury
GRADUAL LOSS OF OVARIAN FUNCTION EXACERBATES AGE-DEPENDENT COGNITIVE DYSFUNCTION IN AN ALZHEIMER’S DISEASE MOUSE MODEL

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Over two-thirds of individuals with Alzheimer’s Disease (AD) are female, implicating biological sex as a major risk factor for the onset and progression of AD. Biological differences between males and females – most notably the gradual loss of ovarian hormones during the perimenopausal transition – are thought to be critical factors contributing to the greater female risk. To investigate the possibility that menopause exacerbates the development and progression of AD, we are inducing transitional menopause (TM) in female mice with the ovotoxin 4-vinylcyclohexene diepoxide (VCD). We are using the APPSwDl/mNos2−/− AD (CVN-AD) mouse model, which mimics familial AD with the expression of mutated APP and creates a human-like immune environment through lowered NOS2 expression. CVN-AD mice exhibit many of the neuropathological features of human AD, as well as exacerbated AD-like neuropathogenesis and resistance to therapeutic intervention in females. Both wild-type C57BL/6 and mNos2−/− mouse lines serve as controls. To evaluate cognitive function and the impact of TM in female CVN-AD mice, we are using a Barnes Maze task to evaluate spatial learning and memory. As expected based on their neuropathogenic progression, 4-month old CVN-AD mice do not differ from control mice on latency to locate the escape hole, while 14-month old CVN mice are significantly impaired. However, when we analyzed the strategies used to locate the escape hole we found that both young and old CVN-AD mice used a non-spatial, serial-search strategy, whereas control mice were more likely to navigate to the hole directly using spatial cues. We also determined that while TM did not adversely alter spatial learning in control mice, loss of ovarian hormones in CVN-AD mice drastically impaired their ability to locate the escape hole. TM also increased the likelihood that CVN-AD mice used a serial search strategy. Our findings support previous reports that CVN-AD mice show progressive, age-related spatial learning deficits, and reveal that young CVN-AD mice are likely performing well on the Barnes Maze task by using a compensatory, non-spatial strategy to locate the escape hole. Moreover, our study demonstrates that a gradual, menopause-like loss of ovarian hormones exacerbates AD-like cognitive decline. Ongoing and future studies will investigate the effects of TM on other aspects of AD-like disease progression, and the response of females to therapeutic interventions at various stages of the menopausal transition.

Theme: Neurodegenerative Disorders and Injury
ROLE FOR COMPLEMENT IN OPTIC NERVE REGENERATION FOLLOWING OPTIC NERVE CRUSH

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Cell survival and axon regeneration are often limited in the central nervous system (CNS) following injury or disease, creating a significant need for novel therapeutic interventions that improve regeneration. The mouse optic nerve crush model mimics neurodegenerative diseases and traumatic CNS injuries by inducing progressive death of retinal ganglion cells (RGCs), the neurons whose axons were severed and do not regrow. Complement proteins of the innate immune system have been found to have neurotoxic, neuroprotective, and neurodegenerative roles in various injury and disease models, though their role in cell survival and regeneration following optic nerve crush have not yet been studied. Zinc plays a crucial role in a variety of cellular functions, accumulates in amacrine cells and RGCs after optic nerve injury, and its removal by zinc chelation (TPEN or ZX1) increases RGC survival and regeneration after optic nerve injury. We hypothesize that complement is required for RGC survival and regeneration after optic nerve injury. We tested this hypothesis by assessing RGC survival and axon regeneration in mice with deletion of complement proteins C1q and C3 and the C3 receptor CR3, as well as wild-type control mice 14 days after optic nerve crush plus zinc chelation treatment (TPEN). Our data demonstrate that while neither C1q, C3, nor CR3 affect RGC survival, C1q, C3, and CR3 are each required for RGC axon regeneration treatment. Further investigation into the role of the innate immune system in CNS axon regrowth may lead to the development of interventions to improve regeneration following injury.

Theme: Neurodegenerative Disorders and Injury
Parkinson’s disease (PD) is a progressive neurodegenerative disorder due to the loss of dopamine neurons within the nigrostriatal pathway. The destruction of these neurons through inflammation may be a result of enhanced glial cell responses. Curcumin, a compound derived from turmeric, has shown to not only provide protection to dopamine neurons, but also reduce the inflammatory response of glial cells in a 6-hydroxydopamine nigral lesion model. Furthermore, previous research from our lab has demonstrated that curcumin has both protective and restorative properties in this acute model of PD. To determine the scope of protection offered by curcumin, the current study was designed to investigate whether curcumin could exert a similar protective effect in a striatal 6-hydroxydopamine rat model of PD. Each experimental animal received an injection (i.p.) of 75 mg/kg curcumin for 5 days/week while the control animals received an equal volume of the vehicle, DMSO. Due to the progressive nature of this lesion, behavior data using the rotarod and foot-fault test were obtained for seven weeks post-surgery. Dopamine cell survival within the substantia nigra is being assessed via stereology of tyrosine hydroxylase immunoreactive neurons.

Theme: Neurodegenerative Disorders and Injury
EFFECTS OF DEXTRAN SULFATE SODIUM-INDUCED COLITIS ON AN APPNL-G-F MOUSE MODEL OF ALZHEIMER’S DISEASE

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Alzheimer’s disease (AD), the most common form of age-related dementia, is a neurodegenerative disease pathologically characterized by the extracellular deposition of plaques, intracellular neurofibrillary tangles, and gliosis in the brain. Inflammatory bowel disease (IBD), including Crohn’s disease and colitis, are other forms of chronic inflammation with increased prevalence in the elderly. Due to the bidirectional communication of the brain with the gastrointestinal tract via the gut-brain axis, we hypothesized that the AD brain pathologically reacts to the inflammation initiated in the colon. To test this idea, we used dextran sulfate sodium (DSS)-induced model of colitis in the APP$^{NL-G-F/NL-G-F}$ transgenic mouse model of AD. Both C57BL/6 wild type control and APP$^{NL-G-F/NL-G-F}$ mice demonstrated severe colitis-like symptoms following DSS treatment. Surprisingly, DSS treated mice resulted in no differences in reactive microgliosis with mild differences in astrogliosis in both APP$^{NL-G-F/NL-G-F}$ and wild type mice. However, DSS treatment resulted in increased protein levels of cyclooxygenase 2 (COX-2) and the amyloid precursor protein (APP) in the APP$^{NL-G-F/NL-G-F}$ compared to wild type mice. These findings support the hypothesis that intestinal inflammatory changes affect the brain during AD perhaps through increasing inflammatory prostaglandin and amyloid β (Aβ) levels.

Theme: Neurodegenerative Disorders and Injury
ACUTE TRANSCRIPTOME RESPONSE OF THE MIDBRAIN TO INJURY IN THE MUMMICHOG (FUNDULUS HETEROCLITUS)

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Unlike adult mammals, adult fish produce new cells throughout their central nervous system (CNS) during the course of their lives. Furthermore, they maintain a tremendous capacity to repair damaged neural tissue. Much of the focus on understanding brain repair and regeneration in fish has been directed at neuronal tissue in regions of the brainstem, forebrain, and retina; however, the mesencephalon (midbrain) has received little attention. Using the mummichog (Fundulus heteroclitus) as a model species, we developed a reliable method in which to administer an accurate mechanical lesion to the adult fish midbrain optic tectum and underlying tegmentum. We then began characterizing the adult fish midbrain response to injury using a whole transcriptome approach. Specifically, RNA Seq was used to examine differential gene expression of the midbrain in response to a mechanical lesion at an acute recovery time of 1hr post-injury. Comparisons of whole transcriptomes, derived from isolated RNA of intact and injured midbrain tissue, identified nearly 600 differentially expressed genes with False Discovery Rates (FDRs) of either < 0.1 (181 genes) or < 0.4 (404 genes) using both 'condition' and ‘replicate + condition’ models. The majority of differentially expressed genes were upregulated (visualized via MA plot), and based on functional annotation, showed that the injured tissue was engaged in cellular processes such as proliferation and neurogenesis. Using qPCR, we validated the upregulation of two highly differentially expressed genes, *pim-2* and *syndecan-4*, as well as an example of a non-differentially expressed gene, brain *insulin-like growth factor* (*igf-1*). In related work, we examined the temporal profile of the mummichog midbrain reparative process from acute to chronic times of recovery. We found that brain *igf-1*, though not differentially expressed at 1hr post-lesion, was significantly downregulated at 48hr post-lesion in the injured midbrain tissue relative to non-injured midbrain tissue. Overall, these data indicate that whole transcriptome approaches would allow for a comprehensive molecular profile of the reparative process in a brain regenerative-capable adult vertebrate and thus, give insight into the progression of cellular processes involved in successful brain repair. Supported by NIH-INBRE (P20GM103499), and the Furman University Research and Professional Growth (RPG) Award and The Furman Advantage (TFA).

Theme: Neurodegenerative Disorders and Injury
A NEUROTROPHIC FACTOR MIMETIC IMPROVES MEMORY RETENTION IN AN AGED, TRANSGENIC MOUSE MODEL OF ALZHEIMER’S DISEASE

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Alzheimer’s disease (AD) is associated with an increased accumulation of amyloid beta (Aβ) peptide in the brain, which is believed to lead to cognitive impairment. Neurotrophic factors, which aid neuronal growth and survival, have shown promise for treating AD symptoms. We tested the effectiveness of a growth factor mimetic (BTX-1039) in the treatment of spatial memory deficits. The transgenic mice used in this study have three mutations that lead to over-production of the 770 isoform of the human amyloid beta-precursor protein and associated Aβ in a C57BL/6J background strain. We conducted separate experiments with 8-month-old and 12-month-old mice. Mice were divided into six groups based on strain (transgenic or wild type) and drug dose (vehicle, 60 mg/kg, or 100 mg/kg). The sex ratio for each group was approximately 1:1. Mice received daily i.p. injections of 0.20 ml saline or BTX-1039 dissolved in saline for 14 consecutive days prior to starting behavioral testing. We used a Morris water maze protocol that consisted of 6 days of place-learning trials (submerged platform), 1 day of probe trials, and 3 days of cued trials (platform visible). For both age classes, the transgenic mice showed significantly longer path lengths during the place-learning trials relative to the wild type mice, indicating that the transgenes impaired spatial learning. However, there was no effect of the drug on place learning. For both age classes, the groups showed no differences in cued trials, indicating no effect of the transgenes or the drug on stimulus-response learning. For the probe trials, we observed a nearly significant impairment in memory retention among the transgenic mice relative to the wild type mice at 8 months of age and a significant impairment in the transgenic strain relative to the wild type at 12 months of age. For the 8-month-old mice, the transgenic strain injected with saline was the only group that did not perform significantly above chance levels during the probe trials. For the 12-month-old mice, transgenic groups injected with either saline or 60 mg/kg of the drug performed at chance levels during the probe trials. In summary, the results indicate that the transgenes impaired spatial memory acquisition and retention, whereas the drug improved retention specifically among the transgenic mice. This suggests that there may be some therapeutic value for BTX-1039 in the treatment of memory impairment associated with AD. For the 12-month-old mice, brains were collected after behavioral testing, and histological staining is underway to quantify Aβ plaques. This will allow us to determine whether the observed effects of the drug on memory retention correlate with Aβ levels.

Theme: Neurodegenerative Disorders and Injury
ALTERATIONS IN MOTIVATIONAL BEHAVIOR AND EXECUTIVE FUNCTION IN ADOLESCENT RATS FOLLOWING PEDIATRIC TRAUMATIC BRAIN INJURY

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Traumatic brain injuries (TBIs) affect 2.5 million individuals in the United States each year, ranging from mild concussions to severe trauma or death. With 500,000 yearly emergency room visits being attributed to childhood-acquired brain trauma (under 14 years of age), patients endure long-lasting cognitive impairments, as well as psychopathological consequences. When head trauma occurs during a critical period of neuronal development and maturation characterized by increased educational and environmental demands, survivors of childhood TBI display reduced learning rates, delays in making age-appropriate developmental gains, as well as behavioral and emotional disturbances. The overarching aim of this proposal was to assess clinically-relevant motivational and cognitive-behavioral dimensions in adolescent rats who received a TBI as pediatrics, by using an instrumental learning task and an attentional set-shifting test (AST). We hypothesized that rats subjected to TBI will display task-dependent impairments in motivated behavior and executive function. We employed a multimodal approach to determine instrumental learning and cognitive flexibility capabilities after moderate parietal lobe (2.2 mm tissue deformation depth at 4 m/sec) or sham controlled cortical impact injury to the right hemisphere in pediatric Sprague-Dawley rats (PND 17). After ten days of recovery, they were trained on a fixed-ratio schedule of 1 for 12 consecutive days in operant chambers fitted with three nose-poke holes and a food trough, by learning to poke for sucrose pellet reinforcement in the center hole when illuminated. Each session lasted 100 trials or 30 min, whichever occurred first. Outcome measures included the number of total trials completed, task-irrelevant pokes (left or right nose-poke holes), and latency for pellet retrieval following instrumental nose-poking. Rats were then trained/tested on AST at PND 42-43, which involves a series of increasingly difficult stages, including simple and compound discriminations, stimulus reversals, and intra/extradimensional set-shifts. Dependent measures included the number of trials to reach criterion, as well as total and perseverative errors. While testing is currently ongoing, upcoming statistical analyses will employ repeated-measures ANOVA followed by Newman-Keuls post hoc for individual test days when appropriate. These findings will advance our understanding of long-term higher-order cognitive and motivational deficits in adolescent survivors of childhood brain trauma.

Theme: Neurodegenerative Disorders and Injury
IMPACT OF SEVERAL PD-ASSOCIATED GENES ON THE TOXICITY OF α-SYNUCLEIN IN A YEAST MODEL

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Parkinson’s disease (PD) is characterized by α-synuclein misfolding and the death of midbrain neurons. PD can be described as familial, or sporadic, both of which are influenced by a multitude of environmental and genetic factors. Familial PD is directly caused by a mutation in one of at least ten genes, including SNCA, DJ-1, VPS35, and ATP13A2. SNCA, which encodes α-synuclein, has six identified missense mutations (A30P, E46K, H50Q, G51D, A53E, and A53T) that each cause aggressive PD. Sporadic PD is linked with several risk genes and loci, including VPS13, the Sac I domain of SYNJ1, and the Swa2 domain of DNAJC6. Using our previously established budding yeast model system for α-synuclein, we first show that wild-type (WT), E46K, A53T, H50Q, and A53E α-synuclein are toxic to yeast and show varying degrees of membrane binding and aggregation, while A30P and G51D α-synuclein are relatively non-toxic and shows cytoplasmic diffuse localization. What is still not well understood is whether the other PD-causing and risk genes mentioned above can influence toxicity and localization properties of WT α-synuclein and these six familial PD mutants. To test the hypothesis that they do influence α-synuclein, WT and familial mutant forms of α-synuclein were studied in haploid yeast strains that were singly deleted for these six PD-linked genes (all of which are linked to loss-of-function in PD). Results show that some gene deletions increase (Δhsp31) or decrease (Δatp13, Δvps35) α-synuclein toxicity and alter its localization in a highly familial mutant specific way, while others more broadly increase α-synuclein toxicity or aggregation (Δvps13, Δsac1), while still others show no effect (Δswa2). Our findings suggest that WT and each familial mutant of α-synuclein create cellular toxicity and alter localization in distinct ways and that each is likely regulated by different subsets of genes, opening doors for mutant-specific mechanistic insight into the varying modes of α-synuclein toxicity.

Theme: Neurodegenerative Disorders and Injury
Poster #64

INSIGHT INTO PARKINSON’S DISEASE FROM YEASTS: COMBINED IMPACT OF COVALENT MODIFICATIONS AND FAMILIAL MUTATIONS ON Α-SYNUCLEIN

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Parkinson’s Disease (PD) is a neurodegenerative disorder linked to the loss of dopaminergic neurons in the midbrain. A key pathological marker of PD is the presence of Lewy bodies, which are mainly composed of misfolded α-synuclein protein. α-Synuclein is a highly post-translationally modified protein. While phosphorylation and nitration of α-synuclein are well studied in the context of PD pathology, less is known about sumoylation, which is proposed to be neuroprotective based on limited studies. The majority of sumoylation takes place on the lysine-96 and lysine-102 sites of α-synuclein, and it increases the protein’s solubility. The goal of this research was to better understand the role of sumoylation in regulating α-synuclein toxicity, and we performed four studies towards it. First, we evaluated the effects of blocking sumoylation on α-synuclein in the well-established budding yeast model for PD and found that α-synuclein becomes more aggregated, gains toxicity, and loses localization at the plasma membrane. Second, we evaluated the effects of altering sumoylation pathways by using yeast strains with reduced (ulp1ts) or excessive sumoylation (smt3ts), and found that α-synuclein aggregates more with reduced sumoylation, but becomes less toxic with increased sumoylation. Third, we asked how altering phosphorylation of α-synuclein would alter sumoylation’s protective role and found that blocking phosphorylation reduced the protein’s toxicity. Finally, we evaluated whether blocking sumoylation and altering phosphorylation on familial PD mutant versions of α-synuclein would exacerbate its toxicity and found that such altered modifications did not have a significant impact. In the future, we will conduct further studies to understand how sumoylation affects other variants and modifications of α-synuclein.

Theme: Neurodegenerative Disorders and Injury
Multiple sclerosis (MS) is a complex disease, encompassing a wide range of physical, social, and psychological aspects; common symptoms include deficits in information processing speed, executive function, and visuospatial integration. Up to 65% of individuals also experience some type of cognitive dysfunction, with 5-10% having moderate to severe symptoms. The objective of this research is to characterize cognitive dysfunction in the marmoset experimental autoimmune encephalomyelitis (EAE) model. One of the most suitable primate models of MS, EAE in the common marmoset, reflects more closely the clinical, anatomical, and neuropathological aspects of MS in humans than any of the other current EAE models. In contrast to previous MS research, our study incorporates quantitative functional deficits in cognition, and investigates whether exercise results in improved performance on cognitive tasks and/or delays disease progression. Cognitive function is tested in all subjects with tasks involving executive control (tested via the object retrieval task with detours) and visuospatial integration (via the staircase task). We are also utilizing structural magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI), to measure differences in myelin density, as well as histological data to determine how neural physiology is altered by the disease and by exercise. Results of the study have applications for investigating novel therapeutic strategies, including combined pharmacological treatments and lifestyle factors.

Theme: Neurodegenerative Disorders and Injury
The auditory system of the adult cricket, *Gryllus bimaculatus*, which is necessary for both mating and avoiding predators, is comprised of two ears, located on each foreleg. Auditory afferents from each foreleg innervate ipsilateral ascending neurons (AN-2s), which then carry auditory information to the brain. After unilateral injury to an ear, AN-2 dendrites extend across the midline, a boundary they normally respect, to form functional synapses with the auditory afferents of the contralateral ear. This type of plasticity is highly unusual, and we are examining the consequences of this compensatory plasticity at the anatomical, physiological and behavioral level. The Horch lab has also found that the extent of the plasticity is highly variable, and we hope to understand the causes and consequences of this variability. By measuring the behavioral, physiological and anatomical changes after deafferentation within individual crickets, we can begin to understand how and if form influences function in this process. Using predator (bat) ultrasound stimuli, we are first able to elicit a negative phonotactic response that is quantifiable. We then use a modified suction electrode to record from and then iontophorese neurobiotin into auditory axons in the brain of the same cricket. The dye is trafficked down the axons into the AN dendrites in the prothoracic ganglia, allowing the collection of high-quality images of isolated ANs. This approach will create a more correlated data set that can relate behavioral, anatomical and physiological changes in the same cricket to elucidate a mechanism for compensatory growth.

Theme: Neurodegenerative Disorders and Injury
Brain-Computer Interface (BCI) devices use neural activity to create alternate methods of communication or control. A user’s intent is measured by their neural signals, which are translated to a computer program that provides a control mechanism in real-time. Of particular importance to this study is the alpha wave, which is associated with increased relaxation and an idling state of the visual cortex. This experiment used a wireless EEG headset to detect changes in alpha wave activity, blinking, and gyroscope activity to control a small robot through a series of tasks. In the present study, we examined the ability of participants to navigate an obstacle course, and determined whether performance improved with repeated training over a two-day procedure. Each participant had individualized, calibrated threshold values for the various BCI controls (alpha wave activity, blinking, and gyroscope activity), and was trained to operate the BCI-controlled robot. Following training, participants were asked to navigate the robot through an obstacle course designed to mimic a real-world scenario. Results from this study demonstrate that participants perform as well on the first day of testing as they do on the second day of testing, which occurred at least 24 hours later. Unlike other types of BCI devices, all participants who attempted the obstacle course were able to successfully complete it following training. High user success rates, and short training times, suggest that this low cost, wireless BCI device has potential to reach a broader population for use with a variety of applications.

Theme: Neurodegnerative Disorders and Injury
INVESTIGATING THE EFFECT OF TRAUMATIC BRAIN INJURY ON JAK/STAT ACTIVITY IN A DROSOPHILA MODEL OF GLIAL TAUOPATHY

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Traumatic brain injury (TBI) is a complex injury that triggers a sequence of destructive and neuroprotective cellular responses characterized by an extended period of secondary brain damage. Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease found in individuals with a history of TBI that is characterized by the aggregation of hyperphosphorylated tau in neuronal and glial cells. The molecular mechanisms that link TBI with the formation of tau pathology, and the pathological consequences of tau pathology, are largely unknown.

Glial cells are activated by a variety of brain insults, including TBI, yet the mechanisms that control their activation are only beginning to be defined. The janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway is a conserved signaling cascade involved with cell proliferation and cell death, and is also activated in reactive glial cells in response to TBI. This study aimed to determine the effect of TBI on JAK/STAT activation in a Drosophila model of TBI that inflicts rapid acceleration and deceleration of the fly. In addition, we explored the extent to which glial tau expression alters TBI-induced JAK/STAT activation. We found that the JAK/STAT pathway was activated 24 hours after one episode of TBI, and that the response was more robust in male flies, compared to female flies. Moreover, TBI-induced JAK/STAT activation was seen across all ages of male flies, whereas this response was only present in older female flies. The presence of tau in glial cells did not affect the transient JAK/STAT activation following one episode of TBI, however it did dramatically reduce JAK/STAT activation in a repetitive TBI paradigm. These findings demonstrate that TBI activates JAK/STAT signaling in Drosophila in a sex-specific fashion, and that the upregulation of JAK/STAT signaling after repetitive TBI is impaired by the presence of glial tau. Together, these studies identify age and sex-specific effects on the glial response to TBI and identify a role for tau in disrupting the activation of glial cells in response to injury.

Theme: Neurodegenerative Disorders and Injury
Prevalence of Alzheimer’s Disease (AD) in the U.S. is predicted to triple by 2050, thus necessitating disease models that facilitate ‘bench-to-bedside’ translation of therapies. Human induced pluripotent stem cells (iPSC) surpass common animal models of AD in this regard as they negate species differences. Dermal fibroblasts derived from humans with genetic risk factors for AD may be prompted back into the pluripotent state, and then derived into highly specific cell types while retaining the patient’s genetic profile. iPSC-derived cortical astrocytes from non-affected siblings were used to design an iPSC-based astrocyte inflammation response assay, as aberrant neuroinflammation is thought to lead to synaptic degradation in AD. Astrocytes were activated by the inflammatory microglial factors IL-1α, TNF-α and C1q. IL-6 release from astrocytes was examined by ELISA, and revealed IL-1α is both necessary and sufficient for significantly increased IL-6 release. qPCR for IL-6, ICAM1 and LCN2 showed that IL-1α, TNF-α and C1q are all necessary for the induction of a complete inflammatory profile. In an iPSC-derived in vitro model of neuroinflammation in AD, astrocytes should thus be stimulated with all three microglial factors, and IL-1α concentrations modulated to attenuate neurotoxic IL-6 release. iPSC are a powerful model of neurodegenerative diseases for three reasons: They (1) carry the genetic information of the diseased patient, (2) can be differentiated into relevant cell types with high specificity and (3) facilitate ‘bench-to-bedside’ translation of therapies due to the absence of species differences.

Theme: Neurodegenerative Disorders and Injury
Poster #70

TIME IN THE HIPPOCAMPUS: A NOVEL APPROACH TO EXAMINING THE FUNCTION OF TIME CELLS

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Space and time are both essential features of episodic memory, for which the hippocampus is critical. The involvement of the hippocampus in spatial processing was first described following the discovery of neurons, known as place cells, which fire with spatial-specificity (O’Keefe and Dostrovsky, 1971). More recently, the existence of hippocampal neurons that fire at successive moments in temporally structured experiences has been reported. These cells were dubbed time cells because it was suggested that they represented time (MacDonald et al., 2011). While spatial tasks have been used for the study of place cells, the tasks used for the study of time cells do not use time as an independent variable and therefore the behavioral relevance of this cell firing is unclear. In order to directly study the role of the hippocampus in processing elapsed time, we created a novel time duration discrimination task suited for the study of the function of time cells in memory. Twelve rats were tested on a figure-8-maze and experienced a 10- or 20-second time delay at the end of the center arm. During this delay, a 2000Hz tone played for the 10- or 20-second duration. Rats learned to make a decision to turn left or right out of the delay box depending on the associated tone duration (10 seconds = left turn; 20 seconds = right turn). Once the rats reached criterion performance of 90% correct on two out of three consecutive days, they received either an excitotoxic hippocampal lesion or a sham lesion surgery. After recovery, rats were tested to determine hippocampal involvement in discriminating time duration. Results from the time discrimination task will be discussed in terms of the involvement of the hippocampus in the processing of elapsed time.

Theme: Neurodegenerative Disorders and Injury
THE TOLL-LIKE 2 AND 4 RECEPTOR ANTAGONIST (+)-NALTREXONE REVERSES NEUROPATHIC PAIN AND ASSOCIATED SPINAL INFLAMMATION IN MALE AND FEMALE RATS IN A MODEL OF MULTIPLE SCLEROSIS

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Up to 92% of patients with multiple sclerosis (MS) report frequent and disabling neuropathic pain. In MS, neuropathic pain develops after demyelination, neuroinflammation, and damage to axons in the central nervous system. Although several treatments for MS-related neuropathic pain exist, many patients’ symptoms are refractory to current treatments. Recent research has provided evidence that toll like receptors 2 and 4 (TLR2/4) are implicated in propagating the inflammatory response of MS. Moreover, previous research in our lab has shown that TLR2/4 antagonists are effective at reversing neuropathic pain in various rodent models. In this study we thus investigated the effects of the non-opioid TLR2/4 antagonist, (+)-naltrexone, on mechanical allodynia and transcription levels of spinal TLR2/4-related inflammatory markers (i.e. TLR2/4, NLRP3, IL-1β, TNF, IκBα) as well as the Th17 cell signalling molecule, IL-17, using the experimental autoimmune encephalomyelitis (EAE) model of MS. Male and female Dark Agouti rats were induced with EAE and 14 days later began 14 days consecutive treatment with subcutaneous (+)-NTX or saline. (+)-Naltrexone treatment successfully reversed neuropathic pain in both male and female rats compared to the rats receiving saline treatment. Moreover, (+)-naltrexone treatment resulted in significantly lower inflammatory mRNA markers (i.e. TLR2/4, NLRP3, IL-1β, TNF, IκBα, and IL-17) in the spinal cord relative to the saline-treated animals. Lastly, administration of intrathecal interleukin-1 receptor antagonist (IL-1ra) on day 15 and 29 post EAE induction demonstrated that ongoing spinal IL-1β signaling is necessary for EAE-induced mechanical allodynia both early and late in disease development. Collectively, our findings provide the first evidence supporting both TLR2/4 and intrathecal IL-1β antagonism as effective interventions against EAE related chronic neuropathic pain in both males and/or females and suggests decreased spinal IL-1β and other related inflammatory signals as important mechanisms by which (+)-naltrexone exerts its therapeutic effects.

Theme: Neurodegenerative Disorders and Injury
Multiple sclerosis (MS) is a debilitating and lifelong disease of the central nervous system. MS leads to demyelination of neurons and neuroinflammation, ultimately leading to an inability for neurons to communicate effectively. The primary symptom associated with MS involves paralysis to varying degrees; however, patients with MS also experience many additional secondary neurological symptoms, such as neuropathic pain, deficits in cognition/social interaction, and depression including anhedonia. Importantly, the most common preclinical rodent model of MS, experimental autoimmune encephalomyelitis (EAE), causes paralysis similar to MS which can confound testing in many standard assays of rodent behavior, particularly in assays that are optimized to study many of the aforementioned secondary symptoms of MS. In this study, we utilize a low-dose EAE model in which rats and mice develop minimal motor impairment throughout disease progression, allowing for extensive and repeated testing of complex behaviors. Male and female Dark Agouti (DA) rats, female C57Bl/6J mice, and female SJL mice were administered reduced doses of standard EAE-inducing agents (myelin oligodendrocyte glycoprotein [MOG] for DA rats and C57BL/6J mice and proteolipid protein (PLP) for SJL mice). Paralysis and mechanical allodynia (central neuropathic pain) were then assessed for up to 2 months post-EAE induction. Results indicate that by reducing the MOG or PLP doses used, a low-level EAE disease progression can be induced that minimizes confounding motor impairment yet allows robust expression of pain behavior in both rat and mouse EAE models. Future studies will utilize our newly-developed low-dose EAE models to study additional complex behaviors that model secondary symptoms associated with MS.
Alzheimer’s disease is a neurological degenerative disease that appears to be due to accumulation of metabolic waste products that include phosphorylated tangles and amyloid beta plaques. However, the etiology on how the waste products accumulates is not clearly understood. Recently a waste clearance system, called the glymphatic (glia lymphatic) system, is proposed to be disrupted and may lead to progression of Alzheimer’s disease (AD). The current hypothesis is that insulin insensitivity may alter cell waste clearance. Aquaporin-4 (AQP4) is a channel present in astrocytes that is critical to move fluid through the brain to remove waste. When stress or injury occur such as in diabetes type II, the AQP4 proteins may redistribute away from the blood brain barrier where it is typically located. By examining the expression of AQP4, the effects of waste accumulation can be processed and applied towards the understanding of AD. To test the effect of diabetes on AQP4 channel distribution, Streptozotocin (STZ) was injected to destroy pancreatic islet cells. Additional animals were fed high fructose diet for 12 weeks to induce diabetes type 2. Following treatment, animals were tested for cognitive function with the Morris water maze and object recognition. Following behavioral analysis, brains were collected, fixed and sectioned. Fluorescent immunocytochemistry detected AQP4 and phosphorylated Tau in control and diabetic brains. The distribution of AQP4 in the hippocampus and the presence of phosphorylated Tau tangles were measured. In addition, the size of the hippocampus was measured through unbiased stereology.

Theme: Neurodegenerative Disorders and Injury
Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder characterized by resting tremor, slowness of movements, rigidity, gait disturbance and postural instability. Since the discovery of markedly decreased dopamine concentrations in the striatum in 1960s [1], the basal ganglia (BG) are the major clinical research targets in PD. At the same time, pathological changes in the cerebellum following dopaminergic degeneration have been reported in patients with PD and animal models, and anatomical, pathophysiological and clinical evidence suggest that the cerebellum may contribute substantially to the clinical symptoms of PD [2,3,4]. To further understand the possible cerebellar pathology in PD we have used the MPTP-treated rhesus monkey, a well-established non-human primate model of PD, to study possible anatomical changes in the cerebellum of one control and one parkinsonian MPTP-treated monkey. The volume (Cavalieri analysis), and the total number of Purkinje (PK) neurons in the motor areas of the cerebellum have been estimated using serial sagittal sections, calbindin immunostaining, and an unbiased stereological approach (optical fractionator; StereoInvestigator). In this analysis, we have found a 38% reduction in the volume, and a 20% decrease in the number of the PK neurons in the cerebellum of the parkinsonian monkey compared with the control. Although additional analysis (increasing the number of animals) are needed, these preliminary results suggest anatomical changes in structure of the cerebellum, and potential pathological changes in the cerebellar-thalamic-cortical functional network in parkinsonian animals. 1. Hornykiewcz O, 2006. J Neural Transm 70: 9–15. 2. Ghez C and Fahn S, 1985. In: Kandel ER, Schwartz JH, eds. Principles of neural science. New York: Elsevier. p. 502-22. 3. Wu T and Hallet M, 2013. Brain 136: 696-709 4. Mirdamadi JL, 2016. J Neurophysiol 116: 917–919.

Theme: Neurodegenerative Disorders and Injury
TUMOR RESECTION AND PROGNOSIS IN GLIOBLASTOMA

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Background: Glioblastoma (GBM) is the most frequently occurring malignant primary brain tumor in the adult population. Despite advancements in surgical and medical therapy, it continues to have a poor prognosis. Previous studies have shown that there is a positive correlation between extent of tumor resection (EOR) and overall survival in GBM patients. EOR is subdivided into either gross total resection for complete removal or subtotal resection for partial removal of the tumor. The decision to perform one resection type versus the other is made by the surgeon based on location of the tumor and risk of neurological deficits from the resection. In this study, we assessed whether resection improves both overall survival and 6-month progression-free survival in glioblastoma patients. In addition, we planned to evaluate the association between tumor location, genetic markers, and survival in GBM.

Methods: The patient population included males and females diagnosed between ages 19-83. Institutional review board approval was obtained in order to conduct this retrospective chart review. A total of 45 cases between January 2014 until December 2017 from the tumor registry offices at Jersey Shore University Medical Center and JFK Medical Center were reviewed. Information obtained included: age at diagnosis, Karnofsky performance status (KPS), primary site of the tumor, genomic characteristics of the tumor, resection status of the tumor (i.e. gross total resection, subtotal resection, or biopsy only), and date of tumor progression, if applicable. We also collected data on radiation and chemotherapy treatment. Various statistical tests were conducted, such as Fisher’s exact test for two way comparisons of median overall survival between surgical groups, and log-rank test to determine statistical significance between surgical resection groups.

Results: A total of twenty-nine subjects received a gross total resection. Ten subjects received a subtotal resection, and six subjects received a biopsy only. The median overall survival in months for those having either gross total or subtotal resection was 15.5 months [IQR: 8.2, 23.0] as compared to biopsy only, which was 2.5 months [IQR: 1.2, 4.6], (p=0.004). The median progression-free survival (PFS) in months for those having gross total resection, subtotal resection, or biopsy only was 7.3 [IQR: 4.6, 9.4], 19.3 [IQR: 7.2, 23.0], and 2.5 [IQR: 1.2, 3.6], respectively. There was a significant difference in length of survival between subjects who received gross total resection, subtotal resection, or biopsy only (p= 0.017). In addition, those who had a subtotal resection had significantly higher median PFS than those who received a biopsy only (p = 0.010). There was no significant difference between tumor location, radiation, chemotherapy, and survival outcomes. In this study sample, 31.1% of subjects have MGMT promoter methylation, 6.7% of subjects have the IDH2 mutation, and 22.2% of subjects have the 1p/19q co-deletion.

Conclusions: Gross total and subtotal resection significantly improves overall survival and progression-free survival in GBM. Our small subtotal resection group had a better survival outcome than our total resection group, but as our total number of patients in each group was small, the significance of this finding remains unknown. Our numbers were also too small to
determine a relationship between specific genetic biomarkers, prognosis, and surgical treatment of GBM, which other studies have explored.

Theme: Neurodegenerative Disorders and Injury
A ROLE FOR FOREBRAIN PRIMARY CILIA IN FEAR CONDITIONING AND EXTINCTION IN MICE

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Primary cilia are present in almost all mammalian cells, but their function remains unclear. Primary cilia are microtubule-based organelles that are maintained by an intraflagellar transport system (IFT). A critical protein in this system is IFT88, encoded by the gene (Ift88) of the same name. This project focused on the role of primary cilia in the forebrain, specifically a putative function of primary cilia memory and cognition. In these experiments we crossed mice with a floxed Ift88 allele with a NEX-CRE strain (both strains on a C57BL/6 background), which expressed CRE only in forebrain projection neurons. The resulting offspring would lose primarily cilia only in forebrain neurons late in embryonic development. The hypothesis of this study was that mutated mice would show impaired extinction memory and therefore take longer to extinguish fears because of the lack of primary cilia in the forebrain. To test this hypothesis, mutated and control littermate mice were put through a five-day fear conditioning and extinction protocol. On day one, mice were conditioned with a tone/shock stimulus. Day two consisted of a five-minute context test in which there were no tones or shocks. On days three through five mice were subject to just a tone, and the amount of time they spent freezing was recorded. There was a significant interaction between genotype, day, and tone showing that mice with the Ift88 knockout were slower to extinguish their fear of the tone. These data lead to the conclusion that the Ift88 gene in the brain has a role in fear extinction, and supports previous evidence that forebrain primary cilia play a role in memory and cognition.

Theme: Neurodegenerative Disorders and Injury
TRANSCRANIAL DIRECT CURRENT STIMULATION LESSENS NEGATIVE IMPULSIVITY EFFECTS OF CONTROLLED CORTICAL IMPACT TRAUMATIC BRAIN INJURIES IN RATS

West Virginia University

Traumatic brain injuries (TBI’s) are a growing health concern that can result in major cognitive deficits such as attentional impairments and reduced impulsive control. Previous studies have suggested reduced dopamine signaling may mitigate such cognitive dysfunctions following TBI. One way to increase dopamine levels in the brain is through cathodal transcranial direct current stimulations (tDCS). Therefore, utilizing tDCS on an injured brain may lessen the impacts of the injury. This study aimed to investigate the effectiveness of tDCS as a treatment for impulsivity defects resulting from TBIs in rats. Twenty-four Long-Evans rats were trained on the 5-choice serial time task (5-CSRT), an assessment of attention and motor impulsivity, until reaching a stable baseline. The task consists of the animals responding rapidly to the presentation of a brief (0.5 seconds) light stimulus in one of five holes (attentional component) and withholding their response until a stimulus is presented (motor impulsivity component). Premature (impulsive), incorrect, and omitted responses are punished with a 5 second timeout where the house lights were illuminated. After a stable baseline was reached, a frontal controlled cortical impact injury (+3.0, +0.0, -2.5 @ 3 m/s) was administered to 14 of the 24 rats, and the remaining 10 served as shams. Testing resumed after one week of recovery. After six weeks to allow behavior to stabilize, cathodal tDCS treatment was administered at 800 μA for ten minutes per session, and seven days in a row. The start of tDCS was counterbalanced within subjects (each received sham and actual stimulation). We found that TBI chronically impaired both attention and impulsivity. Treatments of tDCS appeared to selectively reduce instances of impulsivity but had no effect on attention. The decreased impulsivity after tDCS treatments in injured rats may be due to increased dopamine levels from tDCS treatment.

Theme: Neurodegenerative Disorders and Injury
IMMUNOHISTOCHEMISTRY REVEALS DISTINCT PATTERNS OF BRAIN-WIDE AMYLOID PRECURSOR PROTEIN EXPRESSION AND AMYLOID-BETA PATHOLOGY IN THREE MOUSE MODELS OF ALZHEIMER’S DISEASE

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Alzheimer’s disease (AD) is one of the world’s most dire neurodegenerative conditions and is characterized in part by accumulation of amyloid-beta (Aβ) protein plaques in extracellular spaces, causing synaptic impairment and cell death. A variety of AD mouse models overexpress mutant forms of human amyloid precursor protein (APP), leading to the formation of Aβ plaque pathology. These models exhibit different spatiotemporal patterns of AD pathology that may be related to differences in their genetic makeup, and the degree to which different models mimic patterns of human pathology is unknown. Building off our team’s ongoing in vivo characterization of Aβ plaque distribution in three such mouse models (APP/PS1, Tg2576, and hAPP-J20) using methoxy-X04 labeling, serial 2-photon tomography, and an automated brain segmentation and registration pipeline, we here used slice immunohistochemistry and fluorescence confocal microscopy to examine trends in APP expression and Aβ deposition in specific brain regions. Interestingly, a sexually dimorphic trend of APP expression emerged in all three genetic models, with female mice showing higher APP expression in all brain regions, especially those associated with later pathology. Furthermore, antibody staining of Aβ in conjunction with prior methoxy-X04 labeling revealed distinct plaque compositions between the models, with hAPP-J20 mice showing significantly lower ratios of dense-core to diffuse plaques. Further investigation is necessary to elucidate more comprehensive mechanistic understandings of the sex differences and variations in pathology between mouse models of AD.

Theme: Neurodegenerative Disorders and Injury
ELUCIDATING THE IMPACT OF NEURONAL DAMAGE ON HERPES SIMPLEX VIRUS TYPE-I (HSV-1) LATENCY

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HSV-1 is a neurotropic virus that establishes and maintains latency in sensory neurons. Physiological and emotional stressors have been shown to reactivate the virus from this latent stage. One physiological stressor that has not been studied extensively in relation to HSV-1 latency is neuronal damage. To this end, our work tested the hypothesis that physical damage to rat dorsal root ganglia neurons, modeling blast-induced neurotrauma (BINT) at the cellular level, would prompt the reactivation of HSV-1 from its latent stage. To test this, neurons were first cultured and then infected with the F strain (MOI=1.5; multiplicity of infection) of HSV-1 in the presence of the antiviral compound acyclovir (100µM) to model a latent-like state. Current cellular morphological results have revealed successful production using this in vitro model of HSV-1 latency. Moreover, dorsal root ganglia neurons have remained viable up to 2 weeks post-infection in the presence of acyclovir. Ongoing quantitative PCR analysis is determining levels of the viral gene Latency Associated Transcript, the only robustly expressed gene in neurons latently infected with HSV-1. Additionally, axotomy studies are being conducted to test if damaging axons would reactivate HSV-1 from latency in this primary neuronal model. Results from this work would demonstrate the impact neural trauma has on reactivating the virus, potentially increasing the likelihood of HSV-1 crossing the blood-brain barrier and promoting neurodegeneration.

Theme: Neurodegenerative Disorders and Injury
THE EFFECTS OF REPEATED STRESS ON BEHAVIOR IN C57BL/6J MICE: GENDER DIFFERENCES

Saint Francis University

The harmful effects of stress have been demonstrated to aggravate psychopathological conditions such as post-traumatic stress disorder (PTSD), anxiety and depression. A gender-based comparison of the epidemiology of stress shows that women are twice as likely as men to receive the diagnosis of stress disorders. While it has been suggested that women have a different response to stress compared to men, which makes them more susceptible to the effects of stress, it is possible that different stressors exert differential effects on the behavioral phenotype of males and females. The current project was designed to evaluate the effects of repeated stress exposure on male and female mice, tested using a battery of animal models of human behaviors. Thirty-six C57Bl/6J mice (18 males and 18 females), were randomly divided into stress and control groups. The ‘stress’ group was exposed to repeated forced swim stress sessions, and tested using tests for activity, anxiety, memory, social behavior and behavioral despair. Results demonstrated that exposure to repeated stress exerts differential effects on the behavior of male and female mice. While stress exposure led to an increase in anxiety and depression-like behaviors in both genders, the effects were more pronounced in males compared to females. On the other hand, stress exposure increased the social-preference of female, but not male mice. Further studies will assess the biological/molecular mechanism which may underlie the differential effects of repeated stress on the behavior of male and female mice, and may expand the repertoire of stressors evaluated in the current study.

Theme: Neurodegenerative Disorders and Injury
IDENTIFYING PHYSIOLOGICALLY RELEVANT TARGETS OF TDP-43 MEDIATED TRANSLATIONAL INHIBITION IN A DROSOPHILA MODEL OF ALS

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The mRNA binding protein TDP-43 forms cytoplasmic inclusions as part of the pathogenesis of amyotrophic lateral sclerosis (ALS), a genetically heterogenous neurodegenerative disorder affecting motor function and survival. A potential causative mechanism for the ALS phenotypes is inhibition of key synaptic or trafficking related proteins via mRNA sequestration and translational repression [1]. The objective of the project is to identify physiologically relevant targets of TDP-43 mediated translational inhibition. We overexpressed TDP-43, either wild type or a mutant variant, in Drosophila motor neurons [2] then performed immunoprecipitation experiments to detect mRNAs enriched in TDP-43 complexes. Tagged Ribosome Affinity Purification was also utilized to identify translational alterations occurring in motor neurons exhibiting TDP-43 proteinopathy. Several mRNA candidates linked to synaptic function and trafficking were identified as potential primary targets due to their high association with TDP43 and depletion with ribosomal subunits. Additionally, DAVID (Database for Annotation, Visualization and Integrated Discovery) analyses identified several translationally dysregulated processes including oxidative phosphorylation and other metabolic processes, which likely contributes to the deficiencies in cellular energetics found in ALS. We will report our progress on validating candidate targets in Drosophila and other model systems including patient cells and tissues; the identification of targets of TDP-43 mediated translational inhibition will offer insight into both ALS as well as several other neurodegenerative disorders with which TDP-43 is associated [3,4].


Theme: Neurodegenerative Disorders and Injury
TRAVEL AWARD WINNER
Haley Rhodes sponsored by FUN Member Jessica Boyette-Davis
Award Sponsor: FUN

PAIN PERCEPTION IN NON-SUICIDAL SELF-INJURY

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Previous research shows that people who engage in non-suicidal self-injury (NSSI) have key differences in their baseline biopsychological state compared to the general population, and these differences may help motivate NSSI behavior. Specifically, they have altered opioid signaling and increased symptoms of psychological dissociation. It was hypothesized that the pain-inducing experience of NSSI releases β-endorphin, which alleviates aversive dissociative symptoms and thus makes the behavior rewarding. The present study investigated this potential mediating effect of pain on β-endorphin release and dissociative symptoms, and compared these effects between those who do (n = 9; 2 males, 7 females) and do not (n = 18, 4 males, 14 females) engage in NSSI. Participants (n = 27) provided self-reports of dissociative symptoms and affect before and after a painful stimulus (Cold Pressor Test; CPT) as well as ratings of pain intensity at their pain threshold and tolerance during the CPT. No differences were found for baseline dissociative scores (p = .898), baseline affective arousal (p = .373), or pain intensity at threshold (p = .603) between the two groups. However, the time required to reach pain threshold was significantly negatively correlated with baseline affective arousal in the self-injuring group (r = -.727, p = .026) but not in the nonself-injuring group (r = .150, p = .579). Additionally, ELISA immunoassays were used to quantify pain-induced β-endorphin release. Analysis revealed differential release of β-endorphin in self-inurers and nonself-injurers according to their pain-induced changes in dissociative symptoms and affective state. This study demonstrates the importance of investigating the pain-induced biopsychological changes that underlie and motivate the harmful condition of NSSI.

Theme: Sensory Systems
IDENTIFYING THE MOLECULAR COMPONENTS OF COLD NOCICEPTION IN DROSOPHILA MELANOGASTER

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Nociception, the central nervous system’s processing of noxious stimuli, gives rise to conscious perception of pain and protective reflexes. This study aims to explore the molecular mechanisms of nociception by researching the potential role of Drosophila melanogaster Innexin gap junction proteins in cold nociception. Invertebrate Innexins are functionally analogous to mammalian Connexins. To screen for a possible role of these Innexins in cold nociception, the expression of each Innexin was knocked down by cell-specific expression of innexin RNAi constructs in the class III dendritic arborization (da) neurons. Wild type (WT) third instar Drosophila larvae exhibit a characteristic “cringing,” or shortening, response when exposed to noxious cold (6°C). Larvae were subjected to a cold plate and their behavior was video recorded. 100 larvae were tested for each genotype. The larval images were processed using Image J software to quantify the “percent cringe”. By comparing the percent cringe of the protein-lacking larvae to the WT, the involvement of the knockdown protein in the cold nociceptive pathway was inferred. A control was established using Oregon-R WT larvae (positive for WT cringe response). Tetanus toxin, a potent neurotoxin, was expressed specifically in class III da neurons and significantly inhibited cringing. This provided evidence that class III da neurons function in cold nociception and moving forward this test served as a negative control. To date, every Innexin has been tested with at least one RNAi construct, and six of the eight Innexins have been tested with two different RNAi constructs. In class III da neurons, down regulation of four Innexins - Inx2, Inx5 (with both RNAi constructs), Inx1 and Inx3 (only tested with one construct) - significantly inhibited cringing. This provides evidence that these four Innexins function in the cold nociception pathway of third instar larvae, and also, therefore, that this pathway utilizes electrical synapses. Down regulation of Inx4, Inx6, Inx7, and Inx8 significantly inhibited cringing with one RNAi construct but not the other. To clarify the potential role of these Innexins, mutant flies lacking a functional Innexin will be tested to eliminate confounding variables that may exist with the RNAi constructs.

Theme: Sensory Systems
This experiment was aimed at critically informing current empirical theories of perceptual awareness. The general design was based on Dehaene et al.’s (2001) visual masking study. Dehaene et al. (2001) used a sandwich masking technique to render visual stimuli clearly seen or unseen on different trials while controlling for overlapping brain activity elicited by the masks (subtracting blank control trials from target-present trials). While this design was clever, elegant, and the results were intriguing, the stimuli were always task-relevant such that neural activity linked with conscious perception may have been confounded with neural activity associated with performing the task. In the current study, we used this same sandwich masking technique to manipulate perceptual awareness, but we also manipulated task-relevance, resulting in a 2x2 design. The critical stimuli were line drawings of animals and objects that always appeared for 33ms. In the unseen condition, 100ms masks immediately preceded and followed the stimuli, while in the seen condition, these same masks were separated from the stimuli by 200ms blank periods. On 20% of trials, a colored oval was presented instead of a critical stimulus. During task-relevant blocks, subjects made trial-by-trial reports as to whether they saw an animal, an object, or nothing; in task-irrelevant blocks, subjects completed a color discrimination task, withholding response when no color was presented. We compared event-related potentials (ERPs) elicited by the critical stimuli in seen versus unseen trials, separately for the two task conditions. The results from the task-relevant condition replicated the findings of Dehaene et al. (2001), while the results from the task-irrelevant condition differed, particularly at time-points beyond 300ms. Specifically, the P3b wave, which has been proposed as a neural marker of perceptual awareness, was absent in the task-irrelevant condition. These results suggest that long-latency ERP differences between seen and unseen stimuli are more related to the reporting task than to perception per se.

Theme: Sensory Systems
ASSOCIATING GNRH NEURONS WITH TASTE AND Olfactory Systems in Teleost Fish

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The proper development of mammalian nervous system requires that cells and their axons navigate a complex and dynamic environment as the brain grows and matures. Cells respond and adapt to regulatory extracellular cues by exchanging activated surface membrane proteins and targeting damaged or unnecessary surface membrane proteins for lysosomal degradation. The small GTPase Rab7A operates within both autophagic and endocytic pathways as a master regulator of lysosomal degradation and membrane trafficking. We have used a conditional Rab7A knockout in neural progenitor cells and their post mitotic progeny to look at the consequences of improper trafficking events in the endocytic and autophagic pathways. Immunofluorescence of cortical layer markers in the conditional knockout reveal perturbations in layer V development and the subplate. Furthermore, loss of Rab7A resulted in aberrantly projecting axon bundles in the lateral and medio-dorsal cortex, highlighting a potential role for Rab7A in subplate-mediated axon guidance. Ultrastructurally, knockout cortices exhibited intracellular accumulations of vesicular machinery and evidence of failed fusions with LAMP1-positive lysosomal structures. Disrupted trafficking translates to proliferative deficits in ventricular zones of the cortex. This work suggests novel functions for Rab7A during key processes of cortical development.

Theme: Sensory Systems
THE EVOLUTION OF FOOD PREFERENCES IN THE NEMATODE C. ELEGANS

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Food choice is critical for survival because organisms must choose food that is edible and nutritious and avoid pathogenic food. Odors are some of the most important cues that animals use to find and distinguish among foods. The nematode *Caenorhabditis elegans* shows strong innate preferences for the odors of different bacterial species, their major food source, but little is known about how these preferences evolve. *C. elegans* is strongly attracted to the odor of the pathogenic bacterium, *Serratia marcescens*. This initial attraction likely facilitates ingestion and infection of the *C. elegans* host. However, *C. elegans* coevolved with *S. marcescens* SM2170 for 30 generations avoids ancestral SM2170, but does not avoid coevolved SM2170 (Penley and Morran, 2017). We hypothesize that the coevolved SM2170 strains counteract avoidance by releasing odor blends that attract coevolved *C. elegans*. We are testing this hypothesis by examining differences between volatile organic compounds (VOCs) released by ancestral and coevolved strains of *S. marcescens* SM2170 (#1 and #3). VOCs were identified through Gas Chromatography Mass Spectrum analysis of SPME (Solid Phase Microextraction) of bacterial samples. We found that all tested strains released dimethyl disulfide and dimethyl trisulfide, but only the ancestral strain released 2-butanone. Future work is needed to characterize further the differences among VOC profiles of ancestral and coevolved SM2170 strains and how specific VOCs contribute to the preferences of coevolved *C. elegans*.


Theme: Sensory Systems
Grapheme-color synesthetes experience consistent and automatic associations between graphemes (letters or numbers) and colors. The current study investigates whether synesthetic color perception provides an advantage in a visual search task. Electrophysiological and behavioral data were collected across three experiments. In experiment 1, synesthetes and matched controls performed a visual search task to find a target letter amongst 7 distracter letters positioned in a circular array. For each synesthete, 2 letters with the same color association (e.g. both red) were designated as targets while the distracter letters were all different (e.g. non-red) colors. The stimuli (black letters) and task (find a target letter and report it) were identical for both groups. Comparing event–related potentials (ERPs) recorded on electrode sites contralateral vs. ipsilateral to the target, allowed us to measure the “N2pc” component, a well-studied marker of selective attention ~200-300 ms post-target stimulus. We found faster reaction times, along with a larger and earlier N2pc for synesthetes vs. controls, suggesting that their synesthetic perception of color may have speeded their attention toward the target. In experiment 2, non-synesthetes were tested with the same paradigm as exp. 1, but with an added colored-letter condition, where every letter was presented in a unique color on the screen. The colored grapheme condition was intended to simulate the synesthetic color perception of synesthetes in exp. 1. When color was available as a search cue, non-synesthetes had faster reaction times along with larger and earlier N2pcs than when they were presented with uncolored (black) letters. The magnitude of these behavioral and neural differences was very similar to that observed for synesthetes in exp. 1. Experiment 3 served to replicate the results of exp 1 with a new population, and to investigate visual search performance with stimuli that do not elicit synesthetic percepts. The procedure was the same as exp. 1 with the addition of two conditions: an unfamiliar graphemic condition (Georgian alphabet) and a non-grapheme condition (stick figures). We found faster reaction times, along with larger and earlier N2pc for synesthetes vs. controls across all three conditions, suggesting that their synesthetic perception of color may not be the main factor speeding their attention toward the targets. Synesthetes may possess a generic advantage in visual search tasks due to enhanced early processing of visual stimuli in general.

Theme:  Sensory Systems
THE effect of HCN channel activity on migration and maturation of adult-generated neuroblasts

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Adult neurogenesis occurs in the subventricular zone of the lateral ventricles throughout life. Neural stem cells proliferate and give rise to neuroblasts that migrate to the olfactory bulb where they differentiate into interneurons. Hyperpolarization-activated cyclic nucleotide-gated cation channels (HCN) have a significant effect on neurite outgrowth and layer formation in the developing olfactory bulb. The tetrameric ion channels consist of four subunits (HCN1-HCN4). We are investigating the role of HCN channel activity-dependent mechanisms in migration and maturation of neuroblasts in the olfactory bulb. Mice lacking the HCN1 subunit or wildtype mice are injected at birth with a plasmid expressing either GFP or a plasmid that alters HCN2 levels. We hypothesize that HCN2 gain/loss-of-function will affect neuroblast migration and maturation. Preliminary data indicates that loss of HCN2 interferes with olfactory bulb layer targeting and dendritic branch formation. This study may have important implications for activity dependent mechanisms of adult neurogenesis.

Theme: Sensory Systems
Feeding increases after exogenous cannabinoid administration, and our body contains endocannabinoids (ECB) that may act on feeding and taste. ECB receptors are found in taste buds, and ECB activation increases whole taste nerve responsiveness to sweeteners in mice. My study hypothesizes that ECB agonism increases taste-guided intake of sucrose by rats and will allow rats to more easily discriminate between the taste of sucrose and water. Two experiments were conducted, each with 5 male Sprague-Dawley rats: 1) 30-min intake of 0.03M sucrose by nondeprived rats after injection with ECB reuptake inhibitor AM404 (2.5mg/kp ip) or vehicle (30% EtOH in water) and 2) sucrose-water discrimination after AM404 and vehicle across 5 sucrose concentrations by rats trained in a 2-response operant taste detection. As hypothesized, AM404 doubled sucrose intake. This suggests that ECB reuptake inhibition is able to impact taste-guided behavior in a need-free state and that the chosen AM404 dose was effective in changing taste-guided behavior. In contrast to our hypothesis, AM404 did not enable rats to more easily discriminate between sucrose and water. This suggests that ECB reuptake inhibition does not impact behaviorally assessed sucrose detectability and that the effect of AM404 on sucrose intake may be the result of its other motivational or hedonic impacts on behavior. Our data contrast with the increase in whole-nerve sweetener sensitivity after ECB receptor agonism in mice, which may be due to an incongruency between electrophysiological and behavioral results, differences between rats and mice, and/or influence of direct vs. indirect ECB receptor agonism.

Theme: Sensory Systems
THE EFFECTS OF ACUTE STRESS ON RESPONSES TO NOCICEPTIVE AND NON-NOCICEPTIVE STIMULI

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Stress-induced analgesia is characterized by a reduction in response to painful stimuli. However, a sufficiently stressful stimulus may exhibit a pro-nociceptive effect in addition to its anti-nociceptive effect. Previous studies suggest that endocannabinoids reduce responses to nociceptive stimuli and increase responses to non-nociceptive stimuli. Serotonin (5HT) had also been shown to contribute to these effects. In these experiments, the medicinal leech (Hirudo Verbana) was shocked twice per minute for fifteen minutes. This electrical stimulus reliably raised 5HT levels in the CNS, suggesting a stressed-state. Response to nociceptive stimuli was measured using a Hargreaves Apparatus to test latency to thermal nociceptive stimuli. The non-nociceptive response was measured by Von Frey fibers which test response to mechanical stimulation. These tests were then repeated after injection of diacylglycerol lipase (DAGL) inhibitor, tetrahydrolipstatin (THL), which blocks the synthesis of 2-arachydonoylglycerol (2-AG), DMSO, and SB366791, a TRPV channel antagonist. Injections of 5HT were used to mimic the stress stimulus and a 5HT receptor antagonist, methysergide, was used to block this effect. The electric shocks did not alter responses to the nociceptive stimulus but did cause sensitization to the non-nociceptive stimulus. Endocannabinoids had no effect on the response to either stimuli. Injections of 5HT caused sensitization to the non-nociceptive stimulus and were concentration dependent. The 5HT injection had no effect on the nociceptive stimulus. Injections of methysergide blocked sensitization to non-nociceptive stimuli after stress. The lack of change in response to the nociceptive stimulus may indicate a need to alter the pattern of electric stimulation.

Theme: Sensory Systems
SEXUAL ATTRACTION IN THE SILKWORM MOTH: A TRACTABLE OFLCATION DEMONSTRATION TO EXHIBIT THE NEUROPHYSIOLOGY OF SEX-SEARCH BEHAVIOUR IN BOMBYX MORI

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Demonstrating how the nervous system can sense, perceive and react to stimuli is a cardinal component of neuroscience outreach. One of the most difficult senses to research and demonstrate is olfaction. This is due to the unknown diversity of odorant molecules, multiple areas of the brain involved and research being primarily performed in rodent models. Here we present a robust experiment to demonstrate the sex-search behaviour in the silkworm moth, *Bombyx mori*. When reproductively mature, *B. mori* has no nose or mouth, and will only live for 5-10 days. During this time period, females emit two pheromones to attract the males: bombykol and bombykal. Males have pheromone-specific odorant receptor neurons in their antenna that are used to sense and locate females in the odor plume. It been demonstrated that males are capable of sensing bombykol at concentrations equating to a distance of 11 kilometers. We have designed a binary-choice assay to observe behaviour and a tractable electroantennogram technique using a SpikerBox (Backyard Brains, Ann Arbor, MI) to observe antenna physiology in response to pheromones and general odorants in male and female silkworm moths. This accessible experiment will allow students and audiences to observe sexually dimorphic behaviour and physiology in the simplest, and most rigorously studied, sex-pheromone system.

Theme: Sensory Systems
ASSESSING THE EFFICACY OF CHEEK FISTULA AS OPPOSED TO INTRAORAL CATHETERS FOR INFUSING ORAL SUCROSE TO INDUCE CFOS EXPRESSION IN THE ROSTRAL NUCLEUS OF THE SOLITARY TRACT IN RATS

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Oral sucrose exposure induces the expression of cFos (an early active gene) in the rostral nucleus of the solitary tract (rNST), which is the first central relay for taste information (Harrer & Travers, 1996). cFos expression (in the form of Fos protein) can be used as an index of neural activity, as changes in rNST Fos levels could provide insight into the mechanism underlying changes in taste-guided behavior. Oral exposure to induce rNST Fos typically requires surgically implanted intraoral (IO) catheters. We experienced complications from IO catheters (e.g., persistent infections, headcaps dislodging), and the catheters must be cleaned daily to maintain patency. We hypothesized that we could achieve 1) more surgical success and 2) cFos expression similar to Harrer & Travers (1996) by infusing sucrose through cheek fistulae, which require a less invasive surgery and are more easily maintained (Hintiryan et al, 2006). We therefore compared recovery times and extent of full recovery leading to testing of rats given either bilateral IO catheters or cheek fistulae (the former group also equipped with a 4th ventricular cannula for other purposes). Rats in both surgical groups (IO catheters n= 48; cheek fistulae n=20) were anesthetized with ketamine (100 mg/kg ip) and xylazine (10 mg/kg ip) and lengths of heat-flared polyethylene-50 tubing set with a Teflon washer was, using a 19 G needle, bilaterally inserted into the oral mucosa just anteriolateral to the 2nd molar. Intraoral catheters were tunneled subcutaneously, exited adjacent to the scalp, and affixed to the skull with bone screws and dental cement, whereas cheek fistulae exited through the cheek and were held in place with another Teflon washer. All animals received analgesic (carprofen 5 mg/kg sc) and antibiotic (gentamicin 8 mg/kg sc) on the day of surgery and for each of 3 days following, as well as access to softened food until body weight returned to presurgical levels and/or stabilized. Rats from 3 study phases for both surgeries are included in analysis. Similar to Hintiryan et al (2006), we found, on average, that rats given cheek fistulae recovered in 15 days compared to 26 days needed by rats given IO catheters (p<0.001), 65% of those with cheek fistulae reached their full presurgical body weight compared to 37% of those with IO catheters (p=0.057), and 85% of rats with cheek fistulae were able to be tested as opposed to 79% of those with IO catheters (p>0.05). After recovery from surgery, all of the rats were acclimated to oral infusions of water and tested with either water or 1.0 M sucrose (7.2 ml across 30 min). The rats were then anesthetized with sodium pentobarbital (100 mg/kg ip), perfused, and their brains sliced at 50 µm on a freezing microtome. We are currently analyzing brainstem slices immunohistochemically processed to visualize cFos expression to assess if sucrose exposure through cheek fistulae results in more rNST Fos than water exposure, similar to that seen when IO catheters are used (as per Harrer & Travers, 1996).

Theme: Sensory Systems
MILD WATER RESTRICTION OF FEMALE RATS DOES NOT IMPACT ESTROUS CYCLE STABILITY

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Many behavioral studies in rodent models impose environmental manipulations, like food or water restriction, to motivate the animal to respond. The level to which these manipulations are effective or the consequences they have on physiology and behavior may differ based on sex. For example, water restriction is necessary to motivate rats to respond in operant assessments of taste detection. Since sex differences related to hormone level changes across the estrous cycle have been observed in taste detection (e.g., Matia et al 2016), it is imperative to determine if water restriction itself influences the estrous cycle. The present study examined if mild water restriction akin to that used in operant taste detection studies would interrupt or destabilize the normal estrous cycling of female rats. We used 24 Sprague-Dawley female rats from 12 litters across 2 study phases in an A-B-A between-subjects design. Sister-pairs of rats were divided into two experimental groups, one of which was never water restricted and one of which was restricted to 10 ml of water per day Mondays through Fridays during the B phase of the experiment; all animals received ad libitum access to water during the two 3-week A phases and over the weekends during the 3-week B phase. The estrous cycles of all the rats were tracked every week day during the 9 weeks. The estrous cycle stage was determined by 2 observers blind to the experimental group via microscopic analysis of stained cells collected via vaginal swab. Five-day cycle periods were scored in a binary fashion as either stable or not, where an unstable cycle period was defined as 3 or more consecutive days of a single stage. Differences in the percent of rats presenting with unstable cycles overall and for each 3-week phase was assessed between experimental groups (water-replete vs. water-restricted) using independent samples t-tests. No significant differences between groups in percent instability were found over the course of the study both during and not during the water-restriction phase; however, nearly all of the rats presented with at least 1 week of instable cycles across the 9 weeks. This suggests that water restriction has no direct effect on estrous stability in rats, and that mild water restriction can be used as motivation in behavioral tests in both sexes of rats without fear of its impact on the estrous cycle. However, it may be advised that estrous cycle stability of individual female rats should be determined prior to testing, so that the impact of estrous cycle instability can be minimized in behavioral studies.

Theme: Sensory Systems
Arthropods use withdrawal and escape responses to retreat from threats. Looming stimuli, which can represent the approach of a predator, evoke different escape responses in the spider *Phidippus regius* and the cricket *Acheta domestica.* The purpose of this research is to investigate the underlying modalities and mechanisms of escape in *Phidippus regius* and *Acheta domestica.*

For both arthropods, looming stimuli were presented by a 3” black polyurethane ball projected (1 m/s, 45°) toward the animal from eight circumferential directions. The arthropod was placed into an arena of white canvas surrounded by white roof flashing. Escape responses were recorded using high-speed video (300-650 fps) and tracked in software. For the jumping spider, *Phidippus regius,* the aim was to determine the strategy used to escape from looming stimuli. Preliminary findings showed looming stimuli consistently evoked translation, but not turning. The angle of translation depended significantly on stimulus direction. Typically, following initial translation the spider executed one or two movements that appeared linked to the stimulus, often ending in a position that faced the looming object. Importantly, three of the spiders sometimes jumped away from the looming stimulus, a movement previously reported only associated with prey capture. These results suggest jumping spiders may use specific, diverse, multi-stage strategies to escape from looming stimuli. Further, their name-sake behavior, jumping, may be employed for both predation and escape. In *Acheta domestica,* the escape response is primarily directed by cercal detection of incoming wind stimuli; however, the escape response is often accompanied by the pointing of an antennae toward the incoming object. To date, little research has been done on antennae pointing and its relationship to the properties of the stimulus and escape movement. The goal of this research focuses on determining the characteristics and sensory modality responsible for antennae pointing and its relationship to the escape response. Preliminary findings showed that pointing usually (~95 %) accompanied the escape response and occurred for all directions of stimuli. Stimuli presented from the posterior end of the cricket often resulted in running or jumping with little movement of the antennae, while anterior stimuli resulted in a turn and run, with more frequent antennae pointing. When antennae pointing occurred there was an attempt by the cricket to maintain the pointing throughout escape. Additionally, pointing appeared to occur in only one antenna at a time, with a preference for the one ipsilateral to the incoming stimulus. These findings highlight the diversity of responses and behaviors in arthropods during escape from looming stimuli.

Theme: Motor Systems
MECHANISMS UNDERLYING VARIABLE RESPONSES TO ISOFORMS OF THE NEUROPEPTIDE C-TYPE ALLATOSTATIN (AST-C) IN THE AMERICAN LOBSTER, HOMARUS AMERICANUS

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The cardiac ganglion of the American lobster (*Homarus americanus*) is a model central pattern generator. Among the peptides that modulate the system are three isoforms of C-type allatostatin (AST-Cs I, II, III). AST-C II has an amidated C terminus, whereas AST-C I and AST-C III have non-amidated C termini. Individual responses to perfusion of all isoforms vary and are characterized by either increases or decreases in contraction amplitude (Dickinson et al., 2018). Responses to AST-C I and AST-C III are more similar to each other than either response is to that elicited by AST-C II. We are testing two hypotheses regarding the mechanisms underlying these variable responses: (1) differences in expression levels of four putative AST-C receptors, (2) amidation of the C-terminus, which may influence binding to AST-C receptors. To investigate the former, we recorded physiological responses of the heart to AST-Cs, then extracted RNA from the cardiac ganglion of each lobster. Using Illumina RNASeq technology, cardiac ganglia RNA is being sequenced. The receptor transcripts will be mapped onto a preexisting cardiac ganglion transcriptome to determine if differences in receptor expression exist among lobsters with varying physiological responses. To investigate the latter, the responses to amidated and non-amidated versions of each isoform are being compared.

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Theme: Motor Systems
NITRIC OXIDE FEEDBACK IN THE THERMALLY ROBUST LOBSTER CARDIAC SYSTEM

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The heartbeat of a lobster is locally generated by the cardiac ganglion (CG) consisting of four pacemaker neurons and five motor neurons. Also, the cardiac muscle controls the CG through a negative feedback loop mediated by nitric oxide and a positive feedback loop mediated by stretch. The CG is a central pattern generator, which produces a stable rhythm in the absence of external input. The cardiac ganglion must be thermally robust due to the wide temperature variations experienced by lobsters in nature. This thermal stability is notable considering the high thermal sensitivity of the ion channels that control the CG. It is possible that the negative feedback loop mediated by nitric oxide contributes to this stability: if the ganglion over stimulates the heart, the increased calcium concentration in the cardiac muscles stimulates the increased nitric oxide release which inhibits the ganglion. To test this hypothesis, lobster hearts were subject to temperature ramps that increased the temperature of the heart until a point of reversible failure, after which the hearts were returned to baseline temperature. These ramps were conducted in the presence and absence of the nitric oxide synthase blocker L-nitroarginine. Preliminary results suggest that nitric oxide feedback increases thermal stability in less stable hearts while decreasing thermal stability in more stable hearts.

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Theme: Motor Systems
THE EFFECTS OF PRELOAD STRETCH AND AFTER-LOAD PRESSURE ON CARDIAC OUTPUT IN THE HEART OF THE AMERICAN LOBSTER, HOMARUS AMERICANUS

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The cardiac ganglion controls cardiac output, frequency, and force amplitude of the heart of the American lobster, *H. americanus*. While contraction during systole causes volume reduction in the decapod heart, the nature of the restoring forces during diastole are not as well understood. We have measured the role of the dorsal abdominal artery in providing these restoring forces during diastole in an intact system and have found that its elastic properties contribute to heart expansion during relaxation. Additionally, we investigated how preload stretching on the wall of the heart and after-load pressure imposed on the dorsal abdominal artery alters cardiac output, frequency and force amplitude of the heartbeat. We observed that frequency responses to after-load pressure varied with preload stretch. One hypothesis is that cardiac output is regulated by stretch-feedback from pressure in the dorsal abdominal artery in ways reminiscent of the baroreceptor reflex found in vertebrates.

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Theme: Motor Systems
Coordinated movement is an essential skill for an organism’s survival, and can be crippling when it is absent, as seen in disorders like ataxias and Parkinson’s disease in humans. Electrical signaling through gap junctions between cells has been shown to support coordinated movement, and gap junctions localized to movement circuitry have been identified in the model organism Caenorhabditis elegans (C. elegans). We hypothesize that gap junctions in C. elegans and the innexin proteins that form them contribute to their ability to produce accurate movements. To test this hypothesis we genetically suppressed expression of specific innexin proteins using RNA interference (RNAi) feeding. We compared head movement in swimming behavior assays between control groups and RNAi-fed worms to assess the importance of innexins both individually and in combination with respect to motor function. Assessed were innexins 3, 4, 6, 8, 9, 12, 18, and 8 + 9 combined in C. elegans strain NL2099. Assays following RNAi-feeding were run on both parent and progeny generations. Control experiments were run using the HT115(DE3) blank plasmid in tetracycline-resistant E. coli. We used WormLab© software for video analysis and to measure changes in head angle movement in the nematodes. The findings of this study will improve understanding of the impact innexin proteins have on coordinated movement and will aid in identifying important molecular components in cellular signaling with respect to well-timed and coordinated organismal movement.

Theme: Motor Systems
ALTERING THE GAIN OF THE INFRALIMBIC TO ACCUMBENS SHELL CIRCUIT ALTERS ECONOMICALLY DISSOCIABLE DECISION-MAKING ALGORITHMS

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Recent theories in neuroeconomics suggest that multiple decision-making processes are responsible for distinct valuation systems that mediate motivation and self-control behaviors. These systems are thought to arise from separate neural circuits that may work to regulate one another and allow for new information to direct changes in reward driven behavior. The infralimbic cortex (IL) to nucleus accumbens shell (NAcSh) pathway has been associated with reward and valuation. However, the distinct role of this circuit and how it works to mediate self-control remains unknown. Using optogenetics, we altered the gain of the glutamatergic IL-NAcSh projections in mice trained on a task that separated decision making processes across space and time. A behavioral metric was used to identify two distinct valuation functions (deliberative and foraging) and to show the effects of stimulation that can induce lasting plasticity changes. Mice receiving gain-lowering stimulation showed significant, lasting changes in foraging behavior, while deliberative function remained unchanged. Our results suggest that altering cell-type-specific plasticity in the IL-NAcSh pathway selectively mediates a distinct valuation modality involved in foraging while having no effect on deliberative processes. These findings support the claim that different valuation algorithms arise from separate circuits.

Theme: Integrative Physiology and Behavior
THE EFFECTS OF SLEEP DEPRIVATION ON STRESS GRANULES IN CAENORHABDITIS ELEGANS

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Stress granules (SGs) are non-membrane bound aggregates of messenger ribonucleoprotein. It has been shown in cells in vitro that suppression of the mammalian target of rapamycin (mTOR) pathway and its non-mammalian orthologue target of rapamycin (TOR) is associated with an increase in SG formation. It has also been shown that the mTOR pathway is suppressed in response to sleep deprivation in mice. Despite the possible connection via the TOR/mTOR pathway, there has not been any previous evidence directly linking sleep deprivation with SG formation. Our present investigation uses the microscopic, transparent, and genetically tractable nematode, Caenorhabditis elegans as a model for examining SG formation in response to sleep deprivation. C. elegans experience two different types of sleep, developmentally-timed sleep and stress-induced sleep. Developmentally-timed sleep occurs between the different larval stages of the worm, while stress-induced sleep occurs in response to a stressor, such as heat or UV shock. These different types of sleep are mediated by different mechanisms, and mutant strains have been developed that are deficient in the mediators of each type of sleep. We developed two novel strains of C. elegans that model each type of sleep deprivation and have RFP-labeled PAB-1 protein, a key component of SGs, expressed in the pharynx. Through analysis and quantification of SG levels in the sleep-deprived mutant and the sleeping wildtype, we analyzed the impact of sleep deprivation on SG formation. Preliminary imaging shows a sustained increase in SG formation after heat stress in wildtype animals, and current work involves imaging sleep-deprived animals. This work explores novel mechanisms by which sleep deprivation affects neuronal function.

Theme: Integrative Physiology and Behavior
LAMINAR DIFFERENCES IN VELOCITY MODULATION OF HIPPOCAMPAL LOCAL FIELD POTENTIAL

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Local field potential oscillations, which arise from large-scale synaptic activity over a population of neurons, are hypothesized to play a fundamental role in organizing the timing of single neuron action potentials to promote the formation of cogent cell assemblies (Lisman & Idiart, 1995). During active behavior and REM sleep, the hippocampal LFP is dominated by gamma oscillations (50-120 Hz) and the 4-12 Hz theta rhythm (Vanderwolf, 1969; Bragin et al., 1995). As the frequency and power of both these oscillations are modulated by running speed and other behaviors (Montgomery and Buzsaki, 2008; Ahmed and Mehta, 2012; Zheng et al., 2015), and these oscillations are primarily generated by ionic flux related to synaptic activity, then it stands to reason that increases in power are a consequence of more afferent input into the hippocampus. While there is a well-defined organization of afferent input across the hippocampal laminae (Amaral and Witter, 1993), to our knowledge, there has yet to be an explicit investigation of changes in local-field potentials across layers of the hippocampus as a function of velocity. A simple hypothesis is that increased running velocity is associated with a uniform increase in all synapses independent of afferent location (e.g., radiatum power increases as much with velocity as the lacunosum-moleculare). Alternatively, the entorhinal cortex containing path integration-related information may eclipse the CA3 input field. These mutually exclusive hypotheses were tested in the current study.

Theme: Integrative Physiology and Behavior
In the current study we investigated the interaction of hypothalamic paraventricular nucleus (PVN) glucagon-like peptide-1 (GLP-1) and ghrelin signaling in the control of metabolic function. Using indirect calorimetry, we initially assessed the effect of PVN des-acyl or acylated ghrelin on the respiratory exchange ratio (RER) of adult male Sprague Dawley rats. RER was measured over 2 h of the active cycle. Results indicated that acylated ghrelin elicited a robust increase in RER representing a shift in fuel utilization toward enhanced carbohydrate oxidation and reduced lipid utilization. In contrast, treatment with comparable dosing of des-acyl ghrelin failed to elicit significant alterations in metabolic function. In separate groups of rats we subsequently investigated the ability of exendin-4 (Ex-4), a GLP-1 analogue, to alter acylated ghrelin’s metabolic effects or the combined action of ghrelin. Rodents were treated with either systemic or direct PVN Ex-4 followed by acyl ghrelin microinjection. Our results indicated that both systemic and PVN treatment of Ex-4 significantly reduced RER, and that, importantly, Ex-4 pretreatment itself reliably inhibited the impact of ghrelin on RER. PVN Ex-4 was also found to suppress the combined metabolic action of ghrelin paired with neuropeptide Y (NPY). Overall these findings provide compelling evidence that GLP-1, ghrelin, and NPY signaling interact in the neural control of metabolic function within the PVN.

Theme: Integrative Physiology and Behavior
Poster #103

GHRELINERGIC SIGNALING IN APPETITIVE MOTIVATION AND ETOH REWARD: FOCUS ON NEURAL CIRCUITS IN THE CNS

Reed College

The present study examined the functional role of ghrelinergic neurons in the control of appetitive behaviors, specifically food intake and alcohol reward. Neurons expressing ghrelin 1a receptors in distinct regions of the hypothalamus, or within the mesolimbic reward system, were targeted using brain cannula mapping protocols. Specific anatomical structures included the arcuate (ArcN) and paraventricular (PVN) nuclei of the diencephalon, as well as the midbrain ventral tegmental area (VTA) and its forebrain projections sites, the nucleus accumbens core (NAcC) and shell (NAcS). In eating behavior testing, our results indicated that ghrelin significantly increased food intake when administered directly into the ArcN and PVN while other areas of the hypothalamus were non-responsive. In EtOH testing, microinjection of ghrelin into the VTA and both the core and shell regions of the accumbens produced robust effects on consumption. In contrast, hypothalamic treatment did not alter alcohol intake. Finally, using a conditioned place preference paradigm, we demonstrated that accumbal injection of the ghrelin 1a receptor antagonist, JMV 2959, attenuated the ability of alcohol to elicit behavioral responses consistent with conditioned place preference. Overall, these findings identify key regions of the brain mediating the differential effects of ghrelin on appetite and alcohol reward. Our findings support the hypothesis that ghrelin functions as an orexigenic peptide robustly within medial regions of the hypothalamus whereas its stimulatory effect on alcohol consumption is mediated via 1a receptor mechanisms with the VTA and NAc. Both the core and shell regions of the accumbens were responsive to ghrelin’s stimulatory action.

Theme: Integrative Physiology and Behavior
Poster #104

GHRELIN AND ALCOHOL REWARD: A META-ANALYSIS AND GENE EXPRESSION INVESTIGATION

Reed College

Current research indicates that the gastric derived peptide ghrelin may play an important role in the modulation of reward and alcohol consumption. In the present study we systematically assessed the literature on ghrelin and alcohol using meta-analysis methods. The results of the analysis were coded according to animal model, route of administration, drug or peptide manipulation, housing condition, habituation paradigm, sex of subjects, weight, age, diet, whether the alcohol was sweetened or not, type of sweetener, light/dark cycle, vehicle used, and the structure where the drug or peptide was microinjected if centrally administered. Our assessment resulted in a d value of 0.4397, indicating a moderate effect of ghrelin on alcohol consumption in these models. However, the d values varied from 0.004 to 1.64, suggesting that habituation methods significantly affect ghrelin’s modulation of alcohol reward and consumption. Therefore, although there is clear and consistent evidence for a ghrelinergic role in mediating the reinforcing properties of alcohol, more work is needed to assess the magnitude of the effect. In order to further explore this relationship, we have begun to systematically assess the impact of alcohol consumption and exposure on ghrelin 1a receptor expression throughout the mesolimbic system. Towards this aim we exposed female rats to long-term alcohol habituation in a two-bottle choice paradigm and measured 1a receptor expression levels in nucleus accumbens (NAcc) and ventral tegmental area (VTA) by qPCR. Our preliminary analysis indicates that 1a receptor expression is indeed affected by alcohol consumption and withdrawal. Future investigations in male and female rats with varying levels of exposure are needed to determine the full effect of ghrelin on the CNS.

Theme: Integrative Physiology and Behavior
Poster #105

MEASURING ANXIETY IN DANIO RERIO FOLLOWING TREATMENT WITH CAFFEINE, APOMORPHINE AND BLUE LIGHT

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Our laboratory uses Danio rerio (zebrafish) as a model organism and focuses on assessment of anxiety or memory in the fish. While these fish have long been used by developmental biologists, their utility for neuroscientists has only recently become apparent. Zebrafish are easily maintained and have the ability to absorb substances through their gills, eliminating any stress induced by the traditional method of injections. Zebrafish, as vertebrates, have complexity that is useful for behavioral models. Their social structure and conditioned behaviors are being carefully characterized. Relevant to these studies, the Danios’ behavior in a novel dive tank has been demonstrated to correspond to their anxiety. (Anxious fish spend more time near the bottom of the tank while control fish spend more time near the surface.) Using this behavioral assay, fish were treated with increasing concentrations of caffeine or apomorphine prior to placement in the novel tank. The treated fish were less anxious at lower doses but more anxious at higher doses compared to untreated control fish. In a separate study, fish were exposed to blue light (455-475nm wavelength) for 7 days and then assessed for anxiety. This wavelength was selected since blue light has been demonstrated to interfere with melatonin production and circadian rhythms. Danio rerio exposed to blue light were more anxious than control fish as determined by the novel dive tank test. Taken together, these studies are consistent with results seen in other systems and support the continued refinement of this model organism.

Theme: Integrative Physiology and Behavior
Head direction (HD) cells, found in many areas of the rodent Papez circuit, are thought to reflect the spatial orientation of the animal. Each HD cell fires maximally when the head is oriented towards a particular direction, known as the preferred direction of that cell. Each HD cell has a unique preferred direction and it is thought that the combined activity of the HD cell population indicates the directional orientation of the animal. HD cells are known to use visual landmarks in the maintenance of a stable preferred direction. The superior colliculus (SC) is a brain structure that has been shown to be important in attention and orienting to visual landmarks and has indirect connections with limbic areas containing HD cells. Up until now, however, the role of the SC in the generation and maintenance of the HD signal has not been investigated. In order to determine the relationship between SC functioning and the HD cell network, HD cell activity was recorded before and after inactivation of the SC. Female, Long Evans rats were implanted with a recording electrode in the anterodorsal thalamic nucleus to record HD cells. In addition, a bilateral cannula was implanted in the SC to allow for inactivation. Following the isolation of an HD cell, rats were recorded in a baseline condition where they foraged for sugar pellets dropped into a circular enclosure to determine the preferred direction of the cell relative to the visual landmarks of the enclosure. The animal was then administered a unilateral or bilateral infusion of muscimol (500 ng) or isotonic saline into the SC. After allowing 30 minutes for absorption, the animal was returned to the recording enclosure and the HD cell was again recorded. As reported by previous investigators, unilateral inactivation of the SC resulted in a neglect syndrome, with the animal showing a strong turn preference during foraging to the side opposite of injection. For saline infused animals, the directional activity of recorded cells remained stable for the post-infusion sessions. In contrast, cells from animals receiving SC inactivation showed a decrease in directional-specific activity, with the remaining activity being unstable relative to the location of the visual landmarks within the apparatus. In addition, some cells showed a continuous drift in preferred direction within recording sessions, an effect that is not normally seen in HD cells. While unilateral inactivation produced significant attenuation of the HD signal, our preliminary results show a much more dramatic degradation following bilateral infusions, to the extent that the HD cells essentially became nondirectional. This was the first investigation of the relationship between the functioning of the SC and the HD system and our findings provide evidence that these brain structures work cooperatively to enable stability in the brain network that mediates directional orientation.

Theme: Integrative Physiology and Behavior
Eyeblink conditioning procedures were used in this study to assess the ability of rats to discriminate between two different color stimuli (i.e., blue and red cues, counterbalanced). One stimulus was paired with a periorbital stimulation unconditioned stimulus (A+) and the other stimulus was not (B-). Each rat received fifteen 100-trial sessions of A+/B- discrimination training and some of the rats also were given lesions of the fornix prior to training. Therefore, one aim of our study was to assess whether fornix damage impaired a rat’s ability to discriminate between two colors. Our findings showed that rats with fornix damage are able to discriminate between the two colors, but additional control rats (i.e., rats without fornix damage) are needed in order to determine if the magnitude of visual discrimination in lesioned rats is smaller than control rats. Nevertheless, this work, to our knowledge, is the first ever demonstrate successful two-color discriminative eyeblink conditioning in rats. Thus, our data contribute new information to the behavioral neuroscience field that can be used as a foundation for designing and conducting additional experiments that also examine the behavioral and neurobiological mechanisms of visual discrimination.

Theme: Integrative Physiology and Behavior
The peptide vasotocin (VT) and its mammalian homologue, vasopressin, produce effects on social behavior that are highly species and context-specific. We therefore examined whether goldfish respond differently to VT based on social context. Behavioral tests indicated that VT does influence social behavior differently depending on the context: in male goldfish, VT inhibited social approach towards other males, but not towards females in the presence of sex pheromones. We have also begun to characterize the distribution of V1a-like receptors in the brain that mediate these behavioral effects with immunohistochemistry, and to determine if social context alters receptor expression and thus, potentially, behavioral responsiveness to VT.
CARNIVORES AND RODENTS EVOLVED DIFFERENT PATTERNS OF PROPORTIONAL BRAIN STRUCTURES THAN OTHER TAXA

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Examining the proportion of brain devoted to various neuroanatomical structures across taxa provides an interesting lens into how mammalian orders diverged through evolution. By using proportions, all brain regions are on the same scale, which allows us to compare the pattern of relationships across various taxa. This will enable us to determine whether the same pattern of brain evolution occurred across taxa or if they have diverged. We examined each of the following regions as proportions of the whole brain: isocortex, subcortical structures (striatum, thalamus and hippocampus), and cerebellum. Images were obtained from the Comparative Mammalian Brain Collection (http://neurosciencelibrary.org/). Considering all of the animals together, the proportional subcortical structures were all positively and significantly correlated with each other. The proportional isocortex, however, was significantly inversely correlated with all other structures. The proportional cerebellum was significantly correlated with the proportional hippocampus and thalamus but inversely correlated with the isocortex. We then sought to see if this pattern was uniformly present in the various taxa. We examined these brain regions across primates (n =11), carnivores (n=17), artiodactyls (n = 8), rodents (n = 7) and “others” (n=18). Some notable exceptions to the overall pattern appeared. Given that the proportional isocortices are fairly large are in carnivores, we expected an inverse relationship between proportional isocortex and subcortical structures. However, this inverse relationship was largely lost, although the proportional subcortical structures stayed fairly well correlated in carnivores. The pattern of relationships in carnivores suggest that the subcortical features evolved in concert but independent of proportion of brain devoted to isocortex. The relationships among the proportional subcortical structures among rodents are essentially absent. Rodents have a relatively large proportional hippocampus, striatum, and thalamus compared to other taxa. Whereas these structures seem to evolve in concert in other taxa, they don’t seem to do so in rodents. Finally, the relationships between the proportional cerebellum and any other structure are largely lost when we examine the individual taxa. The cerebellum appears to be subject to very different sets of evolutionary pressures both across and within taxa than are the other structures. Clearly brains evolve in different patterns across taxa—most particularly carnivores and rodents, which are on very different paths in brain evolution.

Theme: Integrative Physiology and Behavior
EFFECTS OF PARTIAL HIPPOCAMPAL LESIONS IN RATS ON THE TRAVELING SALESMAN PROBLEM

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The Traveling Salesman Problem (TSP) is a spatial navigational task that differs from many other behavioral techniques because it allows the observation of behavior in a more naturalistic setting. The goal of the task is not to verify if an animal can do a certain behavior, but to record how the animal behaves in natural foraging conditions. This task may involve a variety of cognitive functions, such as spatial processing, memory, attention, route planning, and decision making. Given the established role of the hippocampus in both spatial processing and spatial memory, we examined how hippocampal damage affects rats’ performance in the TSP. The rats were pretrained on the TSP, which involved learning to retrieve bait from targets in a variety of spatial configurations. Matched for performance, rats were then divided into two groups, receiving either a partial hippocampal lesion or a control sham surgery. After recovering from surgery, the rats were tested on eight new configurations. A variety of behavioral measures were recorded, including distance traveled, number of revisits, span, and latency. The results showed that the sham group outperformed the lesion group on most of these measures, with the lesion group demonstrating more pronounced impairment on the more complex configurations. Based on histological tissue analysis of each group, we determined that the hippocampus appears to be involved in finding efficient routes, particularly in complex versions of the TSP.

Theme: Integrative Physiology and Behavior
FLUOXETINE ADMINISTRATION ON ADOLESCENT RATS AND ITS EFFECT ON THE ACQUISITION AND EXTINCTION OF A CONDITIONED TASTE AVERSION PRESENTED IN ADULTHOOD

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Fluoxetine (FLX), a selective serotonin reuptake inhibitor (SSRI), is the first drug approved for pediatric cases of Major Depressive Disorder. Adolescence marks a period when brain regions are undergoing critical developments that will have a lasting impact on many neurobiological functions thus it follows that SSRI exposure during this period could result in long-lasting effects. Indeed in rodent models, FLX treatment during adolescence results in changes in mood-related behavioral changes when animals were tested later in life (Iñiguez et. al. 2010). This study seeks to extend these findings in a learning context. Conditioned taste aversion (CTA) was used to assess the effect of early-life FLX administration on acquisition and extinction of a CTA in adulthood. Male and female rats received FLX (20 mg/kg i.p) once daily for 15 consecutive days from postnatal days (PND) 35-49. During this time, FLX-injected rats gained body weight more slowly than saline-injected controls, and this effect was more pronounced in male than female rats. In adulthood (from PND60), all rats were presented with 0.15 M saccharin followed by an i.p injection of lithium chloride (LiCl) to induce visceral malaise. Four saccharin (conditioned stimulus) and LiCl (unconditioned stimulus) pairings occurred across the CTA acquisition phase. Next, saccharin was presented without LiCl, the aversive stimulus, and intake was measured across 18 consecutive days of the extinction phase. The groups did not significantly differ in rates of CTA acquisition or extinction. Although previous research has shown changes in mood-related behaviors as a consequence of early-life FLX administration, the current findings show early-life administration does not appear to influence the rate of CTA learning.

Theme: Integrative Physiology and Behavior
CHARACTERIZATION OF THE RELATIONSHIP BETWEEN PHENOTYPE, DIET, AND MITOCHONDRIAL FUNCTION IN A DROSOPHILA MODEL OF EPILEPSY

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Drosophila mutants known as “bang-sensitive” have been utilized as models for neurological conditions including epilepsy, sensorineural deafness, and age-dependent neurodegeneration. These mutants also have a shortened lifespan. While the mechanisms producing these phenotypes are unique to each strain, some of the gene products suggest mitochondrial dysfunction as a possible underlying cause. Diet is an important factor in determining energetics. For example, a ketogenic diet has been shown to be an effective treatment for refractory epilepsy in humans, and diet can manipulate lifespan. We wanted to more clearly define the connection between diet, mitochondrial function and phenotype in this fly model system. Bang-sensitive mutant strains were reared on a standard molasses, yeast and cornmeal (MYC), which is a low protein/high carbohydrate diet, or a protein-rich yeast sugar (YS) diet. The mutants display a lower percentage of seizures on the YS food, but also reduced viability and lifespan. Several biochemical methods were utilized to define the effects of diet. Cytochrome oxidase (CO) in mutants is reduced as compared to wildtype, with increased CO levels in all flies raised on the YS food. In this case, alterations in mitochondrial function correlate with improvement in epilepsy phenotype. In addition, citrate synthase activity and other metabolic indicators suggest that both the mutant phenotype and diet interact in complex ways to produce the defects in excitability and aging.

Theme: Integrative Physiology and Behavior
IMIQUIMOD (IMQ) MEDIATED PSORIATIC ITCH IS NOT DEPENDENT ON TRPA1 OR TRPV1, WHILE DERMAL INFLAMMATION IS DEPENDENT ON BOTH

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Summary: Plaque psoriasis is a chronic inflammatory skin disease that affects 2-3% of the world population. This disorder is characterized by scaly, thick skin, intense ongoing itch, and itch from light touch (such as clothing contacting skin, called “alloknesis”). Imiquimod (IMQ) is a topical treatment for basal cell carcinomas and warts that has been used to create a mouse model of plaque psoriasis. IMQ-treated mice show significant increases in spontaneous scratching and alloknesis that were unaffected by antihistamines. Previous studies have shown that histamine-mediated itch is dependent on the TRPV1 ion channel, while non-histaminergic itch is dependent on TRPA1. The present study sought to investigate the role of TRPV1 and TRPA1 in the imiquimod model of psoriatic itch.

Aim of Investigation: The purpose of this investigation is to determine if the increase in spontaneous scratching behavior and other signs of itch of mice treated with IMQ depends on TRPV1 or TRPA1 representing the histaminergic and non-histaminergic itch pathways, respectively. We additionally investigated sex differences in the behavioral manifestations of chronic itch following IMQ treatment.

Methods: In male and female wildtype and TRPV1- and TRPA1-knockout mice, IMQ (5%; Aldara) or vehicle was applied topically to the rostral back for 5 consecutive days. Spontaneous hindlimb scratch bouts directed to the treatment area were measured on Day 0, 1, 3 and 5. To assess alloknesis, a 0.07g von Frey monofilament was applied to the perimeter of the IMQ application area 5 consecutive times, and the alloknesis score consisted of the number of immediately evoked hindlimb scratch bouts directed to the stimulus site divided by 5. Hyperknesis was assessed by counting the number of hindlimb scratch bouts elicited by intradermal injection of chloroquine (CLQ; 100 µg/10 µL) in rostral back skin 1 week prior to IMQ application and again on Day 4 of IMQ treatment. Transepidermal water loss (TEWL) was measured before and after IMQ treatment. Dermal infiltrate, a measure of skin inflammation, was measured using Giemsa for staining of mast cells and immunohistochemical staining for dermal inflammatory makers CD31 and CD45.

Results: All groups receiving IMQ treatment exhibited a significant increase in TEWL score as well as skin scaling compared to vehicle controls. For IMQ-treated mice, TEWL scores were significantly lower in both TRPV1 and TRPA1 knockout mice compared to wildtypes. IMQ treatment also resulted in a significant increase in spontaneous scratching in wildtype males but not females compared to vehicle controls. Interestingly, there were no significant differences in spontaneous scratching among the IMQ-treated male wildtype, TRPV1 and TRPA1 knockout mice. Curiously, IMQ treatment resulted in a significant increase in spontaneous scratching in femaleTRPV1 knockouts (but not female TRPA1 knockouts or wildtypes). Skin biopsies revealed a significant increase in dermal inflammatory markers CD31 and CD45 and skin...
thickening following IMQ (but not vehicle) application in wildtypes. These markers were significantly lower in both TRPV1 and TRPA1 knockout mice treated with IMQ. Before IMQ treatment, alloknesis scores were zero in all mice. All IMQ-treated mice exhibited a significant increase in alloknesis scores. In these treated mice, male TRPV1 and TRPA1 knockout groups exhibited significantly lower alloknesis scores compared to wildtypes. There were no significant differences in alloknesis scores among IMQ-treated female wildtype and knockout groups. Prior to IMQ treatment, CLQ evoked significant increases in scratch bouts in all mice, including TRPA1 knockouts. Following treatment with IMQ or vehicle for 4 days, CLQ still elicited a significant increase in scratch bouts in all treatment groups, with no significant between-group differences. This indicates that hyperknesis did not develop as a result of IMQ treatment and was independent of TRPV1, TRPA1 and sex.

Conclusions: Our results corroborate previous findings that IMQ treatment increases spontaneous scratching behavior and alloknesis. However, our data indicate that hyperknesis does not develop in this model. IMQ treatment also resulted in an increase in dermal inflammatory markers as well as TEWL, demonstrating a robust increase in skin barrier dysfunction. Interestingly, these markers were reduced in knockout mice lacking TRPV1 or TRPA1 which, however, did not exhibit any reduction in spontaneous scratching or alloknesis. This suggests that TRPV1 and TRPA1 are more important for inflammatory skin reactions than they are for sensitization of itch signaling. Finally, IMQ treatment more robustly affected male vs. female mice, suggesting a sex difference for the expression of chronic itch in this model.

Theme: Integrative Physiology and Behavior
The dorsal striatum, an input nucleus of the basal ganglia, is generally thought to be involved in action selection and control of behavioral strategies. A key modulator of striatal function is the neurotransmitter dopamine (DA), which is released by projections from the substantia nigra pars compacta (SNc). The dorsal striatum can be subdivided into two subregions based on cortical inputs and differing functional roles. Specifically, the dorsomedial striatum (DMS) is thought to be involved in goal-directed actions, whereas the dorsolateral striatum (DLS) is believed to be involved in habitual behaviors. However, the behavioral function of nigrostriatal dopamine projections to the DMS and DLS remain largely unknown. To investigate the roles of nigrostriatal dopamine in these subregions, we selectively expressed the light-activated cation channel channelrhodopsin-2 (ChR2) in the SNc of DAT-cre mice. This manipulation allows us to selectively stimulate dopamine neurons using laser light. Next, we bilaterally implanted mice with fiber optic implants in either the DMS or DLS, allowing for subregion-specific stimulation. Mice first performed a ten-day intracranial self-stimulation (ICSS) task where they were given access to two levers in an operant chamber and where behavioral flexibility was assessed following DLS and DMS self-stimulation. Consistent with the canonical roles of DMS and DLS in habit formation, DLS mice were less efficient altering their behavior following changes in ICSS contingency, indicating accelerated habit formation. Mice were next placed in an open field assay to explore the effect of stimulating DA on locomotion. Stimulation of DA projections in both subregions led to changes in velocity, such that mice moving quickly pre-stimulation began moving slowly with stimulation, and vice-versa. Finally, mice were placed in a two chamber, real-time conditioned place preference (RTCPP) paradigm, where moving to one chamber led to laser stimulation and being in the other chamber led to no stimulation. Contrary to DA’s reported role in place preference, mice did not show a preference for the stimulated chamber, regardless of the stimulated subregion. These results suggest differential roles for nigrostriatal dopamine in action selection and reinforcement, where activating dopamine release across subregions similarly reinforces action and invigorates locomotion, but where DLS stimulation may specifically increase behavioral inflexibility.

Theme: Integrative Physiology and Behavior
Obesity has evolved into a global epidemic in just the past few decades, with a significant increase of obese people in the United States. Overeating and lack of physical activity are among the main contributors to obesity. Leptin is an adipocyte derived hormone involved in the regulation of body weight, food intake, and energy expenditure. Leptin regulates feeding behavior by acting as a satiety signal in the brain. Obese individuals are hyperleptinemic compared to lean subjects; and the hyperleptinemia can lead to leptin resistance which is thought to be caused by alterations in leptin receptors or by an inability of leptin to cross the blood brain barrier (BBB). Intranasal (IN) drug administration bypasses the BBB, allowing compounds to directly access the brain and avoid first-pass elimination via the liver. On the other hand, the neurotransmitter serotonin has been known to be involved with satiety and recently has been shown to affect the efficacy of leptin at controlling food intake. For this reason, it is believed that leptin and serotonin may conjointly affect food intake. In view of these observations, we hypothesized that IN leptin administration will reduce both food intake and bodyweight and that leptin treated animals will show higher levels of neuronal activation in the hypothalamic arcuate nucleus and the dorsal raphe nucleus as well as greater serotonin turnover in the brain. To test our hypothesis, we treated rats with 25 μl of either leptin (0.2 mg/kg BW) or vehicle that was applied to both nostrils in drops, at the beginning of the dark cycle for one week. At the end of the treatment, rats were either intracardially perfused with 4% paraformaldehyde to determine c-fos expression or sacrificed via rapid decapitation to determine serotonin turnover in the hypothalamus, hippocampus, and olfactory bulb 2 hours after IN leptin administration. Plasma levels of leptin were analyzed before and after IN leptin administration. Leptin treated animals did not show any increase in plasma leptin levels, indicating that IN administered leptin did not leak into the periphery. IN leptin did not affect body weight and daily food intake; however, IN leptin significantly decreased food intake on the first day of treatment in a transient manner. Nonetheless, this effect was not replicated on the last day of treatment. High performance liquid chromatography (HPLC) was performed to measure serotonin (5-HT) and its metabolite (5-HIAA) concentrations. No significant changes in serotonin turnover between the two groups were detected; whereas c-fos expression in the arcuate and dorsal raphe nuclei were higher in leptin-treated animals compared to vehicle-treated animals. Collectively these results suggest that acute IN leptin is able to regulate food intake; however, chronic treatment lacks this ability. Additionally, one week of IN leptin administration activates hypothalamic and raphe neurons, but failed to regulate food intake and serotonin turnover.
Poster #116

EEG ALPHA POWER DECREASES DURING APPROACH OF PAIN FOR REWARD IN PARTICIPANTS WITH CHRONIC PAIN

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Pain does not occur in a homeostatic vacuum and chronic pain sufferers do not have the choice to avoid or relieve pain. Approach-avoidance in the context of pain is a decision-making conflict consisting of approaching pain to achieve a reward or satiate a drive. This research was designed to elucidate the neural mechanisms that accompany approach-avoidance behaviors among chronic pain sufferers. We hypothesized (1) a chronic pain group would avoid pain stimuli more than a painless control group and (2) a pain group would demonstrate increased prefrontal asymmetry, driven by increased left hemisphere activity related to avoidance. Participants included 31 right-handed people aged 19-55 (4 males) that reported current pain for more than 6 months (chronic pain) or reported being pain free controls. iMotions software recorded electroencephalogram activity from B-alert hardware containing 20 active electrodes (referenced to mastoid leads) attached to the scalp during a hypothetical approach-avoidance task. Participants chose to approach varying levels of pain (low-moderate-high) to receive varying levels of a monetary reward (low-moderate-high). MatLab was used for post-processing (filters at .02-50Hz with manual artifact rejections). Fast Fourier Transforms were calculated in Cartool (delta 0-3Hz; theta 4-7Hz; alpha 8-12Hz; beta 13-30Hz; gamma 31-50Hz). Prefrontal asymmetry was calculated in Microsoft Excel as the natural log of right side alpha (F4)- natural log of left side alpha (F3). SPSS was used to compute independent samples t-tests. The pain group avoided pain stimuli significantly less than controls, \( p < .05 \). The pain group did not demonstrate increased prefrontal asymmetry (\( p > .05 \)), but had significantly less alpha frequency activity in 5 electrodes (F4, C3, Cz, C4, P3 & PoZ) during high threat trials, \( p < .05 \). High threat trials altered decision-making during the pain approach-avoidance task. This was powered by a widespread decrease in alpha band cortical activity, rather than the hypothesized hemispheric lateralization. High-threat level approach-avoidance stimuli produced a more salient threat to homeostasis. The broader impact of this research lies the development of a body of literature to continue exploring pain as a multidimensional, complex disruption of homeostasis.

Theme: Integrative Physiology and Behavior
Poster #117

THE EFFECTS OF PRE-EXPOSURE AND SEX ON ETHANOL-INDUCED PLACE AVERSION IN ADOLESCENT CFW MICE

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Alcohol abuse is a problem that can be arise in adolescence and have long-lasting impacts in adulthood. The mouse model is effective in researching the rewarding & aversive effects of ethanol when utilizing place conditioning. Using aversion place conditioning, we measured differences between adolescent male and female outbred CFW mice. The mice were pre-exposed with 2g/kg of ethanol, then conditioned with 4g/kg during conditioning trials. After 4 conditioning trials, we introduced extinction and requisition trials in order to determine the strength of the conditioning. Our results showed conditioning trials created aversion for all but one group, and requisition had all groups showing aversion. However, we did not find any differences between male and female mice throughout the entire experiment. A pilot data collection will be initiated looking at dopamine and glutamate receptor expression in the brains of both male and female mice to measure neurological differences. No behavioral differences observed indicate that both males and females are impacted by the aversive effects of ethanol equally.

Theme: Integrative Physiology and Behavior
DOES THE CARDIAC GANGLION OF THE AMERICAN LOBSTER, HOMARUS AMERICANUS, HAVE A FUNCTIONAL CIRCADIAN TIMEKEEPING SYSTEM?

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All organisms have intrinsic, genetically encoded pacemaking systems, i.e., biological clocks, that allow them to synchronize their physiology and behavior with the environment. The circadian pacemaker, which cycles on an approximately 24-hour time period and is tuned to the solar day, is perhaps the best known clock system. The heart of the American lobster, Homarus americanus, is neurogenic, with contractions controlled by the cardiac ganglion (CG), a central pattern generator. Previous research in decapods has suggested that the cardiac output in Homarus may be driven by an intrinsic peripheral circadian system. In support of this hypothesis, transcripts encoding all five proteins generally recognized to form the ancestral-type arthropod core clock (i.e., clock, cryptochrome 2, cycle, period, and timeless) were recently identified from a H. americanus CG-specific transcriptome (Christie et al. 2018. Marine Genomics. In press). However, previous studies have not provided clear physiological evidence of a functional circadian rhythm in cardiac function. To determine if there is a functional network present that controls cardiac output, we are maintaining the isolated CG under constant light and temperature, and recording the neuronal output of the isolated CG, over a period of 24-96 hours (one to four circadian cycles). Data collected to date are inconclusive, and do not allow us to determine whether or not the cycle frequency oscillates with a 24-hour periodicity. Even if the heartbeat frequency does not oscillate on a 24-hr cycle, it is possible that the circadian-related transcripts nonetheless cycle over a 24-hour period, as they are known to do in other systems. To determine whether this is the case, we plan to isolate RNA at time intervals in lobsters held under constant conditions.

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Theme: Integrative Physiology and Behavior
The freshwater snail, *Lymnaea stagnalis*, has been a valuable research model in the study of learning and memory. Epicatechin, a flavonol found in high amounts in dark chocolate, red wine and green tea, enhances memory formation in *L. stagnalis* in operant conditioning experiments (Fernell, Swinton, and Lukowiak, 2016). Flavonols have also been shown to enhance spatial memory in rodents and working memory in humans. We tested the effects of epicatechin on memory formation in *L. stagnalis* using a taste aversion protocol. Our hypothesis was that epicatechin would enhance the snails’ memory of sucrose when paired with aversive stimuli (electric shocks). A pair of needle electrodes separated by 21.5 mm delivered shocks to the water on each side of the snails (snails were not touched by the electrodes). The built-in 10-volt stimulator of a Biopac® MP 36 data acquisition unit produced the shocks; stimulus strength and duration were controlled using Biopac® BSL software. We performed a stimulus strength-duration experiment in which the criterion response was a snail’s full-body withdrawal lasting 10-14 seconds. In pre-conditioning feeding response tests, we counted the number of bites per minute (bpm) snails made in response to agar pellets containing 100 mM sucrose. A conditioning session consisted of 25 shocks (8 V, 55 msec) with 5 seconds between each shock, causing withdrawal and termination of feeding. An experimental group was exposed to 15mg/L epicatechin for 40 minutes immediately after conditioning. A control group was conditioned in the same manner but was not exposed to epicatechin. Feeding responses to sucrose pellets were measured 24 hours post-conditioning. There were significant reductions in bpm ($p < .0001$; 2-tailed paired t-tests) for the epicatechin-treated group, but the control group had no significant change in feeding response ($p > .1$), supporting our hypothesis. We are currently testing snails raised in low levels of epicatechin (1.5mg/L) to determine whether the availability of environmental flavonols also facilitates memory formation.

Theme: Integrative Physiology and Behavior
Imagine two rooms. In the first room, you are always given a piece of cake. In the second room, you are never given cake. If this pattern repeats, you will come to prefer the first room even when no cake is provided. This preference of one place over another because it has become associated with something pleasurable is known as “conditioned place preference (CPP),” and is a paradigm commonly used to measure the rewarding properties of objects, events, or experiences. While CPP is primarily used in studying drug abuse, there is evidence that certain social behaviors, including aggression, can induce CPP. Thus, the focus of this study was to examine if aggressive behaviors in male zebra finches are rewarding and can induce CPP, using the mate competition paradigm. In mate competition, same-sex dyads are presented an opposite-sex stimulus across a wire partition and compete aggressively for access to the potential mate. The CPP protocol utilized an apparatus with two distinct chambers and included 3 phases: 1) baseline preference trial, 2) mate competition in nonpreferred chamber, and 3) probe preference trial. A two-way repeated-measures ANOVA was used to compare baseline preferences to preferences during the probe trial. The ANOVA yielded no significant within-subjects effect for subjects that displayed aggressive behaviors. However, there was a significant within-subjects effect for subjects that sang at least once during mate competition (p=0.048). Thus, these findings suggest that aggressive behaviors are not sufficient to induce CPP while, in contrast, courtship song is sufficient to induce CPP. Given that zebra finches are gregarious animals, aggressive competition for a mate may not be a rewarding behavior, considering that they innately prefer to live harmoniously with conspecifics. Courtship song, however, is likely reinforced because courtship behaviors are important for securing a high-quality mate and developing pair bonds.

Theme: Integrative Physiology and Behavior
Poster #121

DOES L-DOPA INCREASE IMPULSIVE RESPONDING IN MALE BETTA SPLENDENS

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Previous research has revealed that male *Betta splendens* demonstrate preference for a larger-delayed food reward over a smaller and immediately available food reward. This particular instance of behavioral “self control” occurs without specialized training procedures (e.g. delay fading, secondary reinforcement, etc.) typically employed in studies of other non-human species where self-control is demonstrated. Other researchers have discovered that oral administration of the dopamine precursor, L-dopa, to humans increases delay discounting in humans and subsequently reduces human participants’ self-control for non-food rewards. In the present study, male *Betta splendens* are dosed with oral L-dopa to determine if L-dopa increases impulsive responding for food reward in *Betta splendens*. Subjects were expected to become more impulsive in their behavior when exposed to the appropriate dose of L-dopa. After testing several doses, subjects in a titration pilot receiving 60 mg/kg L-dopa made more consistent impulsive choices for a smaller immediate food reward when compared with males receiving vehicle only. The preliminary findings of the current replication study are presented and discussed in terms of dopaminergic mechanisms influencing reward choice in animals.

Theme: Integrative Physiology and Behavior
Ellagic acid (EA), a phenolic acid found in a wide variety of fruits and nuts, has been shown to possess many health benefits. In addition to antioxidant, anticancer, and neuroprotective properties, Mansouri et al, 2014 reported that ellagic acid produced moderate analgesia when administered alone and a synergistic effect when co-administered with morphine. They also reported that ellagic acid retarded the development and expression of morphine tolerance. The first phase of our experiment was dedicated to verifying the analgesic properties of ellagic acid at doses reported by Mansouri et al., 2013. We found that ellagic acid was completely insoluble for injection at doses reported, and upon careful examination we found inconsistencies in reporting effective doses and molar weights between the 2013 and 2014 papers. Therefore, we proceeded to investigate the analgesic properties of ellagic acid, using the tail flick test, at a dose reported by Mansouri et al, 2013 to be effective when administered orally. Oral administration of ellagic acid (dose 300 mg/kg presented in a food supplement paste) failed to produce an increase in tail flick latency to radiant heat stimuli. Our preliminary results also failed to support the synergistic effect of ellagic acid and morphine on tail flick latency. The extreme insolubility of ellagic acid will weaken its use as a therapeutic coagent with opioids for pain management, unless there is a way to increase the bioavailability through oral administration.

Theme: Integrative Physiology and Behavior
GROOMING DISPLAYS ROBUST FACE VALIDITY IN THE NEONATAL CLOMIPRAMINE EXPOSURE RAT MODEL OF OBSESSIVE COMPULSIVE DISORDER

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Obsessive Compulsive Disorder (OCD) is a chronic neuropsychiatric illness that affects 2-3% of the United States population and is characterized by persistent anxiety producing thoughts accompanied by overwhelming urges to perform repetitive ritualistic behaviors. Modern pharmacological treatments for OCD are only effective in 40-60% of patients, have an 8-10 week delayed onset, and are associated with problematic side effects. Animal models of this psychiatric disorder offer an invaluable tool whereby new therapeutic avenues can be explored. Although multiple behavioral assays have been suggested to reflect the repetitive and compulsive symptoms of OCD, no single behavior has been universally accepted as a model of the disorder. The objective of this study was to explore grooming as an alternative to other types of anxiety-related behaviors in the assessment of the OCD-like phenotype in a novel animal model of OCD induced by neonatal exposure to clomipramine, a serotonin/norepinephrine reuptake inhibitor. Prior studies have demonstrated that the neoclomipramine rodent model has both face and predictive validity in the Hole Board (HB) and Elevated Plus Maze (EPM). In the current study, for the first time, grooming was selected for analysis in this novel model given its frequent over-expression in OCD patients. Grooming, rearing, and HB behaviors were repeated assessed over 3 trials (separated by 1-2 weeks) in adult male Sprague-Dawley rats that had been injected neonatally (Day 9-16) with either 15 mg/kg clomipramine (neoCLOM, n=20) or with saline (neoSAL, n=20). Neo-CLOM “OCD-like” rats consistently exhibited increased grooming versus neo-SALINE control rats across all 3 Trials (*p 1 = 0.049, **p 2 = 0.0071, **p 3 = 0.0037). In contrast, no significant differences between the experimental groups were observed for rearing or head poking measures. Likewise, analysis of the rats’ behaviors in the EPM showed no significant differences between these two groups of rats and data from the multiple trials had considerable variability. In conclusion, this study demonstrates that an analysis of grooming offers valuable advantages over other anxiety-related measures as a behavioral assay for animal models of OCD. Grooming behaviors robustly demonstrate phenotypic differences between experimental “OCD-like” and control rats, is consistently expressed across trials, and has high face validity with the human disorder.

Theme: Integrative Physiology and Behavior
OVERCOMING THE ONE TRIAL TOLERANCE OF RAT BEHAVIOR IN THE ELEVATED PLUS MAZE (EPM)

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Rodent models of anxiety disorders often employ the Elevated Plus Maze (EPM). A higher ratio of time spent in the CLOSED versus OPEN arms of the maze is associated with a higher level of anxiety. A major limitation in using the EPM, however, is the “One Trial Tolerance” phenomena (File, Mabbutt, & Hitchcott, 1990). This refers to dramatic drop in the animals’ exploration of the OPEN arms following the initial exposure to the maze. It has thus been suggested that repeated testing in the EPM for a single rodent is thus experimentally invalid. The aim of this study was to investigate whether manipulation of selected variables of the experimental protocol could increase OPEN arm exploration of male Sprague-Dawley rats (n=20) upon repeated exposure to the arena. For the current study, rats were initially exposed to the arena at an early age (30 days postnatal), observational trials were separated by 10 days, the animals were frequently handled, the housing environments were enriched, and low illumination levels were used when assessing behavior. In accordance with prior studies, OPEN arm exploration was significantly decreased upon the 2nd exposure (Day 40) to the arena. However, by the 4th exposure (Day 60), OPEN arm activity was no longer different from the initial exposure. Moreover, by the 6th exposure (Day 80), OPEN arm activity was actually increased above that measured on the 1st Trial. This study thus demonstrates that procedural modifications of the experimental protocol can enable OPEN arm behavior to be fully restored upon successive exposures in the EPM - thereby overcoming the One Trial Tolerance limitation associated with this commonly used apparatus. Adopting these relatively minor experimental modifications enables the anxiety level of an individual rat to be assessed multiple times in the EPM - allowing for a repeated measures design and minimization of the number of animal subjects required for experimentation.

Theme: Integrative Physiology and Behavior
VALIDATING THE ROLE OF THE DOPAMINE D3 RECEPTOR IN A RODENT MODEL OF PTSD-RELATED HYPERVIGILANCE AND ALCOHOLISM

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Post-Traumatic Stress Disorder (PTSD) is a disorder that may develop in response to exposure to traumatic events. It is most commonly seen among combat veterans, rape victims, and first responders. While the neurological mechanism underlying the development and subsequent symptomology of PTSD is unclear, dysregulation of dopaminergic circuitry appears to be involved. To better understand this potential relationship, we conducted two studies involving the administration of a selective dopamine D3 antagonist SB-277011A (SB) and a model of PTSD-like hypervigilance in C57BL/6J mice. A third study also involved the effects of trauma and later administration of the D3 antagonist on ethanol self-administration in male Sprague-Dawley rats. For our model of PTSD-like behavior, we used a modified version of the Single Prolonged Stress (SPS) protocol, in which an auditory stimulus was paired with a forced swim, restraint, and foot shock. Hypervigilance was quantified by measuring the duration of freezing behavior upon reintroduction to the paired tone in a novel environment. Our first experiment investigated the effects of SB on expression of PTSD-like hypervigilance in female C57BL/6J mice. Mice (n = 34) were tested the day prior to SPS and again 7 days after the procedure. At post-SPS testing, mice were given i.p. injections of saline, vehicle solution, or SB. Freezing responses were measured 30 minutes after administration. We observed significantly greater freeze times in mice that experienced trauma and received only vehicle compared to mice that experienced no trauma and received saline (control), indicating that the SPS procedure was effective. Additionally, there was no significant difference between control mice and mice that experienced trauma and received the D3 antagonist, indicating that the drug attenuated expression of symptoms. In our second experiment, we administered SB during the SPS protocol to investigate the role of the D3 receptor on acquisition of PTSD-like symptomology. Mice were given i.p. injections of either vehicle solution or one of three dosages of SB 30 minutes prior to SPS and again during the procedure. Hypervigilance was measured at one, two, and four-week intervals following SPS. At one-week post-SPS, we observed significantly greater freeze times in mice that received only vehicle during SPS relative to mice that did not undergo the procedure, indicating that SPS was successful in producing hypervigilance. We also observed significantly reduced freeze time in mice that received a high dosage of SB relative to mice that received vehicle. At two- and four-week intervals there were no significant differences observed, although within-group trends indicate this may be due to extinction. Our third experiment sought to investigate the relationship between PTSD and an increased likelihood of developing alcoholism and to evaluate the efficacy of a selective D3 antagonist in addressing this behavior. We modeled this behavior by training SPS-exposed and non-SPS exposed male Sprague-Dawley rats to self-administer non-alcoholic beer. Ethanol was gradually added at 2% increments up to 8% at an FR1 schedule. Once the rats had learned the bar pressing behavior, the reward ratio was increased to FR4. During the FR4 schedule, SB was administered via i.p. injection to half of the SPS-exposed rats. The rats treated with SB did not exhibit significantly different behavior as hypothesized. Theme: Integrative Physiology and Behavior
METHODOLOGY FOR MONITORING A SPECTRUM OF INNATE BEHAVIORS DURING CHEMOGENETIC MODULATION OF MOUSE LATERAL HYPOTHALAMIC NEURONS.

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The lateral hypothalamic area (LHA) is a functionally heterogeneous region of the mammalian hypothalamus implicated in critical homeostatic functions, including feeding, arousal and stress. This functional diversity is likely explained by cellular heterogeneity, which is poorly defined. A large inhibitory neuron population in the LHA, defined by the expression of the vesicular transporter for GABA (VGAT), has been implicated in mediating some of the functions attributed to the LHA, such as vigorous feeding and arousal related behaviors. However, these inhibitory neurons and any putative subpopulations therein have yet to be parsed out functionally. Our project seeks to elucidate the role of transcriptionally-distinct inhibitory LHA neurons in mediating complex behavioral states and discrete innate behaviors. Using mutant mouse lines, coupled with chemogenetic activation techniques, distinct neuronal populations may be selectively targeted based on their expression of specific neuropeptides to determine their behavioral effects in vivo. Our project outlines a methodological approach to assessing a spectrum of innate behaviors, such as rearing, walking, grooming, eating, drinking, digging, etc. using behavioral video-tracking software and a manual scoring protocol. Through this approach, we hope to quantify the finely-tuned behavioral outputs of these LHA inhibitory neurons and provide further insight into the function of this critical though heterogeneous brain region.

Theme: Integrative Physiology and Behavior
Neuromodulators such as serotonin and dopamine (DA) have previously been implicated in behaviors such as sleep and feeding across vertebrates and invertebrates. The majority of the dopaminergic neurons in the fly brain project to an associative learning network called the mushroom body (MB), modulating the synaptic strength of connections within. The MB has been implicated in many motivated behaviors, including decision-making and sleep. Approximately 2,000 kenyon cells (KCs) make up the lobes of the MB and synapse onto MB output neurons (MBONs). Transgenic activation of clusters of DA neurons have been found to result in significant sleep deficits in Drosophila melanogaster without producing a strong rebound post-deprivation. In order to ascertain whether the observed sleep deficits resulting from DA neuron activation resulted from dopaminergic regulation of sleep-homeostasis or a drive to forage we tested these neurons for feeding deficits. Further, we manipulated the levels of DopR1 and R2 in flies with increased dopamine release from specific neuronal subsets to elucidate the circuit basis of sleep-feeding regulation. These results will be presented and provide timely insights into the role of dopamine on sleep-feeding conflict.

Theme: Integrative Physiology and Behavior
Involvement of caffeine cAMP signaling in regards to neural activity and aggression in crayfish, *Procambarus clarkii*.

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Caffeine is a known phosphodiesterase (PDE) inhibitor with an affinity for interacting with dopaminergic pathways in vertebrates and invertebrates (Mustard 2014). PDE inhibitors have been suspected to increase the activity of cAMP cascades producing greater exocytosis of calcium ions by blocking the breakdown of cAMP (Beaumont et. al 2001). Studies have shown that caffeine exposure also decreases action potential threshold in crayfish neuromuscular junctions (Araki et. al 2005; Chiarandini et. al 1970). Behaviorally, crayfish demonstrate a heightened submissive response in conspecific encounters through cAMP signaling on dopaminergic pathways (Momohara et. al 2014, 2016). Our study aims to observe how the physiological effects of caffeine translate behaviorally. Conspecific fights were run using adult, male, *Procambarus clarkii* to measure the aggression before and after the administration of caffeine or 3-Isobutyl-1-methylxanthine (IBMX), a sole PDE inhibitor. Losing crayfish received doses via water bath solution (10 mg/L caffeine or IBMX), while winners were submerged in vehicle. Results for IBMX and caffeine trials indicated enhanced submissive responses in losers, including a decrease in approaches (Two sample T-test; t= -2.479, p= 0.038171, f= .05) and an increase in retreats as opposed to tail-flips (F-test, = 4.0205, p=0.0262, .05), when compared to vehicle in IBMX trials. In caffeine trials, control to experimental fights demonstrated a significant increase between the winner and loser mean score differences (Two sample t-test: t = -2.4522, df = 6, p-value = 0.02482). To further investigate the increase in submissive behaviors, we exposed the abdominal nerve chain for extracellular recording on nerve I of the third abdominal ganglion while dripping IBMX (15 mg/ 100 mL), caffeine (15 mg/100 mL), or vehicle (crayfish saline) solutions. When compared to vehicle, IBMX and caffeine application resulted in a mean increase of two to four events per stimulation, a three-fold rise in the magnitude of the amplitude of the peaks, and an increase in their sharpness by slope measurement. These similarities of effects could suggest that caffeine leads to synaptic enhancement through PDE inhibition which increases submission in losing crayfish.

Theme: Integrative Physiology and Behavior
Across taxa, motivated and goal-directed behaviors, such as aggression, are modulated by transient fluctuations in the neurotransmitter dopamine (DA). More recent data also show that accumulating winning experience during territorial contests can increase competitive ability and upregulate DA neurotransmission. In the present study, we used the Zebra finch model system to investigate the effect of prior winning experience during aggressive competition over mates on future winning ability and dopaminergic regulation of aggressive behavior. Unpaired male dyads were exposed to a female stimulus (or empty cage control) across a wire partition for 15min. Agonistic behaviors during this training experience were quantified, and either the winner of the fight (or a randomly selected individual from control dyads) were placed alone into a holding cage for 2hr. These subjects were then assigned to novel dyads, and again exposed to a novel female (or empty cage control) for 15min. During this second encounter, subject aggressive behavior was also quantified. Immediately afterwards, subject brains were collected and processed for immunohistochemistry for both phosphorylated and total (i.e. phosphorylated and unphosphorylated) tyrosine hydroxylase (pTH and tTH, respectively): the rate-limiting enzyme of DA synthesis. While a win during training didn't influence winning ability during the second contest, relative levels of pTH and tTH within SBN nuclei were significantly modulated by aggressive behavior or during the second contest. Specifically, subjects that fought during the second contest had significantly higher pTH in the medial preoptic nucleus (POM) and bed nucleus of the stria terminalis (BST) than those that did not. This suggests that DA synthesis in these nuclei acutely regulates aggressive competition over mates. Notably, subjects with elevated pTH in BST also had significantly lower tTH in this region. Thus, acute increases in DA synthesis appear to be sufficient to deplete the pool of TH enzyme in the nucleus. Recent fights also elevated pTH in the ventral tegmental area (VTA), but remarkably, also in animals that won only during training with no second contest. This suggests a long-lasting effect on DA synthesis in VTA. In support of this, tTH levels in VTA were significantly lower in animals that won during training than those that were handled. Finally, prior winning experience and fighting during the second contest both significantly lowered tTH in the midbrain central gray (GCl). Overall, these data suggest that DA transmission within these social behavior network nuclei is modulated by prior winning experience as well as recent fights in a region specific manner.

Theme: Integrative Physiology and Behavior
THE ROLE OF PARITY IN THE ECOLOGICALLY RELEVANT MEASUREMENT OF POSTPARTUM DEPRESSION AND ANXIETY IN MICE

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Postpartum depression and anxiety disorders are debilitating diseases that cause disruptions in normal maternal care to offspring and have profound negative effects on offspring long-term development. However, little is known about the etiology of such disorders. Studies in mouse models have been used to attempt to elucidate these factors, but there may be differences in maternal experience (parity) and measurement that have gone unexplored. To better investigate important maternal factors with ecologically relevant measurements, we conducted two studies. The first examined the effect of parity on postpartum depressive-like symptoms and the second modified two classic anxiety apparatuses, elevated zero maze (EZM) and light/dark box (LDB) for lactating dams. In the first study, we found that first-time mothers displayed less depressive symptoms (more mobility) in a forced swim test than experienced mothers. This suggests that parity may be an overlooked factor that should be addressed to determine if behavioral differences in depressive-like behaviors are driven by hormonal or neural differences in first (primiparous) vs. second time (multiparous) mothers. In the second study, both the EZM and LDB were modified to include pups in the open areas and dams were measured for retrieval behavior as well as time spent in the open areas. We found that primiparous mothers reliably retrieved pups in both apparatuses, though faster in the LDB (EZM: M = 542.18 s; LDB: M = 273.27 s). Similar to study one, work is needed to delineate the underlying neuromechanisms in the maternal brain that account for this retrieval behavior. Future work of maternal depressive and anxiety-like symptoms should include ecologically relevant measurements as well as investigate differences in parity.

Theme: Integrentive Physiology and Behavior
Poster #131

BEHAVIORAL ACQUISITION AND ANALYSIS TECHNIQUES IN BILATERALLY BULBECTOMIZED CD-1 MICE

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Olfactory bulbectomized (OBX) mice are a primary animal model for Major Depressive Disorder in humans. Several studies illustrate that OBX mice and rats display a robust hyperactivity phenotype, which is thought to model psychomotor agitation observed in many humans with depression. We performed a pilot experiment using OBX CD-1 mice to collect high resolution data on the emergence and expression of this hyperactivity phenotype. We implanted mice with radio-frequency identification (RFID) tags and constantly recorded their movements using a novel system that includes a platform embedded with 24 RFID readers. Mice lived in large social home cages (groups of four) placed onto this RFID tracking system. Our current analysis demonstrates extensive disruptions to movement patterns, including general increases in activity, as well as circadian disturbances. Our data are in line with previous reports, and constitute a deeper probe into OBX induced hyperactivity, which increases our understanding of this behavioral phenotype.

Theme: Integrative Physiology and Behavior
Poster #132

A NEW USER-FRIENDLY OPEN-SOURCE FLY TRACKER TO STUDY ATTENTION DEFICIENT HYPERACTIVITY DISORDERS IN DROSOPHILA MELANOGASTER

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Alterations in animal locomotive behaviors are important landmarks to study psychiatric disorders like Attention Deficit Hyperactivity Disorders (ADHD). These locomotive behaviors can be captured with video tracking software ranging from commercially available Ethovision to Ctrax, an open source software which requires Matlab. In our lab, we have developed a flexible and user friendly tracking algorithm to analyze single fly movements with behavioral outputs, using computer vision. We then applied this tool to study several locomotive phenotypes such as travel distance, velocity, wall preference in an open arena in the following ways. 1) We were able to validate the tool by comparing the tracking results of wildtype and mutant flies with those from Ctrax and Ethovision. 2) We explored the possible link between diet and ADHD and found that flies developed increased locomotion after initial exploration when bred on high calorie diets. Flies fed on the same diet right after hatching did not develop the same hyperactive phenotypes, suggesting that the effects are probably developmental. This observation is consistent with human studies which suggested epigenetic modification caused by prenatal diets are associated with ADHD. Furthermore, the hyperactivity phenotype was affected by mutations in DAT1, encoding for the dopamine transporter involved in modulating the level of dopamine in neurons, supporting the notion that the observed hyperactivity is ADHD related. Taken together, we have established a new user-friendly and open source tool to study ADHD in flies and this tool can be suitable for both research and educational purposes.

Theme: Integrative Physiology and Behavior
EARLY PRENATAL EXPOSURE OF RATS TO HOMOCYSTEIC ACID LEADS TO LASTING CHANGES IN NMDA RECEPTOR SUBUNIT EXPRESSION AND GABAERGIC INTERNEURON MARKERS

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Homocysteic acid (HCA), a NMDA receptor agonist, is an endogenous metabolite formed from the oxidation of homocysteine. Since hyperhomocysteinemia is a risk factor for several neuropsychiatric disorders, including bipolar disorder and major depressive disorder (MDD), we previously tested the hypothesis that elevated HCA levels in developing rats may induce the development of behaviors associated with MDD and/or bipolar disorder. Our earlier work demonstrated that exposure of postnatal rats to HCA from P3-21 leads to a mixed depressive/manic phenotype that develops post-puberty. Specifically, HCA treated rats exhibit increased risk-taking behavior, reduced social behavior, novelty-induced hyper-locomotion, anhedonia in the saccharine preference test, and reduced spatial learning in the Morris water maze, consistent with a depressive state with manic tendencies. Therefore, in this study, we focused on examining the effects early postnatal HCA exposure had on glutamatergic and GABAergic markers in the hippocampus and the cortex in the adult rat. We hypothesized that early postnatal HCA exposure would lead to excitotoxicity and loss of NMDA-receptor containing GABAergic interneurons which are hypothesized to play an important role in the pathology associated schizophrenia, bipolar disorder and depressive disorder. However, contrary to our hypothesis, we observed that HCA exposure led to an increase in expression of the GABAergic marker, GAD-67 in the cortex of both male and female rats. This finding suggests that perhaps GABAergic interneurons were not appropriately pruned during the critical period. In addition, we observed that HCA exposure led to a significant increase in the NR2A:NR2B subunit expression ratio in the cortex and the hippocampus of male and female rats, but no changes in NR1 subunit expression. Functionally, this would result in NMDA receptors with faster gating kinetics which may be less susceptible to HCA-induced excitotoxicity. Furthermore, we also found that HCA leads to an increase in the expression of BDNF, a neurotrophic factor important for maintenance of GABAergic interneurons. This finding is consistent with the observation that the relative NR2A subunit expression increases as activation of NR2A-specific receptors is associated with an increase in BDNF release. Collectively, these data suggest that early HCA exposure may lead to a shift in the NR2A:NR2B subunit expression, leading to an increase in BDNF expression and GABAergic interneuron survival during the critical period. This research was supported by the Hope College Neuroscience Program, Biology Department and Chemistry Department.

Theme: Integrative Physiology and Behavior
EFFECTS OF ETHANOL PRE-EXPOSURE AND AGE ON THE ACQUISITION AND EXTINCTION OF CONDITIONED TASTE AVERSION IN C57BL/6J MICE

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Adolescents, both human and rodent, consume more alcohol than do adults, a finding believed to result from a combination of a propensity for reward seeking and a decreased sensitivity to the aversive effects of alcohol during adolescence. Previous research from our lab shows that alcohol exposure prior to place conditioning in mice produces tolerance to its aversive motivational effects more quickly in adolescents than adults. In addition, findings from one-trial conditioned taste aversion (CTA) studies indicate decreased aversion in adolescent rats, with higher doses sometimes necessary to produce CTA in adolescent mice and rats relative to adult animals. CTA can develop after pairing a tastant (CS) with an injection of alcohol, and is thought to assess the aversive motivational properties of the drug. In the present study, we combined ethanol pre-exposure with a multiple trial CTA procedure to determine the impact of pre-exposure on both the initial level of ethanol-induced CTA as well as its rate of development. We also investigated age-related differences in the extinction of CTA in animals with or without alcohol pre-exposure. Naive adolescent (PND 21) and adult (PND 56) male C57BL/6J mice received a moderate (2 g/kg) dose of ethanol every other day for four days prior to taste conditioning. On conditioning days, mice received 1h access to a 1.6 mM saccharin solution, then received a moderately high dose of ethanol (3 g/kg) or an equivalent volume saline injection. The conditioning days were followed by extinction trials, whereby the mice were exposed to the saccharin solution with no injection. All mice who received ethanol-paired saccharin exposure show a significant decrease in saccharin consumption over conditioning days, demonstrating the expected development of conditioned taste aversion. Across conditioning days, adolescent mice who received four ethanol pre-exposures learned the CS-US association more slowly than other groups. Retarded acquisition of conditioned taste aversion in the ethanol pre-exposed adolescents suggests that an interaction between age and pre-exposure produces a tolerance to the aversive effects of ethanol. The rate of extinction did not vary significantly on the basis of age or pre-exposure, though a non-significant effect of age showed adolescents tended to consume more saccharin than adults during later extinction trials. More rapid extinction could indicate weaker learning of the taste aversion, or a relative decrease in the magnitude of the aversion.

Theme: Integrative Physiology and Behavior
A MULTIFACETED APPROACH FOR INVESTIGATING SEX-SPECIFIC BEHAVIORAL PROFILES DURING ASSOCIATIVE FEAR LEARNING IN RATS

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Classical Pavlovian fear conditioning has been widely used to study stress learning mechanisms. An animal’s level of fear is typically evaluated by “freezing”, but recent studies have identified a wide array of behaviors, suggesting that freezing is an incomplete measure of fear. Our previous work identified and characterized “darting”, a sexually dimorphic active fear response predictive of improved extinction retention. In this study, we hypothesized that previous stress exposure biases fear behavior toward darting. One week before fear conditioning, male and female Sprague-Dawley rats underwent either a single sham surgery (n = 35) or five days of restraint stress (n = 30) to induce physiological stress or psychological stress, respectively. An additional control cohort (n = 31) was briefly handled. Five days after initial stress exposure, we used the tail flick test (TFT) to evaluate pain sensitivity. Then, the rats underwent fear conditioning on Day 1 via exposure to five unpaired tone presentations (CS) followed by seven footshock-paired tone presentations (CS-US). On Day 2, the animals underwent extinction with 20 unpaired CS presentations. Day 3 tested extinction retention with five unpaired CS presentations. Across the fear conditioning paradigm, velocity data were collected using EthoVision and analyzed with a set of custom Python scripts. Our high-throughput analysis method allows for efficient and accurate evaluation of a variety of behaviors, including darting, freezing, and general locomotion. Importantly, visualization of each animal’s velocity across the entire paradigm allows us to dissect individual differences and predictive factors for these divergent behavior profiles.

Theme: Motivation and Emotion
PERINEURONAL NET REMOVAL DECREASES CUE-INDUCED REINSTATEMENT IN COCAINE SELF-ADMINISTERING RATS

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Repeated exposure to cocaine can lead to the formation of persistent drug memories. Activation of these drug memories are a motivating force behind drug seeking behavior. One of the important brain structures for cocaine-induced drug seeking behavior and memory is the medial prefrontal cortex (mPFC). Here we investigated the effects of chondroitinase-ABC (Ch-ABC) on the reconsolidation of a cocaine-associated memory. Ch-ABC is an enzyme that degrades perineuronal nets (PNNs), a dense extracellular matrix structure surrounding a subset of GABAergic interneurons within the mPFC. Male rats were trained to lever press for cocaine on a fixed ratio 1 (FR1) schedule of reinforcement for 10 days followed by injection of Ch-ABC in the mPFC. Three days following Ch-ABC, rats were given a 30 min memory reactivation session on either an FR1 or a novel variable ratio 5 (VR5) schedule of reinforcement. The next day, rats were tested for memory reconsolidation by measuring lever-pressing behavior for 30 min under extinction and then 30 min during cue-reinstatement. We hypothesized that a novel reactivation session is necessary to induce updating of habituated self-administration drug memories and, in turn, make the memory susceptible to weakening in the absence of PNNs. Ch-ABC did not affect the extinction; however, Ch-ABC reduced cue reinstatement when memory was reactivated by the VR5 session, indicating that memory is reconsolidated only when a novel reactivation session is used. Our results suggest that PNNs in the mPFC may be a target for novel therapies in cocaine addiction.

Theme: Motivation and Emotion
SEX DIFFERENCES IN SUCROSE REINFORCEMENT IN LONG-EVANS RATS

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Background: There are sex differences in addiction behaviors including consumption and relapse. The present study examined sex differences in these behaviors using a rat model.

Methods: Subjects were adult male and female Long-Evans rats. Rats first self-administered 10% sucrose on a FR1 schedule of reinforcement (40 sec timeout to availability of next reinforcer) in 10 daily 2 hr sessions. Sucrose was paired with a 5 sec tone+light cue. Rats were then tested with different concentrations of sucrose (0, 3.75, 7.5 or 7.5, 15, 30%; counterbalanced) with 3 days of 10% sucrose in between each test. Rats next trained with 10% sucrose on a PR schedule for 7 days and then tested on the PR, with the different concentrations as before, with 3 days of 10% sucrose (PR) in between each test. Finally, rats trained again on a FR1 for 3 days and then had 3 extinction tests with 3 FR1 re-training sessions in between each test. Prior to each extinction test, rats were pre-treated with the dopamine D1 antagonist SCH23390 (0, 1, 10 micrograms/kg, 15 min pretreatment, IP; counterbalanced). In separate cohorts of rats, saccharin preference (2-bottle choice; 4 alternating concentrations of saccharin v. water) and sucrose preference (10% sucrose v. water) were assessed. A final study was conducted with rats responding for water on a FR1 schedule (10 days) and then PR (7 days).

Results: Females responded for more sucrose on both FR and PR schedules of reinforcement, even when not accounting for body weight. Females also responded at a higher rate in extinction. ANCOVA analysis revealed that the extinction sex difference was not accounted for by the higher rate of responding during training. The 10 microgram/kg dose of SCH23390 significantly reduced responding of both males and females. In bottle preference tests, males consumed more saccharin across a range of concentrations (0.075, 0.15, 0.3, 0.6%), but there was no sex difference when considering body weight. Males also consumed more 10% sucrose, although females consumed more when accounting for body weight. In both preference test studies, water consumption was similar between males and females. However, females responded more for water on both FR1 and PR schedules of reinforcement.

Discussion: Female Long-Evans rats are more motivated to respond for sucrose and sucrose-paired cues than males. Their increased avidity for sucrose is not explained by sweet taste preference, or a generally higher rate of operant responding (e.g. Day 10 FR1 active lever training for water for males was 17.8% of sucrose-maintained responding; 14.0% for females). These results provide a robust model for further exploring sex differences in sucrose reinforcement in rats.

Theme: Motivation and Emotion
THE EFFECTS OF RALOXIFENE AND MIFEPRISTONE ON PROESTRUS-INDUCED DECREASES IN HEROIN INTAKE

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Preclinical, clinical, and epidemiological studies have consistently revealed sex- and gender-related differences in substance use and the development of substance use disorders. Many of these sex differences can be attributed to gonadal hormones, and the effects of ovarian hormones across the estrous/menstrual cycle have been well documented for many drugs. We recently reported that heroin self-administration decreases significantly (~70%) in female rats during proestrus. Circulating levels of estradiol and progesterone rise and fall in rapid succession during proestrus; consequently, it is not known whether estradiol or progesterone is responsible for the suppression of heroin intake during this phase. The objective of this study was to determine the effects of endogenous estradiol and progesterone on the suppression of heroin intake during proestrus through the use of receptor-selective antagonists in normally cycling female rats. To this end, female rats were implanted with catheters and trained to self-administer heroin (0.0075 mg/kg/infusion) on a fixed ratio (FR1) schedule of reinforcement. The estrous cycle was monitored daily prior to each test session. If a rat was in proestrus, it was treated with either vehicle (peanut oil, sc), the estrogen receptor antagonist raloxifene (1.0, 3.0, or 10 mg/kg, sc), the progesterone receptor antagonist mifepristone (3.0, 10, or 30 mg/kg, sc), or a combination of raloxifene and mifepristone (3.0 and 10 mg/kg, respectively) 30 min prior to the session. Consistent with our previous report, heroin intake decreased significantly during proestrus. Raloxifene blocked proestrus-induced decreases in heroin intake in a dose-dependent manner, whereas mifepristone failed to alter proestrus-induced decreases in heroin intake at all doses tested. Combining moderate doses of raloxifene and mifepristone blocked proestrus-induced decreases in heroin intake, but these effects were not greater than those produced by raloxifene alone. These data indicate that estradiol (but not progesterone) is responsible for proestrus-induced decreases in heroin intake in normally cycling female rats.

Theme: Motivation and Emotion
Poster #139

ULTRA HIGH FIELD FMRI OF HUMAN SUPERIOR COLLICULI ACTIVITY DURING AFFECTIVE VISUAL PROCESSING

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Research on rodents and non-human primates has established the involvement of the superior colliculus in defensive behaviors and visual threat detection. The superior colliculus has been well-studied in humans for its functional roles in saccade and visual processing, but less is known about its involvement in affect. In standard functional MRI studies of the human superior colliculus, it is challenging to discern activity in the superior colliculus from activity in surrounding nuclei such as the periaqueductal gray due to technological and methodological limitations. This study used high-field strength (7T) fMRI techniques to image the superior colliculus at high (0.75mm isotropic) resolution, which enabled isolation of the superior colliculus from other brainstem nuclei. Activation in the superior colliculus while participants viewed emotionally aversive images was greater than that during neutral image viewing blocks and the affective modulation of visual stimuli processing was specific to the superior colliculus. These findings suggest that the superior colliculus may play a role in shaping subjective emotional experiences in addition to its visuomotor functions, bridging the gap between affective research on humans and non-human animals.

Theme: Motivation and Emotion
Reciprocity, a central feature of social interaction present in both humans and animals, allows for the mutual exchange of privileges. It allows individuals to forge alliances, function within groups, and facilitates the avoidance of disadvantageous transactions based on past encounters. Commonly, many social behavioral disorders and psychiatric conditions compromise the ability to reciprocate. Despite the importance of this behavior in maintaining social relationships, the single-neuronal and causal underpinnings of reciprocity remain largely unknown. Here, we investigated the neuronal correlates of group interaction by obtaining multiple-neuronal recordings in the anterior cingulate cortex (ACC) of rhesus macaques. In a three-agent reciprocity-based social task, individuals within groups were able to interact via a rotary table apparatus. This design allowed for the reciprocation of past rewards as well as the development of social preference. In different sessions, we controlled for animal position and gaze contact between actors and their potential recipients. Across sessions, the formation of complex group dynamics allowed for the dissociation of core computations associated with interactive behavior. Behaviorally, monkeys were found to reward the act of reciprocation in conjunction with the use of strategic preferences designed to maximize reward. Additionally, we observed variation in group reward distribution within each session, demonstrating the sensitivity of the group dynamics. At the neuronal-level, we discovered a subpopulation of neurons encoding a signal that tracked the reward received by other agents and displayed differential activity in response to different individuals. Thus, information about specific preferred individuals and their association with reciprocated reward is encoded by neurons in the ACC. These results suggest that monkeys can reciprocate and develop social preferences, and that neurons in the ACC underpin its neuronal mechanisms. Thus, our findings suggest a neuronal mechanism for reciprocity, a fundamental cognitive process underlying social interactions.

Theme: Motivation and Emotion
VOLUNTARY EXERCISE SLOWS EXTINCTION OF CONDITIONED HYPERACTIVITY IN MICE

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Extinction-based treatments alone have shown to be ineffective in treating drug addiction. This experiment, using an animal model of drug addiction (i.e. sensitization), tested if voluntary physical activity enhanced the efficacy of extinction. The experiment consisted of four phases: acquisition, exercise, extinction, and test for sensitization. For the acquisition phase, male, Swiss Webster mice (n=30) either received an injection (subcutaneous, s.c.) of physiological saline (vehicle) or methamphetamine (1.0 mg/kg) for 5 consecutive sessions. Following acquisition, mice were either given access to a home-cage running wheel (runners) or not (sedentary) for the exercise phase that lasted 3 weeks. After 3 weeks of exercise, the extinction phase began in which all mice received daily injections (s.c.) of physiological saline prior to 4 consecutive locomotor activity sessions. The tests for sensitization occurred following the extinction phase. At this time, all mice received a once daily injection of methamphetamine according to an escalating drug-dose regimen (0.25 --> 1.0 mg/kg). The results showed that during the extinction phase, a conditioned hyperactive response was observed for the methamphetamine-conditioned mice regardless of exercise condition. However, methamphetamine-conditioned mice given the opportunity to exercise (i.e., runners) after the acquisition phase, extinguished at a slower rate compared to methamphetamine-conditioned mice not permitted to exercise (i.e., sedentary) after the acquisition phase. Finally, only the high methamphetamine dose (1.0 mg/kg) induced behavioral sensitization; however, runners and sedentary mice did not differ in their sensitized response to methamphetamine. Collectively, these results suggest that post-acquisition exercise retards the rate of extinction of conditioned hyperactivity but does not alter the subsequent sensitized response to methamphetamine. This research was supported financially by the Psychology Department at Dickinson College.

Theme: Motivation and Emotion
Aerobic exercise has been found to reduce anxiety in the general population and patients with anxiety disorders. This is often modeled in experimental rats, however anxiety is typically measured in ‘trait’-like fashion, and fewer studies examine the effects of stressful experiences on anxiety-like behavior in rats. Frustrative non-reward is an operant paradigm that induces emotional reactions in rats, therefore we used frustration to test induced-anxious behavior in running and sedentary rats. Long-Evans rats were allowed free access to a running wheel in their home cage or were in standard laboratory housing for 6 weeks. All rats were training on increasing variable ratio (VR) schedules until they reached criterion on a VR20 schedule, at which point blood was drawn to measure corticosterone (cort) levels and anxiety was assessed on the open field test. Then, we induced frustration, drew blood again, and retested in a contextually separate open field. Running rats were faster to reach criterion throughout operant conditioning and were quicker to advance through VR schedules. Importantly, at baseline conditions, running and sedentary rats both showed similar plasma cort and anxiety levels in the open field. Although both groups of rats behaved similarly during the frustration trial, sedentary rats had higher levels of cort and displayed increased anxiety-like behavior, whereas running rats were buffered from this increase. Brains were extracted and using a classic Golgi staining method, dendritic spine analysis suggests that running increases dendritic spines within granule cells and pyramidal cells through the hippocampus. Our data suggest that running buffers frustration-induced anxiety-like behavior, mediated by enhanced negative feedback of the stress response through dendritic growth of hippocampal neurons.

Theme: Motivation and Emotion
Poster #143

SEX DIFFERENCES IN THE REINFORCING EFFECTS OF NICOTINE UNDER INCREASING SCHEDULES OF REINFORCEMENT

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Introduction: A recent meta-analysis from our laboratory summarized the existing literature on sex differences in nicotine intravenous self-administration (IVSA). The results of this work revealed that IVSA procedural variables, such as the schedule of reinforcement, might influence the magnitude of sex differences in nicotine IVSA. To empirically test this hypothesis, the present study examined sex differences in nicotine IVSA under increasing fixed reinforcement (FR) and progressive ratio (PR) schedules of reinforcement. Methods: To acquire nicotine IVSA, adult female and male rats received extended access (23 hours) to a low (0.015 mg/kg) dose of nicotine for 6 consecutive days under an FR-1 schedule of reinforcement. The rats were then given access to nicotine (0.03 mg/kg) for 5 additional days under an FR-1, FR-3, FR-5, then PR schedule of reinforcement. The rats received 3 days of abstinence between each change in their schedule of reinforcement. Results: Female rats displayed greater levels of nicotine IVSA as compared to males under FR-1 and FR-3 procedures. However, there were no sex differences in nicotine IVSA under FR-5 procedures. Also, females reached higher breakpoints under the PR procedures. Conclusion: These results suggest that in general females display greater nicotine intake under higher reinforcement demands, and our results support our meta-analysis results suggesting that the schedule of reinforcement influences sex differences in nicotine IVSA.

Theme: Motivation and Emotion
Adult neurogenesis in the hippocampus and the ventral hippocampus-medial prefrontal cortex (vHIP-PFC) pathway has been suggested to be important for promoting antidepressant behavior. Previous research in our lab has shown that flexible maze learning increases hippocampus neurogenesis, induces dendritic growth in vHIP and mPFC neurons and reduces depressive symptoms in naïve rats. This study aimed to measure the structural connectivity between vHIP and mPFC and whether training in a flexible maze environment (the flex maze) could rescue rats from an induced depressive phenotype. First, rats were put through a chronic mild stress paradigm for 3 weeks to induce depressive-like behavior and went through flex maze training to see whether it rescued behavior. Adult neurogenesis and dendritic spine density in these rats were measured using doublecortin (DCX) and a classic Golgi stain protocol. Secondly, the vHIP-mPFC pathway was structurally quantified by infusing a fluorescently-tagged adeno-associated virus (AAV) directly into area CA1 of ventral hippocampus. Rats went through flex maze training and then fluorescent concentration of AAV in mPFC sections was scanned to quantify synaptic growth in this pathway. This AAV study was performed using wildtype (WT) rats and a transgenic (TK) rat with ablated adult neurogenesis. For the behavioral experiment, three weeks of chronic mild stress failed to elicit depressive behavior on both the novelty-suppressed feeding and sucrose preference test, so it was difficult to determine an antidepressant effect of flex maze training. However, mazers had significantly less baseline corticosterone than controls by the end of training. Mazers also had higher number of DCX+ cells and density of dendritic spines in the hippocampus. For the pathway study, flex maze training significantly increased AAV concentration in mPFC, but only in WT rats. TK rats without neurogenesis showed no structural gain in the vHIP-mPFC pathway as a result of maze-training. Together, the data suggest that flex maze training induces growth within the vHIP-mPFC pathway, but it is still unclear what clinical significance can be drawn from these structural changes.

Theme: Motivation and Emotion
EFFECTS OF ACUTE SOCIAL DEFEAT-INDUCED PRIMING OF MICROGLIA IN THE VMPFC OF SYRIAN HAMSTER

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Research suggests causal relationships between neuroinflammation and stress-related psychopathologies. Exposure to moderate or chronic psychological stress in rodents leads to increased activation of microglia, the brain’s resident immune cells. The ventral medial prefrontal cortex (vmPFC) is a key limbic region involved in top-down regulation of psychological stress and mediates the deleterious effects of microglial activity following prolonged restraint stress. While there is a growing body of literature indicating that chronic social defeat increases microglial activity in the vmPFC, there has been little research investigating the effects of acute social defeat stress. Here, we used an acute social defeat paradigm in male Syrian hamsters consisting of three, 5-minute aggressive encounters in the home cages of a three, novel resident aggressors. 24-hours later, the effects of defeat-induced priming of ionized calcium-binding adaptor protein (Iba-1) expression, a microglial activation marker, was assessed by a subsequent exposure to 0, 20, 100, or 500 µg/kg (i.p.) injection of lipopolysaccharide (LPS). Four hours after injection, hamsters were euthanized, brains extracted, and expression of Iba-1 was later quantified via immunohistochemistry. Preliminary data suggest that acute social defeat alone leads to increased Iba-1 immunoreactivity in the infralimbic and prelimbic subregions of the vmPFC compared to non-defeated controls. Furthermore, LPS injection increased Iba-1 expression in these regions both in the presence and absence of social defeat stress compared to vehicle controls. Taken together, these results demonstrate that acute social defeat stress stimulates activity of the innate immune system in brain regions that support stress processing and suggest a role for microglia in responses to trauma.

Theme: Motivation and Emotion
Poster #146

THE RELATIONSHIP BETWEEN DOMINANCE STATUS AND COPING STRATEGY

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There is a large amount of variability in how individuals cope with stress. Social dominance is a key factor influencing how animals respond to stress events. In this study, we used Syrian hamsters to test whether the display of an active or passive coping strategy predicted an animal’s future dominance status. We also examined whether a change in dominance status produced a change in active or passive coping strategy. We predicted that hamsters with an active coping strategy would more likely achieve dominant social status compared to hamsters with a passive coping strategy. We also predicted that maintaining social dominance would lead to the development of an active coping strategy. We paired female Syrian hamsters in same-sex dyads and tested animals in daily 5-minute social encounters for two weeks. In both males and females, dominance relationships were formed readily and remained stable during the two-week period. Coping strategies were tested both before and after the creation of dominance relationships via a light/dark transition test, novel object exploration test, and open field exploration test. We found that dominant and subordinate animals did not significantly differ in the amount of time spent investigating a novel object either before or after establishing a dominance relationship. Although animals did not differ in open field activity prior to the formation of dominance relationships, we found that subordinate animals spent less time in the center of an open field compared to dominants after dominance status was established. These findings suggest that while coping styles do not predict subsequent dominance relationships, the maintenance of social dominance alters anxiety-like behavior in female hamsters. Overall, these results support the conclusion that social subordination leads to less activity in an open field test and a more passive coping style.

Theme: Motivation and Emotion
THE DOPAMINE D-1 RECEPTOR AGONIST, SKF 38393, HAS SELECTIVE EFFECTS ON HIPPOCAMPAL LONG-TERM POTENTIATION IN HIGH-RUNNING MICE, BUT NO EFFECTS IN NON-SELECTED CONTROL MICE

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Selectively bred high-running (HR) mice exhibit levels of voluntary running 2.7 times higher than non-selected mice. This seemingly addictive, hyperactive behavior renders them potential models of ADHD and addiction. Previous and current work has shown that HR mice given access to a running wheel demonstrate higher levels of hippocampal long term potentiation (LTP) than HR mice without a wheel. We investigated the role of the dopaminergic system in this effect by applying the dopamine D1 receptor agonist SKF-38393 on hippocampal slices from HR or control mice, with and without wheel access. We stimulated in the CA3 region and took synaptic recordings from the stratum radiatum in the CA1 region. A mild LTP-inducing stimulus was applied after recording in the presence or absence of SKF (1.0μM). The LTP enhancement in HR mice seems to involve D1 receptors, as application of SKF-38393 decreased LTP in HR mice that had running wheel access, but not those without a wheel. In contrast, at 60 minutes post tetanus, we observed that SKF did not alter the magnitude of LTP in control mice regardless of wheel access. SKF had no effect on basal transmission and did not alter paired-pulse facilitation in control or HR mice with/without wheel access. Thus, HR mice are unique in that running wheel access seems to elevate LTP in a post-synaptic, dopaminergic manner. Better understanding of the mechanisms behind high-intensity running and LTP could provide valuable insights into neurological pathways of addiction and hyperactivity.

Theme: Motivation and Emotion
DELTA FOSB IS INCREASED IN THE NUCLEUS ACCUMBENS CORE FOLLOWING A HORMONE-SIMULATED PREGNANCY

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Pregnancy and the surrounding peripartum period is a time of profound physiological and behavioral changes. Whereas some changes in behavior are adaptive, many women experience unwanted peripartum behavioral changes that are indicative of depression and/or anxiety. During pregnancy, the ovarian hormones estrogen and progesterone increase precipitously; following delivery, however, estrogen and progesterone levels decrease abruptly. Although it is assumed that the dramatic drop in ovarian hormones following birth is related to the symptoms of peripartum mood disorders, how this hormone withdrawal impacts the brain is poorly understood. The nucleus accumbens (NAc) is a candidate site of hormone-mediated neural and behavioral plasticity during the peripartum period. We therefore hypothesized that the accumulation of ∆FosB, a transcription factor associated with long-term neural plasticity, would be altered in female mice following a hormone-simulated pregnancy. Females were ovariectomized and administered daily injections of estrogen and progesterone that approximate early and late pregnancy. Following this hormone-simulated pregnancy, one group of females was withdrawn from estrogen, simulating postpartum hormone withdrawal, while the other group continued to receive estrogen injections. Using immunohistochemistry, we found that ∆FosB was increased in the NAc core of estrogen withdrawn female mice. When ∆FosB was examined in D1 and D2 receptor-bearing neurons, we found that estrogen withdrawn females had more ∆FosB in D2 neurons in the NAc core, but there was no significant difference between groups in D1 neurons. Interestingly, these brain changes were associated with a decrease in anxiety like behavior in the elevated plus maze. These data suggest that ∆FosB accumulation in the NAc may be part of a typical mechanism of anxiety reduction in new mothers, and importantly, may be dysregulated in those females who experience peripartum mood disorders.

Theme: Motivation and Emotion
ESTROGEN WITHDRAWAL INCREASES ANXIETY-LIKE BEHAVIOR AND DORSAL RAPHE OXYTOCIN RECEPTORS IN HAMSTERS

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Peripartum mood disorders are the most common complication associated with childbirth and are associated with negative outcomes for both mothers and children. Despite this, the underlying neurobiological mechanisms remain poorly understood. Previous research suggests that the drop in estrogen at parturition may lead to changes in neurobiology and behavior. Indeed, our laboratory has demonstrated that estrogen withdrawal following a hormone-simulated pregnancy leads to more oxytocin-immunoreactive neurons in the paraventricular nucleus of the hypothalamus (PVN) in female hamsters. We therefore hypothesized that estrogen withdrawal would likewise lead to alterations in oxytocin receptor density in efferents of the PVN and concurrent changes in anxiety-like behavior. To test this hypothesis, we used a hormone-simulated pregnancy model in female Syrian hamsters. In this model, females are ovariectomized and given daily injections to approximate changes in ovarian hormones in the peripartum period. Specifically, ovariectomized females were assigned to one of three groups: an oil control group; a hormone-withdrawn group, which received hormone injections for 17 days before being withdrawn from hormones for five days; and a hormone-sustained group, which received the same hormone regimen as the hormone-withdrawn group for 17 days, then continued to receive estradiol for five subsequent days. On days 18-22, all females underwent testing for anxiety-like behaviors in an open field and an elevated plus maze. Following behavior testing, subjects were sacrificed and their brains were processed for oxytocin receptor autoradiography in the medial amygdala, nucleus accumbens, bed nucleus of the stria terminalis, and dorsal raphe nucleus. We found that hormone-withdrawn females spent significantly more time in the closed arms of an elevated plus maze. In addition, hormone-withdrawn females had a significant increase in oxytocin receptor density in the dorsal raphe. Together, these data indicate that estrogen withdrawal may lead to anxiogenic behavior via oxytocin signaling in the dorsal raphe.

Theme: Motivation and Emotion
Strategies for coping with stress can vary dramatically between individuals. The origins of these different strategies are not fully understood, but there is strong evidence supporting a role for environmental factors such as social dominance. This study used a social defeat model to examine potential links between types of coping strategies and dominance status. Female Syrian hamsters were paired in daily social encounters for two weeks to establish a dominant-subordinate relationship. Then, animals were paired with new social partners for two weeks so that half of the animals changed their dominance status, while the others remained either dominant or subordinate. After the creation of dominance relationships, all animals received acute social defeat stress which included 3, 5-min aggressive encounters with a trained aggressor. Following social defeat exposure, we used a social interaction test to assay status-dependent differences in social avoidance. We hypothesized that dominant Syrian hamsters would show greater social interaction at testing compared to their subordinate counterparts as well as former dominant animals that lost their social status. Unexpectedly, subordinate animals spent more time investigating a social target than did dominants. In addition, initial dominance status appears to have a stronger effect on defeat-induced social avoidance than subsequent status changes. These findings indicate that social dominance modulates how female hamsters respond to social defeat stress. We are currently performing ΔFosB immunohistochemistry in the nucleus accumbens to investigate neural activity in brain regions known to modulate stress susceptibility.

Theme: Motivation and Emotion
SOCIAL DEFEAT STRESS INDUCES SIMILAR TRANSCRIPTIONAL PROFILE CHANGES IN CALIFORNIA MICE AS COMPARED TO PATIENTS WITH DEPRESSION IN REWARD-RELATED BRAIN REGIONS

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Social defeat stress (SDS) is a widely used model of chronic psychosocial stress for inducing anxiety-like and depression-like behavioral abnormalities in rodents. It has been difficult to use SDS in female rodents due to a lack of aggressive behavior. However, female California mice show similar levels of territorial aggression as males, allowing us to study the effects of SDS in both sexes. Using this model, we exposed both male and female California mice to SDS. In order to assess the long-term effects of SDS, brains were extracted two weeks following the end of SDS and immediately flash frozen. Using RNAseq, we assessed SDS-induced transcriptional profile changes in the nucleus accumbens (NAc) of both stressed and unstressed male (unstressed = 7, stressed = 6) and female (unstressed = 8, stressed = 7) California mice. Additional analyses were also performed in the prefrontal cortex (PFC) in stressed and unstressed female (unstressed = 8, stressed = 7) California mice. These data sets were compared to transcriptional profiles observed in either the NAc or PFC from human tissue of both men (MDD = 13, control = 13) and women (MDD = 13, control = 9) patients with or without major depressive disorder (MDD). Using rank-rank hypergeometric overlap (RRHO) analyses, we found more overlap in genes that were decreased by stress in female California mice and decreased in women diagnosed with depression. Based on patterns observed using RRHO, we used gene ontology (GO) and KEGG analyses to look for highly enriched GO terms and pathways that were altered by stress and depression. In the NAc, one of the more highly enriched terms affected is G-protein coupled receptor (GPCR) signaling and regulation of GPCR, where both GPCR signaling and regulation of GPCR signaling are down-regulated by stress or in human depression. In the PFC, major terms arising using KEGG analyses are associated with ribosomal translation. In addition, many immediate early genes are downregulated by stress and in patients with depression, suggesting less activity in the PFC in general. Follow-up qPCR analyses are in progress to confirm these findings in separate biological replicates. Together these results suggest that the California mouse model of social defeat may be particularly effective at modeling aspects of depression that involve transcriptional repression as opposed to transcriptional activation.

Theme: Motivation and Emotion
Roughly 7.6% of Americans over the age of 12 suffer from major depressive disorder (MDD) in a given 2 week period, and the World Health Organization ranks MDD as the leading cause of disease burden in high and low income countries. Thus, the need for effective treatment options for MDD is extremely important. Currently the most commonly prescribed antidepressants are serotonin selective reuptake inhibitors (SSRIs) such as fluoxetine (Prozac). While SSRIs have been found to successfully treat many of the symptoms associated with MDD such as mood, rumination, and anxiety, motivational deficiencies remain resistant to this form of treatment. In both clinical studies and preclinical rodent models, fluoxetine has even been shown to actively exacerbate motivational deficits. As the mechanism of action for SSRIs involves the elevation of synaptic serotonin (5-HT) by transport inhibition, it is likely that this fluoxetine-induced dysfunction could be due to overstimulation of one or more 5-HT receptors. While there are several subtypes of 5-HT receptors with numerous additional subtypes, there was particular interest in the 5-HT1B receptor. This is because 5-HT1B Rs in the ventral tegmental area (VTA) have been shown to play a role in modulation of impulsive behaviors possibly due to actions on dopamine (DA) neurons originating in the VTA and projecting to the nucleus accumbens, and also because extracellular concentrations of DA in the striatum are known to impact motivated behavior. For this reason, the aim of this study is to evaluate the possible role of the 5-HT1B R in fluoxetine-induced motivational dysfunction in a rodent behavioral model using a concurrent choice operant procedure to assess effort-based decision making, and coadministration of fluoxetine with the selective 5-HT1B R antagonist, NAS-181. While initial findings involving systemic administration failed to reveal a conclusive full reversal of the fluoxetine-induced low effort bias, future steps will involve intracranial administration directly to the VTA to more thoroughly investigate the role of VTA 5-HT1B receptors in fluoxetine-induced amotivation without interference with serotonergic actions in other parts of the brain.
Evidence in humans suggests that meal-related memory influences later eating behavior. Memory can serve as a vital mechanism for controlling eating behavior because it provides a record of recent intake that likely outlasts most physiological signals generated by ingestion. We have proposed that dorsal (dHC) and ventral hippocampal (vHC) neurons, which are critical for memory, limit energy intake during the postprandial period. In support, our lab found that temporarily inactivating dHC or vHC neurons after the consumption of a sucrose meal decreased the latency to eat the next sucrose meal and increased the size of that meal. If dHC or vHC neurons control intake through a process that requires memory, then ingestion should increase events necessary for synaptic plasticity in dHC and vHC during the postprandial period. To test this, we determined whether ingesting a sucrose solution induces phosphorylation of AMPAR GluA1 subunits at serine 831 (pSer^{831}) and serine 845 (pSer^{845}) residues. We also determined whether increasing the amount of previous experience with the sucrose solution, which would be expected to decrease the mnemonic demands associated with that meal, would also attenuate sucrose-induced phosphorylation. Specifically, we exposed male Sprague-Dawley rats to a sucrose solution for 10 min/day for 3, 5 or 10 days and their brains were harvested 90 min after their last sucrose bout. Quantitative immunoblotting of dHC and vHC membrane fractions demonstrated that sucrose ingestion increased postprandial dHC pSer^{831} and that increased sucrose experience prevented this effect and decreased vHC pSer^{831}. Sucrose ingestion did not affect pSer^{845} in either dHC or vHC. The sucrose-induced increase in dHC GluA1 pSer^{831} is noteworthy because previous research has shown that learning produces a comparable elevation of dHC GluA1 pSer^{831}, which results in increased glutamate AMPA receptor conductance and augmented synaptic strength. These findings indicate that ingestion activates proteins necessary for synaptic plasticity and memory in dHC, which is consistent with the hypothesis that dHC neurons form a memory of a meal.

Theme: Cognition
THE INFLUENCE OF GENETICS ON INDIVIDUAL DIFFERENCES IN NEURAL ACTIVATION PATTERNS IN THE VISUAL AND FRONTOPIARIETAL COMMUNITIES

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Individual differences in brain function play an important role in executive control, and are linked to general cognitive ability as well as characteristics in the areas of personality and emotion processing. These differences arise from both genetic and environmental influences and enable further understanding of how varying complex cognitive traits arise. By collecting task fMRI and behavioral data for a sample of 766 monozygotic (MZ) twins, dizygotic (DZ) twins, siblings (SIB), and unrelated people, the Human Connectome Project (HCP) allows investigation of the degree to which genetics shapes these differences. This study compared activation similarity patterns in the FrontoParietal and Visual networks across these subject groups of varying relatedness. Activation similarity was correlated for the N-back task under conditions of high or low working memory load and across two object stimulus categories. If heritability plays a substantial role in determining neural activation, groups of higher genetic similarity should have more similar activation patterns. Indeed, in both the FrontoParietal and Visual networks, MZ twins showed a higher similarity than DZ twins or siblings, and DZ twins and siblings showed a higher similarity than unrelated participants. Furthermore, this correlation is emphasized under conditions of higher cognitive load in the FrontoParietal network. As such, this study demonstrated that heredity is correlated to neural activation in both examined communities. This provides evidence that genetic influences play a substantial role in the neural basis of individual differences, and may ultimately help to lay the foundation for task-related brain activation to be considered as an endophenotype for psychiatric or neural disorders.

Theme: Cognition
Alzheimer’s disease (AD), the most common form of age-related dementia, is a neurodegenerative disease pathologically characterized by the extracellular deposition of plaques, intracellular neurofibrillary tangles, and gliosis in the brain. Inflammatory bowel disease (IBD), including Crohn’s disease and colitis, are other forms of chronic inflammation with increased prevalence in the elderly. Due to the bidirectional communication of the brain with the gastrointestinal tract via the gut-brain axis, we hypothesized that the AD brain pathologically reacts to the inflammation initiated in the colon. To test this idea, we used dextran sulfate sodium (DSS)-induced model of colitis in the APPNL-G-F/NL-G-F transgenic mouse model of AD. Both C57BL/6 wild type control and APPNL-G-F/NL-G-F mice demonstrated severe colitis-like symptoms following DSS treatment. Surprisingly, DSS treated mice resulted in no differences in reactive microgliosis with mild differences in astrogliosis in both APPNL-G-F/NL-G-F and wild type mice. However, DSS treatment resulted in increased protein levels of cyclooxygenase 2 (COX-2) and the amyloid precursor protein (APP) in the APPNL-G-F/NL-G-F compared to wild type mice. These findings support the hypothesis that intestinal inflammatory changes affect the brain during AD perhaps through increasing inflammatory prostaglandin and amyloid β (Aβ) levels.

Theme: Cognition
IMPACT OF WAKING REST ON MEMORY CONSOLIDATION

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Introduction—Sleep and quiet rest have both been shown to facilitate the consolidation of memory. In two studies, we explored the features of quiet rest after learning that account for its effect on memory consolidation, experimentally manipulating the activity that participants engaged in during the minutes after encoding. Study 1 compared eyes-closed rest to meditation, a state in which one is similarly relaxed with their eyes closed, but is intently focusing on a mental task—in this case breath mindfulness. Study 2 compared eyes-closed quiet rest to both smartphone usage and mental arithmetic. We hypothesized that conditions characterized by minimal mental effort would result in improved memory consolidation, with even very simple instructions to attend to internal states (breath focus, mental arithmetic) interfering with the memory benefit of rest.

Method—Study 1 used a within-subjects design where participants (n= 40) were trained on an Icelandic word pair memory task before and after either meditating or resting. In Study 2 (in progress), three groups of subjects (planned n=50 per group) learned how to categorize 270 abstract dot patterns into three different categories. Following a 15 minute break in which subjects either rested, used their smartphone, or performed mental arithmetic, participants were tested on their ability to correctly categorize these same dot patterns, as well as new dot patterns, and the category “prototypes” from which the dot patterns were created. EEG was collected during all rest conditions, in both studies.

Results—In Study 1, although there was no overall effect of meditation vs. quiet rest on memory, we found that time engaged in breath focus was negatively correlated with subsequent memory (r(31)=0.54, p=0.002), with participants who spent more time attending to their breath during meditation showing a significant memory decrement at later test.

Conclusions—Our observations in Study 1 are consistent with the hypothesis that even simple focused attention to one’s own breathing could impair subsequent memory by diverting cognitive resources away from spontaneous consolidation processes. It may be that consolidation is best facilitated by periods of unconstrained rest in which no object of mental focus is provided, with even minimal instructions to focus attention on internal states interfering with the mnemonic benefits of rest.

Theme: Cognition
The purpose of the present investigation is twofold. First, we aimed to determine whether a female rat’s ability to acquire and retain spatial memory over time is impacted by the rat’s stage of the reproductive cycle. Second, we tested how the presence of an acute stress interferes with female gonadal hormone effects on rat’s ability to acquire and retain spatial information. We used the Open-field Tower Maze (OFTM) task to assess spatial acquisition, as well as short-term (48 hours) retention of memory. We used vaginal cytology to track female rats’ estrous cycle. The estrous cycle lasts four days and is characterized as: proestrus, estrus, metestrus, and diestrus, which may be determined according to the cell types observed in the vaginal smear. We collected vaginal smears for microscope analysis throughout pre-training, training, and testing phases of the experiment. Immediately prior to the acquisition training, half of the rats received a 30-minute acute restraint stress. Exposure to stress did not affect learning of the female rats in estrus and diestrus I (when levels of estrogen were low). However, females in proestrus and diestrus II (when levels of estrogen were high) required a greater number of trials to reach learning criterion following an acute stress exposure compared to females that were not stressed. Furthermore, acute stress presented during acquisition, had an impairing effect on female rats’ ability to retain spatial information over 48hrs. The data collected in this study will hopefully contribute to the information about the effects of hormone replacement therapy on cognitive function.

Theme: Cognition
The prefrontal cortex (PFC) mediates executive functions that enable behavioral control and self-regulation. Cognitive flexibility is one type of executive function that supports the ability to appropriately adapt behavior when a previous response strategy no longer yields the desired outcome. Cognitive flexibility is vulnerable across a range of neurological and psychiatric conditions characterized by abnormal PFC excitability including epilepsy, aging and mood disorders. Recently, vagus nerve stimulation (VNS) has become of significant clinical interest in the treatment of epilepsy and stress-related mood disorders, but the mechanisms underlying VNS benefits remain unknown. Moreover, the possible benefits of VNS on cognitive deficits associated with neurological and psychiatric disorders remains poorly understood. We have previously shown that modulation of PFC inhibitory GABAergic signaling enhances some forms of cognitive flexibility. Given these findings and efficacy of VNS in treatment of epilepsy, we sought to test the hypothesis that VNS can enhance cognitive flexibility and GABAergic signaling protein expression in the PFC. Cognitive flexibility was assessed in male, Brown Norway rats using a touchscreen reversal learning task. Rats first learned that one of two simultaneously presented stimuli was associated with a food reward (A+, B-). After learning the rule to criterion performance, the contingencies were reversed (A-, B+). Rats were trained in task procedures and then underwent surgery to implant a cuff around the vagus nerve, which could be electrically stimulated via a flexible tether system during task performance. After recovery from surgery, the effects of VNS on reversal learning were tested using a within subjects design such that rats received either no stimulation or VNS (120 µsec pulse width, 700 µA, 30 Hz, 0.8 s train duration) on each stimulus presentation. Preliminary data (n=6) indicate that VNS enhances reversal learning as does systemic administration of the GABAergic agonist baclofen (n=8). In a separate cohort of rats, experiments are ongoing to determine if VNS can influence expression of GABAergic signaling proteins in PFC. Rats were tethered in the behavioral chambers daily and experimental rats (n=5) received 100 VNS trains using the same parameters as above, whereas control rats (n=7) were tethered but received no stimulation. After 8 days, rats were sacrificed, and preliminary biochemical analyses suggest that there is approximately 20% increase in expression of GABAergic signaling proteins in PFC. Ongoing studies are testing the effects of VNS on circulating hormones, cytokines and other measures of peripheral health. Our long-term goal is to investigate the ability of VNS to restore cognitive function in vulnerable populations such as advanced aging or in individuals with stress-related disorders.
NOREPINEPHRINE-DEFICIENT MICE EXHIBIT REDUCED ANXIETY IN RESPONSE TO NOVELTY

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Dopamine beta-hydroxylase knockout (Dbh -/-) mice lack the ability to synthesize norepinephrine (NE) and epinephrine, and have markedly different phenotypes than Dbh +/- control mice with normal NE and epinephrine content. Previous studies established that Dbh -/- mice display deficits in locomotion, memory, and behaviors associated with aggression, mating, and maternity. Dbh -/- mice also exhibit a hypersensitivity to psychostimulants, volatile anesthetics, and convulsant agents. Although NE is important for attention and arousal in response to salient environmental cues, no studies have examined innate behavioral responses to novelty in Dbh -/- mice. Novelty detection is important for survival and adaptive behavior and is distinct from memory, although the two cognitive functions are related. This study investigated potential disruptions of novelty-induced behavior in Dbh -/- mice and assessed differences in neuronal activation using c-fos immunoreactivity in various brain regions of Dbh -/- mice and control mice following novelty-related tasks. The behavioral assays conducted included novelty suppressed feeding (NSF), marble burying (MB), and food neophobia, all of which typically elicit an anxiety-like response to novelty. Dbh -/- mice demonstrated reduced anxiety-like behavior in these novelty-related behavioral assays compared to Dbh +/- littermates. Mice were perfused 90 min after the start of NSF and MB tasks, and the brains were removed and processed for subsequent c-fos immunohistochemistry. Analysis of c-fos immunoreactivity across many brain regions previously associated with anxiety and novelty detection suggests that behavioral disturbances in Dbh -/- mice result from functional deficits in a corticolimbic novelty network, likely due to the absence of NE signaling.

Theme: Cognition
Poster #160

REACTIONS TO PERFORMANCE ERROR

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We sought to understand how humans dynamically control attention during cognitive tasks, as well as how adaptively, or maladaptively, individuals respond to their own mistakes. Paired closely with attention, arousal serves as way to understand the general fluctuations and changes in attention. Alpha-suppression, a term that describes decreases in EEG alpha-wave oscillations following error, and pupil dilation have been shown to be indexes of arousal, with more alpha-suppression and larger pupil dilation both associated with heightened arousal, independently. The relationship between error detection, arousal, and behavior, through the mechanisms of alpha suppression and pupil dilation was explored. We hypothesized that pupil dilation following mistakes will be correlated with error-related changes in EEG oscillations, due to common neural mechanisms. To achieve this, eye-tracking software and EEG were used simultaneously to record pupillary and neural activity during a visual attention task. Results showed that greater alpha suppression occurred following error trials as compared to correct trials and that pupil diameter was significantly larger following errors. These results were consistent with the literature and supported a relationship between both indexes of arousal following error-detection. Future research will examine the performance changes following error, to explore a potential predictive relationship.

Theme: Cognition
Previous studies show that active exploration of an environment contributes to spatial learning more than passive visual exposure (Chrastil & Warren, 2013; Chrastil & Warren, 2015). Active navigation and cognitive decision-making in a novel environment leads to increased spatial knowledge and memory of location compared to a passive exploration that removes the decision-making component. There is evidence of theta oscillations present in electroencephalography (EEG) recordings from the hippocampus and pre-frontal cortex (PFC). These low-frequency waves could reflect spatial navigation and memory performance, suggested by their involvement in communication between the formerly named brain regions. Through communication with the hippocampus, theta oscillations could be involved in the integration of new spatial information into memory. While undergoing EEG, subjects in this study either actively or passively explored a virtual maze, identified as the “Free” or “Guided” groups, respectively. After exploring, subjects’ spatial memory of the maze was tested through a task that required navigation from a starting object to a target object. Preliminary behavioral data show increased spatial memory for the Free group, indicated by significantly greater navigation to the correct target object in the memory task. EEG results indicate significantly greater theta oscillations in frontal regions for the Free group during the exploration phase. These preliminary results support those found in previous studies and could indicate a direct correlation between frontal theta oscillations during learning of novel environments and spatial memory.
Amount of water intake and the level of water impurities influence measures of intelligence and cognitive ability in humans (e.g., Fadda et al., 2012; Choi et al., 2015; Janulewicz et al., 2012); however, causation and mechanism cannot easily be ascertained in human populations. Calcium is one of these water impurities, and it plays an important role in synaptic transmission, which has implications in learning and memory. The levels of calcium in drinking water varies between city water and well water. Chemical analysis with flame atomic absorption spectrometry and EDTA titrations showed that well water from Mount Vernon, OH, has ten times more calcium in it than city water from Mount Vernon, OH. This study was designed to determine if a difference in levels of calcium in drinking water could affect a measure of intelligence in rats. It was hypothesized that rats drinking water calcinated to the levels found in well water would have higher levels of intelligence than rats given drinking water with calcium added to levels found in city water. Each pup of 6 brother-pairs of male Sprague-Dawley rats was a maximum of 12 days after weaning given access to one of two water conditions: city water (0.4 mM CaCl2 in reverse osmosis water) or well water (4.0 mM CaCl2). Once these rats reached adolescence (i.e., 42 postnatal days), they were adapted and trained in preliminary runs of the Closed Field Intelligence Test as per Rabinovitch & Rosvold (1951). In this test, the rats must problem solve to find their way through eighteen different maze configurations (6 in the adaption and preliminary runs and 12 novel ones in the testing problems) to receive a food reinforcer. This task measures the rat’s ability to problem solve and remember, which are aspects of intelligence, by forcing them to find their way through different mazes and remember the correct way when they go back through the maze. Once the rats reached the training criterion (minimum of 12 days), they were tested in 8 trials of 12 novel problems across 4 weeks, and the number of errors that they made before reaching and eating the food reinforcer was counted. Repeated measures ANOVA assessing the number of errors each day across trials during the test problems revealed no significant differences between the two water conditions or over time. This suggests that these levels of calcium in the drinking water did not have enough influence on brain activity to change performance of rats in the Closed Field Intelligence test. Going forward, another researcher could study if the calcium that is ingested from the drinking water is actually making its way to the rat’s brain or not.

Theme: Cognition
Choline is an essential nutrient found in foods such as eggs, meat, and green vegetables. It is the precursor to acetylcholine, a key neurotransmitter involved in learning and memory. The presence of choline during development has a profound, positive impact on learning and memory later in life. The present study was designed to test the hypothesis that prenatal choline supplementation enhances and protects memory by altering the way information is organized across brain structures. We focused on object recognition, an ability that relies heavily on the perirhinal cortex. Damage to the perirhinal cortex creates deficits in remembering familiar objects and learning about new objects. Interestingly, however, the amount of times an animal has seen an object may influence the memory’s ability to withstand perirhinal cortex damage, as described by the distributed reinstatement theory. We hypothesize that one way prenatal choline supplementation may augment adult memory function is through more widespread activation of neural structures in response to object exposures. In this way, prenatally choline supplemented would require fewer exposures to an object for it to be widely distributed in the brain and thus resistant to damage. To test this, rodents were either prenatally supplemented with choline or given a standard diet. As adults, rats were placed in a box and allowed to explore an object for 5 minutes every day for either 5 or 30 days. Within 72 hours of the last object day, rats were given either an excitotoxic lesion to the perirhinal cortex or a sham surgery. After recovering, an object preference test was used to measure the rats’ memory of the object. The results provided encouraging support for our hypotheses. Among the standard-fed rats that received a sham surgery only those in the 30-day group showed a novel object bias during the test and there was no novel object bias evident in either the 5- or 30-day standard-fed rats that received PRh lesions. By contrast, both the 5- and 30-day prenatal choline supplemented rats that received the sham surgery displayed a significant novel object bias. Additionally, among prenatal choline supplemented rats that received PRh lesions, unlike the standard-fed PRh lesioned rats, there was a statistically significant novel object bias in the 30-day group. These results suggest that early life choline availability may alter the ways in which object memories are represented in the brain, making them less vulnerable to the deleterious effects of perirhinal cortex damage.

Theme: Cognition
Protein synthesis is required for the consolidation of long-term memories. Sleep deprivation impairs protein synthesis required for hippocampus-dependent memory consolidation. A specific subset of the insulin signaling pathway, AMPK-mTORC1-4EBP2 signaling, in the hippocampus is affected by sleep deprivation. Here, we examined whether similar changes in the insulin signaling pathway occur in brain regions other than the hippocampus. Mice were sleep deprived by gentle handling for 5 hours and then immediately sacrificed. The control group of non-sleep deprived mice were left undisturbed. The cerebellum, frontal cortex, striatum, and hippocampus were homogenized and analyzed by western blotting. Our findings suggest that there are differences in the impact of sleep deprivation on the insulin signaling pathway in these brain regions. We found that there may be an increase in mTOR activity in the frontal cortex after sleep deprivation. In addition, there may be a decrease in AMPK activity and increase in mTOR activity in the striatum. These findings are in contrast to our findings in the hippocampus. To further examine the effect of sleep deprivation on the AMPK pathway, we also tested whether dorsomorphin, a drug that inhibits AMPK activity, can rescue the effect of sleep deprivation on hippocampus-dependent memory. Mice were given 10 mg/kg of dorsomorphin or saline via intraperitoneal injections daily for 3 days. Afterwards, the mice were trained in the object-place recognition task and then sleep deprived for 5 hours. Twenty-four hours later, the mice were tested. Our findings highlight the vital role protein synthesis has on sleep-dependent memory.

Theme: Cognition
PROTEOMICS TECHNIQUES APPLIED TO THE DETECTION OF NEUROPEPTIDE RECEPTORS IN THE AMERICAN LOBSTER, HOMARUS AMERICANUS

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Neuropeptides are a class of signaling molecules that regulate animal behavior by interacting with their respective cell-membrane receptors. The American lobster, Homarus americanus, is a well-understood model to study neuropeptides and their effect on the modulation of rhythmic pattern generators. Mass spectrometry is an essential tool used to identify neuropeptides and their post-translational modifications (PTMs) in H. americanus. PTMs are also important to modulating the activity of neuropeptide receptors; however, few neuropeptide receptors have been detected and characterized by mass spectrometry. One neuropeptide receptor of interest is the C-type allatostatin (AST-C) receptor, a G-protein coupled receptor (GPCR) hypothesized to modulate cardiac function in crustaceans. Interest in this receptor derives from the observation that the AST-C neuropeptide, perfused into the heart of H. americanus, increased heart contraction amplitude for some animals, while it decreased for others. One hypothesis to explain this differential response is a change in the expression of neuropeptide receptors in H. americanus; a second is a change in PTMs regulating the signaling pathway of the GPCR. This study reports on work to detect and characterize the PTMs associated with the AST-C receptor from H. americanus brain tissues using proteomic techniques and H. americanus receptor sequences discovered using data-mining strategies applied to an H. americanus brain transcriptome. H. americanus brain tissues, extracted using two different detergent-based solvents and digested using a filter-aided sample preparation approach, were subjected to high pH fractionation followed by analysis using a chip-based nanoESI Q-TOF-MS/MS system. We compare the two detergent-based extraction approaches and report on the detection of over 400 proteins identified through searching the H. americanus brain transcriptome. Our study identified over 100 putative membrane-bound proteins; however, the targeted AST-C receptor was not detected using this approach.

Theme: Techniques
ABERRANT NEURAL ACTIVITY OBSERVED IN LONG-TERM USE OF MICROENDOSCOPE-BASED, VIRALLY-TRANSDUCED CALCIUM IMAGING IN A FREELY MOVING C57BL/6J

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Spatial memory is a necessary function for everyday life and can be impacted in numerous diseases, such as Parkinson’s disease and traumatic brain injury. Fluorescent calcium indicators are commonly used as an indirect measure of neural activity through a process termed calcium imaging and this neural activity can be correlated with animal position to infer how spaces are represented and stored within the brain. Calcium imaging allows for more neurons to be detected in the same field of view in comparison to electrophysiological recordings and the stability of the field of view allows for neural activity of the same cells to be compared across multiple recording sessions. While the signal-to-noise ratio and kinetics of calcium indicators have improved in the past decade, the effects of long-term expression are not well characterized. While analyzing data from mice virally transduced with GCaMP6f in our spatial memory experimental protocol, cell activity analyses did not yield precise place fields as expected and aberrant neural activity was noted during our calcium imaging sessions. Upon examining our experimental protocol and performing a literature search, we suggest that the abnormal neural activity and imprecise place fields were most likely due to the long-term effects of our calcium imaging technique. The work described here raises a pressing concern related to the long-term use of calcium imaging in measuring brain activity and posits hypotheses for the mechanism of cause of the abnormal activity.

Theme: Techniques
COMBINING NEAR-INFRARED FLUORESCENCE WITH BRAINBOW TO IDENTIFY SPECIFIC GENES WITHIN A MULTICOLOR CONTEXT
Cook, ZT, Brockway, NL, Tobias, ZJC, Pajarla, J, Boardman, IS, Ippolito, H, Weissman, TA
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Fluorescent proteins are a powerful experimental tool, allowing the visualization of gene expression and cellular behaviors in a variety of systems. Multicolor combinations of fluorescent proteins, such as Brainbow, have expanded the range of possible research questions. While Brainbow and other multicolor approaches are useful for distinguishing and tracking cells, the addition of a separately driven color would allow researchers to tag a manipulated gene within the multicolor context, in order to investigate mechanistic effects at a cellular level. A far-red or near-infrared protein could be particularly suitable in this context, as these emit light of a lower energy that can be distinguished visually from Brainbow. We investigated the in vivo brightness, photostability, and cytotoxicity of five far-red/near-infrared proteins in zebrafish: TagRFP657, mCardinal, miRFP670, iRFP670, and mIFP. Our results show that both mCardinal and iRFP670 are useful fluorescent proteins for zebrafish expression that emit in the near-infrared range. We also introduce a new transgenic zebrafish line that expresses Brainbow under the control of the pan-neuronal neuroD promoter. We demonstrate that mCardinal can be used to track the expression of a manipulated bone morphogenetic protein (BMP) receptor within the Brainbow context. The overlay of near-infrared fluorescence tagging onto Brainbow background labeling defines a clear experimental strategy for future research questions that aim to manipulate or track the effects of specific genes within a population of cells that are also delineated using multicolor approaches.

Theme: Techniques
A lack of understanding of science and/or distrust of it is prevalent in today’s society, and it is imperative that scientists take the lead in appropriately communicating scientific results. That was the founding goal of ‘BW Brain Fair: Creating Neural Connections’ – to create a service and outreach program that generates understanding of and accessibility to information relevant to health and human experiences. Brain Fairs are tried-and-true platforms that make science available and fun to community members and students of all ages, while also giving budding scientist scholars the opportunity to develop critical professional skills. In this program, Neuroscience majors of Baldwin Wallace University (BW) develop hands-on activities that explain neural structure and function, demonstrate phenomena of sensation and perception, and discuss biological underpinnings of mental health disorders, as well as writing applications for grant opportunities to fund the activities and analyzing survey data regarding impact. The demonstrations can be taken to local schools, museums, and/or senior centers, with the penultimate goal of hosting a large-scale event on campus that would be open to the entire surrounding community. BW Brain Fair started as a Faculty-Student Collaboration class of 3 students and 1 faculty member; in 2 semesters it has tripled in size. We have engaged our demonstrations at 2 local high school events and BW’s year-end exhibition of scholarship, and surveys reported that the majority of participants agreed or strongly agreed that the activities increased their interest in neuroscience. These activities contributed to our winning BW’s ‘Innovative Program of the Year’ award. Currently in its 3rd semester, the program continues to generate excitement and will transition to purely student leadership this Spring. We seek for ‘BW Brain Fair’ to continue to impact both student participants and community members in a way that begins to bridge the gap between science and society.

Theme: History and Education
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