School of Neuroscience
Summer Undergraduate Research Fellowship
The Summer Undergraduate Research Fellowship in Neuroscience programs are paid summer internships that give students the opportunity to work within a neuroscience laboratory affiliated with the Virginia Tech School of Neuroscience.

The first program, SURF-N (Summer Undergraduate Research Fellowship in Neuroscience), is a 12-week summer fellowship program, giving students the opportunity to experience a neuroscience laboratory, contribute to active research projects under the direction and leadership of School of Neuroscience faculty, and gain valuable experience in data presentation at the end of the summer. Students spend 40 hours per week immersed in a research environment assisting their faculty mentor in an ongoing research project; students are provided with real-world neuroscience research experience and bench skills beneficial to career development.

The second program, NeuroREEF (Neuroscience Research Experience and Engagement Fellowship), is a 6-week full time (40 hours per week) or 12-week part time research experience (20 hours per week). Students participate in hands-on, minds-on learning with a flexible schedule, while assisting their faculty mentor in an ongoing research project. The NeuroREEF program offers the flexibility for students to participate in research full time for one summer session while enrolling in classes for the alternate summer session OR to participate in research part time for both summer sessions while enrolling in classes during one or both summer sessions.

Fellowship Recipients

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Francesca N. Czesak
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Andrew Stublen
Mentor: Dr. Ian F. Kimbrogh
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Katherine Vaughn
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Jackson Willbourne
Mentor: Dr. Lina Ni
*Thermoreceptors in Drosophila Melanogaster Larvae*
When Sex Matters: Different Responses to Traumatic Brain Injury Based upon Sex Hormone Levels

Katie Baros1, Malikah Ajose2, Dr. Carmen Menez-Ballester3, Dr. Stefanie Roth4

1Virginia Polytechnic Institute and State University, Blacksburg, VA
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Introduction
Every five seconds in the United States, someone incurs a traumatic brain injury, and many individuals suffer from long-term damage that exacerbates their quality of life. The damage leads to cognitive decline, memory loss, and physical injury. Efforts in research are attempting to gain more insight on this worldwide epidemic, and scientists have conducted several studies; however, most of this research only involves males. The few studies that include females post-injury, however, are discovering that the subjects respond differently depending upon their sex. Furthermore, the limited studies have suggested that estrogen could work as a neuroprotective factor in the central nervous system after TBI. Nevertheless, scientists have disregarded how estrogen levels fluctuate based upon the stage of the natural menstrual cycle and how this can relate to TBI recovery. The purpose of this study is to investigate if mice in the stages of the menstrual cycle associated with high estrogen levels respond to TBI differently than females with low amounts of estrogen.

Methods:
Using a vaginal smearing technique, vaginal cells were collected, stained with crystal violet, and viewed under the microscope to pinpoint the stage of the mouse estrus cycle. TBIs were inflicted upon the low estrogen females, high estrogen females, and males using a one hundred gram weight. Following the TBIs, righting times were measured in order to determine how the mice immediately recovered from TBI. Seven days post-injury, the mice were sacrificed, and the western blot technique was employed to quantify GFAP expression in the cortex, hippocampus, and cerebellum. Glial fibrillary acidic protein (GFAP) was selected as a means to measure recovery because it is an astrocytic intermediate filament protein involved in the brain’s response to injury.

Findings and Argument:
Our findings show that females with low estrogen levels had higher mortality rates and longer righting times in comparison to females with high estrogen and males, suggesting that females with low estrogen levels recovered worse after TBI than their counterparts. Specifically, low estrogen females had a mortality rate near 60% while high estrogen females had a 20% mortality rate, and males had a 0% rate. In addition, low estrogen females had an almost 75% increase in righting times between the first and third TBI. On the other hand, we were not able to distinguish any significant differences in GFAP expression between experimental groups.

Conclusion:
In reference to the increased righting times and higher mortality rate, low estrogen females have more difficulty immediately recovering from traumatic brain injury in comparison to high estrogen females and males. Contradicting the hypothesis, males had the lowest mortality rate and displayed a decrease in righting times between the first and third TBI. The western blots were used to examine recovery seven days post-injury, and none of the experimental groups showed an upregulation of GFAP after TBI. This promising project has shown the significance of the natural menstrual cycle’s influence on TBI recovery, which can potentially be applied to clinical settings. An improvement for future experiments would be acquiring a larger sample size to increase the power of analysis and obtain more precise results.

Effects of Lead on Brain Regions Involved in Language Production in Male Songbirds

Francesca N. Czesak, Lauren M. Krauss, Kendra B. Sewall

Virginia Tech

Introduction
Lead is an environmental contaminant that poses health risks to children throughout the world. Recent revelations about poor water quality in Flint, MI and in other U.S. cities have renewed concern about childhood lead exposure in this country. Nearly four million households across the United States have been exposed to drinking water with over 15 parts per billion (ppb) of lead, the US Environmental Protection Agency’s action level. Any lead exposure is now considered dangerous to children by the Centers for Disease Control and the World Health Organization because even very low levels of lead can impair neural development, leading to compromised learning, cognition, and language development. Language development in humans may be particularly vulnerable to lead exposure because this form of learning depends upon a critical period of brain development. Language development in humans may be particularly vulnerable to lead exposure because this form of learning depends upon a critical period of brain development. Language development in humans may be particularly vulnerable to lead exposure because this form of learning depends upon a critical period of brain development. Therefore, we examined the consequences of lead exposure during the critical period of vocal development in an animal model, songbirds. Songbirds are an established model for human speech learning; song learning relies upon a critical period of neuroplasticity within a well-defined neural circuit that is analogous to language centers in the human brain (e.g., Broca’s area).

Methods:
To assess the effects of lead exposure on the neural circuitry underlying vocal learning in songbirds, we exposed zebra finches to either pure water (control treatment) or lead in water at levels reported in Flint, MI (100 ppb or 1000 ppb) throughout the first 90 days of development. Then, 4-8 weeks after exposure was ended, we sacrificed and collected the brains of every subject, fixed, froze, sectioned the tissue on a cryostat, Nissl stained, and imaged vocal control brain regions involved in vocal development in an animal model, songbirds. We examined the effects of lead exposure on the neural circuitry involving vocal learning (Area X and LMAN) as well as the principal song control nucleus (HVC), a region involved in both vocal learning and motor control of song production. We sought to determine if the brain region of interest by the telencephalon volume to control for possible overall differences in brain size among groups. We used a one-way ANOVA (GLM, z= 2.623, p=0.0277; Fig. 1). The amount of lead that birds were exposed to (100 and 1000 ppb) had a weight dependent effect on this overall difference. No other brain regions differed in volume between experimental and control groups. The same zebra finches exposed to lead in this study were previously found to have impaired vocal learning (Czesak et al., 2016), and we were able to find compromised song learning in daily life.

Discussion:
The brain region within the song learning pathway that we found to be compromised by lead exposure, LMAN, is centrally involved in the early process of song development. Thus, compromised song production in adulthood...
Mean volume and standard error of LMAN controlling for telencephalon volume from zebra finches provided with control (0 ppb lead), and lead-treated water (at 100 ppb or 1000 ppb) for the first 90 days of life. LMAN is a nucleus in the avian song learning pathway that is central to early vocal development in songbirds.

Introduction

The formation of long-term fear memories requires new protein synthesis in cells in the amygdala, a process referred to as consolidation. While these consolidated memories were once thought to be stable, recent evidence suggests that upon retrieval memories “destabilize” and require new protein synthesis for their storage, a process referred to as reconsolidation. This reconsolidation process allows a temporary time window in which previously stored memories can be modified, which has significant clinical implications for the treatment of various psychiatric disorders. In addition to the need for new protein synthesis, numerous studies have suggested that protein degradation mediated by the ubiquitin-proteasome system (UPS) is a critical regulator of the reconsolidation process. In the UPS, the small protein ubiquitin attaches to a substrate allowing it to be recognized and degraded by a large protein complex called the proteasome. A substrate can acquire multiple ubiquitin tags, though which can link together at different lysine (K) sites and target the substrate for functions both dependent and independent of protein degradation. However, while previous studies have focused on degradation-specific protein polyubiquitination, it is unknown how reconsolidation alters other polyubiquitin tags that are not targeted by the proteasome. Furthermore, the protein targets and functional role of ubiquitin-proteasome activity can vary widely across cellular compartment, though it is unknown how UPS activity changes within the nuclear, cytoplasmic, and synaptic regions in response to memory retrieval.

Methods:

Using cellular fractionation protocols in combination with linkage-specific polyubiquitin antibodies, we examined subcellular changes in ubiquitin-proteasome activity in the amygdala during memory reconsolidation. We trained animals to learn a context shock association and the following day re-exposed them to context (training environment) to reactivate the memory. We dissected out the amygdala from these animals 1 hour after retrieval and collected nuclear and cytoplasmic fractions using a crude sucrose gradient. Levels of different polyubiquitin protein tags were quantified with western blotting and proteasome activity was measured using an in-vitro assay.

Results:

We found increases in overall protein ubiquitination and proteasome activity selectively within the synaptic region (Figure 1). These increases in the synaptic region were associated with elevated K48 protein polyubiquitination, which is a degradation specific tag. Furthermore, we observed increased K63 and M1-protein polyubiquitination in the synaptic region, which are ubiquitin tags that are independent of protein degradation.

Conclusions:

Collectively, these results suggest that upregulation of degradation-dependent and degradation-independent polyubiquitin tags occur selectively at synapses in the amygdala following retrieval (Figure 2). These results may have important implications for the application of reconsolidation-based therapies and strategies for the treatment of various psychiatric disorders.

Figure 1. Mean volume and standard error of LMAN controlling for telencephalon volume from zebra finches provided with control (0 ppb lead), and lead-treated water (at 100 ppb or 1000 ppb) for the first 90 days of life. LMAN is a nucleus in the avian song learning pathway that is central to early vocal development in songbirds.

Upregulation of proteasome activity and protein ubiquitination occur selectively at synapses during memory reconsolidation

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Region-specific regulation of Foxp1 and Foxp2 by dihydrotestosterone in the developing brain

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Introduction:
Language is a quintessential human trait and mutations in the FOXP1 and FOXP2 genes are known to result in communicative impairments in humans, as well as animal models (1). Hormones, specifically androgens, are powerful regulators of the neural circuitry responsible for vocal communication in several species, including fish, reptiles, amphibians, avians, mammals, and humans (2). This plasticity and the degree to which vocalizations can change, in both animals and humans, is linked to the actions of sex hormones during ontogeny (3,4). Moreover, the vocal communication is vital to nearly all animals, because it is the basis for forming complex social bonds, as well as interacting with their social and ecological environments. Decades of research has established rats emit distinct types of ultrasonic vocalizations, which differ depending on the animal’s age, its current state, and the environment of the vocalizations as a function of sex hormones (5,6). To date, no link has been established showing an interaction between androgens and genes known to be involved in vocalization. Research Questions: 1) Investigate the role neonatal administration of androgens has on mediating the transcription factors, Foxp1 and Foxp2, which are known to be important for brain development and vocalization and 2) Explore how androgens may impact vocal communication in adulthood.

Methods:
Ultrasound Vocalizations:
The recording was conducted using Avisoft Recorder software (Version 5.4). Sessions included sampling rate at 300 kHz; format 16 bit. For acoustical analysis, recordings were transferred to Avisoft SASLab Pro (Version 5.2) and fast Fourier transformation (FFT) was conducted. Spectrograms were generated with an FFT-length of 312 points and a time window overlap of 75% (100 Hz, Frame, Hammering Window). A lower cut-off frequency of 15 kHz was used to reduce background noise outside the relevant frequency band. Call detection was provided by an automatic threshold-based algorithm and a hold-time mechanism (hold time: 0.01 s).

Western Blot:
The process follows the methods published in Bowers et al., 2013. In brief, all membranes were blocked in Licor, a protein blocking solution from Licor, then incubated in the primary antibody with Licor buffer overnight at 4°C. One-hour incubation in IRDye-linked secondary antibody. Immunoreactive bands were detected bands using Odyssey FC.

Male Sex Behavior: After a 5-min acclimation, animals performed four tests session each lasting 30-min, or first ejaculation in the testing arena (60 cm long X 30 cm wide X 30 cm high). Took place during the dark phase of the light cycle and under red-light illumination. Testing began with the addition of a hormonally primed receptive female (10 μg estradiol benzoate in 0.1 ml sesame oil 2 days before testing and 500 μg progesterone in 0.1 ml sesame oil 4 h before testing) to the arena.

Findings:
After 3 consecutive days of hormone treatment, starting at birth to postnatal day 3 (PN3), exogenous androgens, but not estradiol, increased the protein levels of the two transcription factors, Foxp1 and Foxp2 (Figure 1). These effects were observed in the striatum and the cortex for Foxp1 (p < 0.001 and p < 0.03, respectively). In contrast, for Foxp2, the increases in protein levels after androgen treatment were observed in the cerebellum and cortex (p < 0.01 and p < 0.004, respectively)

Conclusions:
Androgens, but not estrogens, affect Foxp1 and Foxp2 protein levels in the developing rat brain in a region and age-specific manner. Androgens influence ultrasonic vocalizations in male rats during sex behavior. Sexually experienced males vocalize more frequently than naïve rats. Our future direction will be to analyze mRNA and protein levels of Foxp1 and Foxp2 at PN4 and PN14 as well as after gonadectomy and hormone replacement adulthood. We will also investigate the effects androgens have on the morphology of cells in the neural circuits responsible for vocalization. Lastly, we are exploring the effects of androgens on sex behavior vocalizations in female rats and also investigating the impact androgens have on other genes that might be related to vocal communication.

References:
1. Bowers JM, Konopka G. The role of the FOXP family of transcription factors in ASD.

Figure 1

Figure 2

Figure 3
The putative endocrine disruptor maltol induces pigment aggregation in developing Xenopus laevis tadpoles

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Introduction: The Thompson lab focuses on identifying thyroid hormones and disrupting chemicals that affect brain development using Xenopus laevis tadpoles as an animal model. Previous experiments, showed that maltol, a flavor enhancer that is a putative thyroid hormone disruptor may affect retinal-related signaling, induced substantial pigment aggregation in melanophores. Melanophores are pigmented, light-sensitive cells used for UV protection, camouflage, and thermoregulation. Maltol may act to enhance light-sensitivity in melanophores via a retinal-mediated mechanism. Given that maltol is used in everyday foods such as cocoa, coffee and it is one of the main ingredients in containing the odor of bread, many of these symptoms are caused by loss-of-function mutations in methyl-CpG binding protein 2, MeCP2. MeCP2 functions as a transcriptional regulator, and as such, regulates a wide array of genes. Patients typically present a range of symptomology, including hand stereotypies, seizures, breathing abnormalities, and regression of language, including hand stereotypies, decreased brain volume, neuronal excitation/inhibition balance, decreased neuronal packing.

Methods: Animals: Xenopus laevis wildtype tadpoles stage 46-49 (7-10 days old). Disruptor treatment: Tadpoles were placed on either a black or white background equidistant variables (proportion of brain covered, circularity, roundness, and area), which were used to quantify melanophore pigment aggregation.

Conclusions: Maltol caused an overall increase in pigment aggregation in a dose-dependent manner. The trends in aggregation were identical regardless of background. This suggests that the background adaptation system tadpoles use for camouflage is not affected by maltol. Maltol had no significant impact on expression of POMC and mchr relative to the controls under both light conditions. This is consistent with the previous results that maltol does not impact background adaptation system.

Investigation of Astrocyte Numbers in Rett syndrome

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Rett syndrome (RTT) is a neurodevelopmental disorder, caused by loss of function mutations in methyl-CpG binding protein 2, MeCP2. MeCP2 functions as a transcriptional regulator, and as such, regulates a wide array of genes. Patients typically present a range of symptomology, including hand stereotypies, seizures, breathing abnormalities, and regression of language, cognition, and motor skills. Many of these symptoms are attributed to the biological underpinnings of impaired excitation/inhibition balance, increased later volume, neuroanatomical and morphological complexity, and increased neuronal packing. Additionally, previous studies have demonstrated that neurons...
in RTT are fewer in number and exhibit smaller cell somas. RTT historically has been viewed as a neuro-centric disorder. However, astrocytes have recently been implicated in the pathophysiological progression of this disease. The most numerous glial cell in the brain, astrocytes play an important role in homeostatic mechanisms, such as ionic and neurotransmitter balance. Neurons cultured in the presence of astrocytes that do not express McCp2 do not mature properly. Additionally, in rodent models of RTT, re-expression of astrocytic McCp2 alone restores neuronal morphology, motor abnormalities, and breathing abnormalities. Despite promising experiments and increasing interest in astrocytes, little is known regarding the characteristics of RTT astrocytes.

Findings and Conclusions: During this summer we learned serial tissue sectioning, immunohistochemistry, and stereological microscopy. Additionally, we learned how to apply these techniques in order to accurately determine astrocyte numbers. Our data suggests that, similar to neurons, there may be fewer astrocytes in the hippocampus in RTT syndrome models. However, further studies are needed, including an increased sample size, to ascertain total cellular population sizes in the hippocampus. Additionally, we will perform inter-rater reliability tests to ensure each experimenter is counting each cell population similarly. By doing this, and by performing our experiments with a larger cohort, we will be able to accurately ascertain astrocyte numbers in RTT Syndrome.

The Behavioral Effects of Perinatal SSRI Exposure in Rats

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Introduction: Major depressive disorder is the most common mental illness in the United States (Kessler et al., 2005). Women are 70% more likely to develop depression compared to men, and the highest rates are found in child bearing years (Glover &Clinton, 2016). Selective serotonin reuptake inhibitors (SSRIs) have been the mainstay treatment since the 1980s. However, despite increased usage, little is known regarding the serotonin levels. Serotonin affects many developmental processes in the brain. Thus, SSRI exposure can disrupt these processes, resulting in neurological and behavioral changes (Glover et al., 2016). While it is important to treat maternal depression, human studies have not measured their effects beyond childhood. There is a growing body of evidence from rodent studies that the effects of perinatal SSRI exposure are not confined to early childhood (Simson et al., 2011; Rayen et al., 2014; Glover et al., 2015). Therefore, it is imperative to understand how long-term effects could result from early life exposure to these drugs. Previous studies conducted by our lab with a rat model have shown that perinatal SSRI exposure could lead to an increase of depression-like behavior in adulthood (Glover et al., 2015). The purpose of this study is to confirm this finding with a more common SSRI (citalopram). We hypothesize that perinatal exposure to citalopram will cause an increase in depression-like behavior, but not anxiety-like behavior in a rodent model of RTT.

Methods: We utilize immunohistochemistry to label cellular populations and stereology microscopy to ascertain an unbiased estimate of astrocyte and neuron numbers in a rodent model of RTT Syndrome.

Results: We found some anxiogenic effects of early life SSRI exposure in males. There were no significant differences between groups in the % time rats spent in the center of the open field (Fig. 1A). However, we did find that perinatal citalopram exposure would increase depression-like behavior in the OFT and EPM. We found no significant differences between groups in the % time rats spent in the center of the open field, however, male rats exposed to citalopram exhibited significantly less locomotor activity, and significantly more time grooming than male controls. (D) There were no significant differences between treatment groups in the % time rats spent in the open arms of the EPM, although, there was a significant effect of sex, with males spending less time in the open arms. However, we did find that there was more inhibition in SSRI exposed males in the OFT, showing that the SSRI exposed males had increased anxiety-like behavior. This could suggest differences in the effect of early life SSRI exposure in males and females. This means that it may be important to determine what role this increased anxiety may have affected their performance in the depression-like behavior tests. As for the measures of depression-
like behavior, we did not find any differences in UST or the splash test due to treatment. However, we found a significant effect of treatment in males and females in the FST and the sucrose preference test. This confirms FST results from previous studies and shows that perinatal SSRI exposure can significantly increase both behavioral despair and anhedonia in adult rats.

References:

Examination of Susceptible and Resilient Subpopulations of Females Exposed to Subchronic Variable Stress

Alyssa Johnson, Jennifer R. Rainville, PhD, Georgia E. Hodes, PhD

Introduction
Depression is a debilitating disorder that impacts approximately 150 million individuals worldwide, and women are known to experience depression at twice the rate of men (Hodes et al., 2015a). Stressful events can induce depression in some individuals, with biological factors contributing to differences in stress response (Krishtan et al., 2015). Animal models of stress-susceptibility performed on genetically identical male mice have identified subpopulations of mice that exhibit depression-like symptoms in response to social defeat stress, and mice who are resilient (Krishtan et al., 2015). Previous research focusing on sex differences in stress susceptibility indicates that females become susceptible to six days of subchronic variable stress (SCVS), whereas males do not (Hodes et al., 2015b). Less is known about the individual differences exhibited by females exposed to SCVS. In this study, we examine individual differences between females who became susceptible in response to SCVS, and females who were resilient.

Methods
Sub Chronic Variable Stress Exposure and Behavioral Assessment
Animals
All experiments utilized C57BL/6J female mice from The Jackson Laboratory. Ages ranged from 8 to 12 weeks of age at the start of each experiment. Animals were maintained on a 12 h light/dark cycle with ad libitum food access, except when explicitly stated for behavioral testing. All mouse procedures were performed in accordance with the Institutional Animal Care and Use Committee guidelines of Virginia Tech.

Sub chronic Variable Stress
Animals were exposed to six days of alternating stressors for 1 hour each day. On day 1, shocks were administered to animals at 0.45 mA for a duration of 2 seconds per shock. On day 2, animals were suspended by their tails with tape. On day 3, animals were placed in 50 mL conical tubes with breathing and tail holes and left in their home cages. The stressors were repeated in the same order the subsequent 3 days.

Behavioral Assessment
Behavior testing began on the first day following the final stressor. Animals underwent three tests, in the following order: Splash Test: This test is a measure of self-care. Under red light, animals received 3 sprays on their backs with 10% sucrose solution. Total time spent grooming was measured over a 5-minute period. Novelty Suppressed Feeding: This test is a measure of anxiety-like behavior associated with neophobia. Animals were food restricted overnight. Under red light the following morning, their latency to eat a food pellet in a novel arena was measured. Forced Swim Test: This test is a measure of active vs. passive coping. Under white light, animals were placed in 4L beaker with 2.5L water (25-26°C) for 6 minutes. Each animal’s latency to immobilize was measured.

Results:
Identification of Susceptible and Resilient Populations among Females Exposed to SCVS
Data from 5 SCVS experiments were combined, and females were examined (n=97, 51% control female). Each animal received a stress-susceptibility score. Scores were calculated from averaging z-scores on all behavior tests (Splash, Forced Swim, Novelty Suppressed Feeding) for each animal.

Further analysis led to the discovery that 38 of 49 females exposed to SCVS and 32 of 49 controls responded to at least one of the 3 behavior tests inconsistently. Pie charts indicate the rate at which each test was the inconsistent test (figure 1d).

Division of susceptible and resilient populations: Animals whose stress-susceptibility scores were above 0.2 were labeled “susceptible,” and animals whose z-scores were below -0.2 were labeled resilient. Control animals within the range of -0.2 to 0.2 served as the controls to which the subpopulations of stress states were compared (figure 1a). A comparison of the identified susceptible and resilient control populations on individual tests indicated that the females were not responding consistently across the three tests (figure 1b-d).

Further analysis led to the discovery that 38 of 49 females exposed to SCVS and 32 of 49 controls responded to at least one of the 3 behavior tests inconsistently. Pie charts indicate the rate at which each test was the inconsistent test (figure 1d).
Introduction:
Previous research has shown that differences in metabolic function have been associated with affective disorders like anxiety and depression [2]. To further support this, recent literature has utilized transcriptome profiling to find that there is a difference in expression of genes coding for metabolic function in rats predisposed to an anxious and depressive phenotype [3]. While the existence of a link between metabolism rates and behavioral changes has been identified, the exact molecular underpinnings of this connection has yet to be discovered. The HR/LR model was used to provide a reliable method to produce anxiety and depression-like phenotypes in order to differentiate metabolic activity between the two groups. The HR/LR model describes the selective breeding of rats of similar anxiety and depression-like behavioral phenotype in order to produce offspring that display the same phenotype. LRs (low responders) show more anxious and depressive behaviors where HRs (high responders) show resilient behavior. This model allows for the consideration of innate temperament's role in cellular metabolism. Human fMRI studies have shown altered metabolism within limbic regions of individuals diagnosed with mood disorders [5,6]. Oxidative phosphorylation of the electron transport chain is the main energy-producing pathway in neurons, and cytochrome C oxidase (COX) is the terminal rate-limiting enzyme of the chain. COX activity in the brain is a correlate of overall ATP production and thereby, general cellular energy production.

Methods
Tissue Collection and Preparation: At postnatal day 75 (P75), adult HR and LR males were sacrificed via rapid decapitation. After the brain tissue was extracted and frozen, a cryostat was used to section the brains at 30 μm. These sections were mounted onto slides and stored at -80°C until use. Cytochrome c Oxidase Activity Quantification Assay: The tissue-mounted slides along with dissected bits of known cytochrome c oxidase protein concentrations were incubated in a reaction medium at 37°C for 30 minutes. The reaction medium included cytochrome c as a substrate and 3,3'-diaminobenzidine (DAB) used in the reaction to show color change when oxidized. The dot blot was scanned on a Microtek ScanMaker 9800XL in 16-bit grayscale without corrections. From the dot blot, the optical density of staining was correlated to micrograms of COX reaction medium.

Summary and Future Directions
Prior to or in the absence of stress exposure, there are individual differences that contribute to an animal’s behavioral response. Susceptibility to stress can be consistently divided across behavioral measures, and resilience is not consistent in stressed mice. Future studies will test if phenotype is consistent before and after stress in the same individuals. Brain and blood samples from these individuals will be examined to identify differences in gene expression and cytokines that may contribute to stress susceptibility vs resilience. We are extending this process to examine individual differences in males exposed to SCVS. These data provide a new framework to examine sex differences in the behavioral and biological responses to stress taking into account the context of individual differences. References
Ubiquitin-proteasome activity is localized in the nucleus during long-term memory formation

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Introduction

Long-term fear memory formation requires new protein synthesis in the amygdala, a process referred to as consolidation. Numerous studies have supported a critical role for the ubiquitin-proteasome system (UPS) in the memory consolidation process. In the UPS the small protein modifier ubiquitin attaches to another protein, targeting it for degradation by a large multienzymatic complex that breaks the protein into smaller fragments.

Methods

Using cellular fractionation protocols in combination with linkage-specific polyubiquitin antibodies, we examined subcellular changes in ubiquitin-proteasome activity in the amygdala during memory consolidation. We trained animals to associate a context (training environment) with a footshock and 1 h later dissected out the amygdala. We collected nuclear and cytoplasmic fractions using nonionic synaptic detergent and a crude nuclear fraction using a sucrose gradient. Next, we measured protein polyubiquitination levels in these samples with western blotting and proteasome activity using an in vitro assay.

Results

Following training, overall protein polyubiquitination and the proteasome activity simultaneously increased in the nucleus and decreased in the synaptic and cytoplasmic regions (Figure 1). The nuclear increases were associated with upregulation of degradation-specific (K48) and degradation-independent (K63) ubiquitin polyubiquitin tags, suggesting multiple functions for ubiquitin signaling within this region.

Conclusion

Collectively, these results indicate that the upregulation of degradation-dependent and degradation-independent ubiquitin-proteasome activity selectively occurs in the nucleus following learning (Figure 2), suggesting that the UPS may regulate memory consolidation via unique mechanisms other than protein degradation which varies across cellular compartments.
The anorexigenic effect of gastrin-releasing peptide involves the arcuate nucleus of the hypothalamus in chicks

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Gastrin-Releasing Peptide (GRP) is implicated in various physiological processes, such as regulation of gastric function. The objective of this research project was to evaluate effects of GRP on appetite regulation in birds. In Experiment 1, central (intracerebroventricular) injection of GRP inhibited both food (Figure 1) and water (data not shown) intake in 4-day post-hatch Hubbard x Cobb-500 chicks (Gallus gallus). The reduction in food intake was dose-dependent. In Experiment 2, when measured in fasted chicks, water intake (Figure 2) was not affected by GRP injection. Thus, the effect on water in Experiment 1 was prandial. Next, in Experiment 3 the objective was to elucidate the central mechanism of action and we measured c-Fos immunoreactivity within key hypothalamic and brainstem nuclei. Activated nuclei are likely ones that mediate food intake in GRP-injected chicks.

Within the hypothalamus we measured the following nuclei: arcuate (ARC), dorsomedial (DMN), ventromedial (VMH), paraventricular (PVN) and lateral hypothalamic (LHy) and for the brainstem we measured the area postrema (AP) and nucleus of the solitary tract (NTS). Only the ARC (Figure 3) had an increased number of c-Fos reactive cells in response to GRP treatment. In Experiment 4 we isolated the ARC and, via real-time PCR, measured pro-opiomelanocortin (POMC), neuropeptide Y (NPY), and agouti-related peptide (AgRP) mRNA. POMC mRNA was increased in GRP-treated chicks (Figure 4). In conclusion, central GRP inhibited feeding behavior in chicks, and this effect likely involves increased production of anorexigenic peptides in the ARC and changes in melanocortin signaling.

Examining the role of microbiota in emotional behavior: antibiotic treatment exacerbates anxiety in high-anxiety-prone rats

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Introduction: Gut microbiota play a crucial role in optimizing gut function, but also have an impact on organismal health by exerting broad effects on the immune and central nervous systems. Major depression is among the most prevalent and debilitating mental disorders and yet the molecular underpinnings of this psychoneuroimmunological activity in the brain are largely unknown. One theory proposes that the causal mechanisms of depression and other psychiatric disorders stem from alterations in the gut-brain axis. Alterations to the microbiome have already been linked to psychiatric disorders such as Alzheimer’s Disease, Anxiety Disorders, and Parkinson’s Disease (Dinan et al, 2017). Less is known about whether individuals already exhibiting innate differences in temperament and emotional behavior also have natural differences in gut microbiota. In addressing this question, this study utilized Sprague-Dawley rats selectively bred to display distinct emotion behavior profiles. One high novelty responder (HR) and one low novelty responder (LR) rat of each sex were co-housed. One HR and one LR rat were co-housed and sacrificed. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC). All efforts were made to minimize suffering and number of animals used.

Methods: Antibiotic Treatment

Adult male HR/LR rats were obtained from the 8th generation of our in-house colony. Male rats were (n=16/phenotype) assigned to either antibiotic or control groups by cage. Antibiotic treatment was comprised of ampicillin, neomycin, and vancomycin that was dissolved in drinking water. Treatment began two weeks prior to behavioral testing and continued throughout. Behavioral Testing

To start the open field test (OFT) a rat is placed in a corner of the box and allowed to explore the novel environment for 5 minutes. The latency to approach the center, the time spent and distance traveled in the center, sides, and corners were all quantified. The elevated plus maze (EPM) consists of an elevated platform in the shape of a plus. Two opposite arms were enclosed, and the remaining two arms were open. To begin the test, a rat is placed in the center of the platform facing the closed arm, the latency to enter the open arms, amount of time spent in open arms, closed arms, and overall inhibited behaviors in the EPM were measured. The elevated plus maze was recorded for the duration of the 5-minute test. For the forced swim test (FST) the rats were placed in a clear cylindrical Plexiglas container filled with water. The water was replaced after each trial to ensure that each rat was swimming in clean water. On day one, one rat per cylinder was placed in the water for the 15 minutes pretest phase. 24 hours later, the rats were placed in the water for 5 minutes. Time spent floating (immobile) was scored. The open field test was used to assess anxiety-like behavior.
in rat plasma. Samples were processed according to the manufacturer’s recommended protocol, then run on a Luminex MAGPIX and quantified using MILLIPLEX® Analyst 5.1 software.

**Results:**
Among the 27 cytokines included in the multiplex analyses only 4 showed either differences between experimental groups or significant correlations with behavioral measures: Leptin, IL-1β, Eotaxin, and RANTES. Although there was no significant effect of phenotype, there was a phenotype x treatment interaction on Leptin (F(1,28)=5.213, p=0.0308). Post hoc analysis showed that LR control rats had higher leptin levels compared to HR controls (p=0.0223), and antibiotic treatment significantly reduced leptin in HRs (p=0.0425) and LRs (p=0.0001) (Fig. 1A). For IL-1β, there were no main effects of HR/LR phenotype or antibiotic treatment, although there was a phenotype x treatment interaction (F(1,25)=4.366, p=0.0470); post hoc analysis showed that antibiotic treatment specifically reduced IL-1β levels in HRs (p=0.0264) (Fig. 1B). Eotaxin and RANTES levels did not differ significantly between experimental groups. However, both cytokines positively correlated with anxiety measures in the EPM (Fig.1C-D; Eotaxin: R2=0.1242, p=0.0479; RANTES: R2=0.1234, p=0.0487).

**Discussion:**
We hypothesized that antibiotic treatment would not show differences in HR/LR differences; we instead found that it exacerbated their behavioral differences, with antibiotic treated LRs showing even more anxiety-like behavior. HRs, on the other hand, showed the exact opposite with increased active stress coping in the FST. Acute systemic injections of leptin have been shown to have an antidepressant-like effect in adult mice (Lu, Kim, Frazer, & Zhang, 2006). Future studies should examine the possibility of leptin resistance in the hippocampus of LR rats. A significant limitation of this study is the fact that only male adult rats were examined. A more comprehensive study looking at sex differences as well as different developmental ages might provide more information. However, microbiota can affect emotional behavior.

**References:**

**Vascular Amyloid in an Alzheimer Mouse Model**

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**Figure 1:** Antibiotic treatment reduced serum leptin in HR/LR, and leptin levels correlated with FST immobility (A). Control HRs had higher levels of circulating IL-1β compared to control LRs; however, IL-1β did not correlate with any behavioral measures (anxiety-like behavior in the EPM shown). (B). While we did not find significant HR/LR or treatment-induced differences in eotaxin or RANTES, both chemokines correlated with anxiety-like behavior in the EPM (C-D).

**Discussion:**
Alzheimer disease accounts for 80% of dementia cases worldwide. Traditionally, one of the pathological hallmarks of this disease is Amyloid beta (Aβ) plaques. Aβ is a 36-43 amino acid peptide, formed from improperly cleaved amyloid precursor protein (APP). When APP is cleaved incorrectly in the brain, it forms sticky monomers. These monomers can usually be cleared from the brain and do not pose any hazards to normal brain functioning. However, in cases of disease, these monomers can clump together to form Aβ oligomers, or plaques. In addition to plaques, incorrectly cleaved Aβ can also aggregate on vessels in the brain. Previous research has shown that these amyloid aggregates can displace astrocytic endfeet from blood vessels. This can cause the blood brain barrier to leak and prevent proper regulation of the diameter of vessels in the brain. This regulatory ability of the vessels in the brain is called functional hyperemia, and it enables precise control of where nutrient-filled blood is directed. When vascular amyloid surrounds the vessel and displaces astrocytic endfeet, it has been shown to cause a loss of this ability. This inhibits the brain’s ability to direct nutrients to areas of need and could be a major contributor to the cognitive decline seen in patients with Alzheimer Disease. In addition, any leakage of the blood brain barrier is very unhealthy for the surrounding tissue, as the brain is a very hyperemic organ. If the blood brain barrier permits blood and its proteins to enter the brain’s parenchyma, it could cause brain damage to areas of the tissue. This can affect emotional behavior. Additionally, we studied whether these damaging vascular amyloid plaques could demonstrate a preference for certain kinds of vessels in the brain, based either on vessel size or vessel type. We showed that vascular amyloid does have a preference for arterioles and venules over capillaries, arteries, and veins. However, we were unable to distinguish with certainty whether amyloid displayed a preference for either arterioles or venules due to shortcomings in our DMC imaging.
Introduction
Major depression disorder (MDD), a disease that affects millions of people, is defined as a mood disorder leading to both emotional and physical deficits. The World Health Organization has recognized depression as the single largest contributor to global disability. Depression cases increased 18.4% between 2005 and 2015 in the United States. While there are treatment options, they are not effective for all patients. Women are twice as likely to be diagnosed with depression compared to men. The lifetime prevalence of depression for women is 21.3%, while for men it is only 12.7%.1 During adolescence years, depression disorders peak. Specifically, studies found that when estrogen levels rise significantly during Tanner Stage 3, the onset of MDD in girls increases. However, post menopause the risk of MDD lowers in women until there is no sexual difference.2 These examples suggest that estrogen may play a role in stress susceptibility. This has spurred research on the sex differences in MDD through different models. The sub chronic variable stress (SCVS) paradigm offers the ability to study sex differences in mice models. It includes six days of alternating stressors including tail suspension, foot shock, and restraint tube. After 6 days of alternating stressors, the mice are exposed to three tests, in the following order: immobility, novelty suppressed feeding, and forced swim test. The tests are chosen specifically to model core human depression symptoms. These tests measure stress susceptibility by evaluating various aspects of depression-like behaviors. In previous work, these behavioral tests were shown to result in a stress susceptible phenotype in females but not the males. At the end of SCVS, female mice are stress susceptible while males are resilient.3 This models similarities seen in women with mood disorders. In this study, SCVS was preformed after Letrozole, an aromatase inhibitor, had been administered. Letrozole was used to stop the synthesis of 17β-estradiol from testosterone in the entire body. The mice were started on letrozole water or vehicle two weeks before starting the experiment. Using this paradigm, our research studied the effects of inhibiting estrogen synthesis on stress susceptibility in female mice.

Methods: Sub Chronic Variable Stress Exposure and Behavioral Assessment
Animals
All experiments utilized C57BL/6J female mice from The Jackson Laboratory. Ages ranged from 8 to 12 weeks of age at the start of each experiment. Animals were maintained on a 12 h light/dark cycle with ad libitum food access, except when explicitly stated for behavioral testing. All procedures were performed in accordance with the Institutional Animal Care and Use Committee of Virginia Tech.

Sub chronic Variable Stress
Animals were exposed to six days of alternating stressors for 1 hour each day. On day 1, animals were administered to animals at 0.45 mgA for a duration of 2 seconds per shock. On day 2, animals were suspended by their tails with tape. On day 3, animals were placed in 50 mL conical tubes with breathing and tail holes and left in their home cages. The stressors were repeated in the same order the subsequent 3 days. Behavioral testing began on the first day following the final stressor. Animals underwent three tests, in the following order: Forced Swim Test: This is a measure of self-care. Under red light, animals received 3 sprays on their backs with 10% sucrose solution. Total time spent grooming was measured over a 5-minute period.

Findings and Discussion
Depression is a disabling disorder that affects millions of people. Women have twice the risk of being diagnosed compared to men starting at puberty and continuing post menopause. This led to research on the sex difference of depression. In this study, the effects of inhibiting estrogen synthesis on stress susceptibility in female mice using the SCVS paradigm were evaluated. The novel to home data from NSF showed a significant effect (p=0.0370) between the control and stress vehicle groups (Figure 1). This is similar to previous studies that use the SCVS paradigm and found females to be stress susceptible.2 There was no statistical difference between

References

The Effects of Inhibiting Estrogen Synthesis on Stress Susceptibility in Females
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the control and stress letrozole groups, showing that the stress effect was consistent between the two groups. The results were consistent with previous SCVS experiments. This data suggest that low levels of estrogen may increase stress susceptibility in female mice. The data from splash test and FST were inconclusive. The data collected for the control mice showed higher than normal stress levels, leading to higher baselines. Due to NSF data showing significance, it is believed that the treatment did not cause the higher stress baseline. One possible explanation for the higher stress baseline is the age of the mice. Normally this experiment is done with 8 weeks old mice, while these females were about 4 weeks old when treatment started. Another possible stressor to the mice was being single housed. For the purpose of tracking the amount of letrozole or vehicle consumed, the mice were kept single housed for the entirety of the experiment. This has been found to be a stressor to pubescent mice in previous studies.24 Letrozole use increased body weight. The mice were weighed 5 times during the experiment to track their weight. There was a significant difference between the weight of the letrozole and the vehicle groups, suggesting that the treatment was successful. We also tracked efficacy of letrozole treatment by measuring uterine weight. In all 3 genotypes, the levels of estrogen lead to atrophy of the uterus. A sample (n=5) of uteri was collected for the control and stress letrozole treatment by measuring that the treatment was successful. There was a significant difference between the stress and the control. There were several significant p values between the stress and the control among different genotypes. A recent study in rats has shown that estrogen levels in younger men were associated with depression symptoms.9 Whether looking in the brain or blood, these studies suggest that estrogen has an important role in stress susceptibility and depression in both sexes.

References


Thermoreceptors in Drosophila Melanogaster Larvae

Introduction:

As Drosophila mature and develop, they go through different larval stages or instars. At 25 °C, Drosophila melanogaster hatch from eggs 24 hours after egg laying (AEL). Once in their larval stage, they molt twice, marking the beginning of a new instar each time they molt. 48 hour AEL marks the beginning of second instar and 72 hour AEL marks the beginning of third instar. They remain in third instar for 2.5 to 3 days before entering the pupal stage where they will once again hatch as fully formed adult flies in 3.5 to 4.5 days. This project focuses on identifying thermoreceptors for warm or cold avoidance. The thermoreceptors we are interested in are ionotrophic Receptor (IR) and Transient Receptor Potential (TRP) channels. The TRP channel we are interested is TRPA1. TRPA1 has two alternative promoters. Once transcribed, the mRNA can be alternatively spliced into TRPA1(A) or TRPA1(B). In adult flies, TRPA1(A) has been identified as a chemoreceptor, while TRPA1(B) is a warm thermoreceptor (Kang et al., 2011).

Methods:

Figure 1 shows the 3% w/v agar used to establish a thermogradient using the hot plate (left side) and tray of ice (right side). It also shows the temperature prefer-ence of the larvae by measuring the temperature of the agar and larval distribution on the agar.

We performed rescue experiment by expressing the cytoplasmic TRPA1. The larvae for the experiments were maintained in the Ni lab. Flies were flipped into new food and incubated at 25 °C for 3 hours allowing them to lay eggs. At the end of the 3 hour period, flies were removed from the vial and the vial was incubated at 25 °C. This allowed the Ni lab to accurately measure the age of the larvae. Once at the appropriate age, the larvae were removed from the food by pouring a 20% sucrose solution over it and the larvae would float to the top of the sucrose solution.

We first examined the wild type (Canton S.) larvae at 48, 72, 96, and 120 hour AEL. We then examined the temperature preference with TRPA1(B) mutant. Finally, we performed rescue experiment by expressing the cytoplasmic TRPA1. The larvae for the experiments were maintained in the Ni lab. Flies were flipped into new food and incubated at 25 °C for 3 hours allowing them to lay eggs. At the end of the 3 hour period, flies were removed from the vial and the vial was incubated at 25 °C. This allowed the Ni lab to accurately measure the age of the larvae. Once at the appropriate age, the larvae were removed from the food by pouring a 20% sucrose solution over it and the larvae would float to the top of the sucrose solution.
Then the larvae were washed 3 times in distilled water. After the wash, the larvae were placed on a small plate containing 3% w/v agarose gel to check under a microscope to confirm that the larvae were at the correct instars. Once the instars of the larvae were confirmed, the larvae were placed in the middle of the thermogradient, approximately 24 °C. The thermogradient used in our lab was a 3% w/v agarose gel placed on a metal sheet with a hot plate on one side (the hot side) and a tray full of ice on the other (the cold side) (Figure 1). We found that this set-up adequately kept the agarose gel at 14 °C on the cold side and 30 °C with a continuous temperature gradient throughout the gel.

The larvae were allowed to migrate to their preferred temperature for 10 to 15 minutes. Then the percent preference of the larvae was measured by counting the number of larvae in each section of the thermogradient, dividing by the total number of larvae on the agar, and multiplying by 100. Larvae along the edge of the agar and off the agar were not included in the percent preference.

Results:
We observed that there was a dramatic shift in temperature preference during larval development. The early stage larvae at 48, 72, and 96 hours AEL prefer 24 °C, while the late 3rd instar larvae at 120 hour AEL prefer 18 °C. This shift in temperature preference can be observed in Figure 2. We also observed that TrpA1 knockouts were unable to avoid the warmth. This is consistent with both 72 and 120 hour AEL larvae (Figure 3 and Figure 4).

18 °C. This shift in temperature preference can be observed in Figure 2. We also observed that TrpA1 knockouts were unable to avoid the warmth. This is consistent with both 72 and 120 hour AEL larvae (Figure 3 and Figure 4).

Conclusion:
From our data we were able to conclude that there is a dramatic shift in temperature preference between early stage larvae and late third instar larvae. Figure 2 has shown that wild type larvae at 48, 72, and 96 hour AEL prefer 24 °C while at 120 hour AEL prefer 18 °C. Our pre-liminary data suggest that TRPA1 is the warmth receptor which is supported by Figures 3 and Figure 4.

References:

Figure 2 shows the temperature preference of 48, 72, 96, and 120 hour AEL larvae. The early stage larvae at 48, 72, and 96 hours AEL prefer 24 °C, while the late 3rd instar larvae at 120 AEL prefer 18 °C. CS: Canton S.

Figure 3 shows the percent preference of wild type, TrpA1 knock-out, and TrpA1 rescue larvae at 72 hour AEL. It shows that the increased distribution of TrpA1 knock-out when compared to a control. Such increase was rescued by expressing the wildtype TrpA1.

Figure 4 shows the percent preference of wild type, TrpA1 knock-out, and TrpA1 rescue larvae at 120 hour AEL. It shows that the increased distribution of TrpA1 knock-out when compared to a control. Such increase was not rescued by expressing the wildtype TrpA1.
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