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

Katie Barnes
Mentor: Dr. Stefanie Robel
When Sex Matters: Different Responses to Traumatic Brain Injury Based upon Sex Hormone Levels

Francesca N. Czesak
Mentor: Dr. Kendra B. Sewall
Effects of Lead on Brain Regions Involved in Language Production in Male Songbirds

Rishi K. Devulapalli
Mentor: Dr. Timothy J. Jarome
Upregulation of proteasome activity and protein ubiquitination occur selectively at synapses during memory reconsolidation

Erin Duricy
Mentor: Dr. Michael Bowers
Region-specific regulation of Foxp1 and Foxp2 by dihydrotestosterone in the developing brain

Matthew Emanuel
Mentor: Dr. Christopher Thompson
The putative endocrine disruptor maltol induces pigment aggregation in developing Xenopus laevis tadpoles

Muhannah Hossain
Mentor: Dr. Michelle Olsent
Investigation of Astrocyte Numbers in Rett syndrome

Matthew Hyland
Mentor: Dr. Sarah Clinton
The Behavioral Effects of Perinatal SSRI Exposure in Rats

Alyssa Johnson
Mentor: Dr. Georgia E. Hodes
Examination of Susceptible and Resilient Subpopulations of Females Exposed to Subchronic Variable Stress

Kyle Nickel
Mentor: Dr. Chelsea McCoy, Dr. Sarah Clinton
Altered cytochrome c oxidase activity in rats selectively bred to display anxiety and depression-like behavior

Sabrina A. Orsi
Mentor: Dr. Timothy J. Jarome
Ubiquitin-proteasome activity is localized in the nucleus during long-term memory formation

Patrick B. Rafael
Mentor: Dr. Mark A. Cline
The anorexigenic effect of gastrin-releasing peptide involves the arcuate nucleus of the hypothalamus in chicks

Mohammad Sabbagh
Mentor: Dr. Sarah Clinton
Examining the role of microbiota in emotional behavior: antibiotic treatment exacerbates anxiety in high anxiety-prone rats

Andrew Stublen
Mentor: Dr. Ian F. Kimbrogh
Vascular Amyloid in an Alzheimer Mouse Model

Katherine Vaughn
Mentor: Dr. Georgia E. Hodes
The Effects of Inhibiting Estrogen Synthesis on Stress Susceptibility in Females

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, Mahshid Aya2, Dr. Carmen Muñoz-Ballester3, Dr. Stefanie Robel1
1Virginia Polytechnic Institute and State University, Blacksburg, VA
2Virginia Tech Carilion Research Institute, Roanoke, VA
3School of Neuroscience Virginia Tech

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 other complications, making it a challenge for patients to gain more insight into their own condition. While many studies have been conducted to understand the effects of traumatic brain injury (TBI), most of this research only involves males. The few studies that include females post-injury have attempted to gain more insight into this worldwide epidemic. Therefore, this study is to investigate if mice 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 female and males using a one hundred gram weight. Following the TBIs, 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. The western blots were used to examine recovery seven days post injury. 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

Pratissola C, Zyzyk, Lauren M. Knott, Kundu R. Swain
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 low levels of lead can impair brain development, leading to compromised learning, cognition, and language development, there is 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) through the first 90 days of development. Then, 4-8 weeks after exposure was completed, the brains of each bird were collected. To assess the effects of lead exposure in this study we were previously found to have impaired song learning (area X and LMAN) as well as the principal song control nucleus (HVC). Therefore, we examined the effects of lead exposure in critical period vocal learning have not been rigorously characterized. To this end, we examined the effects of lead exposure on brain regions involved in vocal learning (Area X and LMAN) as analogous to language centers in the human brain (e.g., Broca’s area).

Results:
We found that LMAN was significantly smaller in birds exposed to lead than control birds (GLM, z = 2.623, p = 0.0277, Fig. 1). The amount of lead that birds were exposed to (100 and 1000 ppb) had lead impact 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 song learning (GLM, z = 2.623, p = 0.0277, Fig. 1). The amount of lead that birds were exposed to (100 and 1000 ppb) had lead impact 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 song learning (area X and LMAN) as well as the principal song control nucleus (HVC). A region involved in vocal learning is the supramammillary nucleus (MnS), which is involved in vocal learning and motor control of song production. We found the supramammillary nucleus is a form of learning depends 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).

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
could result from lead damaging this brain area, though we cannot conclude a causal relationship from this study. Future studies will employ more detailed histology to measure the neural densities of each brain region to better resolve the impacts of lead exposure at the cellular level.

Importantly, our findings in songbirds are paralleled by MRI studies in humans, which have shown that children exposed to lead have impaired language and altered patterns of activation in language brain centers. This supports the use of songbirds as a model for studying the effects of lead on critical period learning and brain development, and for drawing inference about impacts on language development in children. This model will allow us to begin testing possible treatments for sublethal lead exposure, including a safe and cost-effective dietary supplement that has been previously suggested to mitigate the impacts of lead exposure, calcium.

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

Rishi K. Devulapalli1, Sabrina A. Orsi1, Rithika Surineni1,2, and Timothy J. Jarome1,2
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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 retrieval and collected nuclear and cytoplasmic fractions using nondenaturing gels and a crude dietary supplement that has been previously suggested to mitigate the impacts of lead exposure, calcium.

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

**Rein Duricy; Soad Elziny; Nadine Schuster; Melodye Taylor; Tate Teyle; Sarah Wartellerton; Miguel Perez-Pouchoulen; J. Michael Bowers**

**Methods:**

**Ultrasonic Vocalizations:**

The recording of vocalizations was performed 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 a fast Fourier transformation (FFT) was conducted. Spectrograms were generated with an FFT-length of 512 points and a time window overlap of 75% (100% Frame, Hamming 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). The call detection process follows the methods published in Bowers et al., 2013. In brief, all membranes blocked in Licor Buffer (Licor) then incubated in the primary antibody with Licor buffer overnight at 4°C. One-hour incubation in 1:1000-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). The male was then placed in the center of the light cycle and under red-light illumination. Testing began with the addition of a hormonally primed receptive female (10 ug estradiol and administered for three days before testing and 500 ug progesterone in 0.1 ml sesame oil 2 days before testing) and under red-light illumination. The male was placed 30 cm above the rats.

**Findings:**男性 vocalizations were significantly no different when compared to their 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 produce significantly higher number of vocal calls (p<0.0001) as compared to naive males.

**Conclusions:**

Androgens, but not estrogens, increase 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 produce significantly higher number of vocal calls as compared to naive males. We also tested vocal communication behavior during mating. We found males with previous sexual experience produced significantly higher number of vocal calls as compared to naive males. Furthermore, the vocalizations were found to be significantly reduce after removal of the gonads. Gonadally intact males produced more vocalizations that after gonadectomy (p<0.0001). We then administered exogenous androgens to the gonadectomized males and found their vocalizations were significantly different when compared to their protein levels in the developing rat brain (p>0.05, Figure 3).

**References:**


2. Bowers JM, Perez-Pouchoulen M, Roby CR, Ryan TE, McCarthy MM. Androgen modulation of Foxp1 and Foxp2 in the developing rat brain: impact on sex specific vocalization.

**Figure 3**

*Female rats and also investigating the impact androgens have on other genes that might be related to vocal communication.*

**Figure 2**

*Female rats and also investigating the impact androgens have on other genes that might be related to vocal communication.*

**Figure 1**

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

Matthew Emanuel, Lau Delon, Qahhaby Hassain, Alexis Figueroa Baiges, Christopher K Thompson

1) School of Neuroscience 2) Biological Sciences 3) TBMH 4) Global Change Center, Virginia Tech, Blacksburg VA

Introduction: The Thompson lab focuses on identifying thyroid hormone disruptors by implementing two-way treatments and analyzing the effects on organ 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 via a retinal-thyroid hormone disruptor mechanism by which maltol acts to control pigment aggregation.

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 from a light source. 0.1261g of Maltol was diluted in 50mL H2O. The stock solution was then diluted into respective concentrations in 200mL of H2O. The stock solution was fixed overnight in 4% PFA. Tadpoles were killed on Day 4, with an overdose of MS222 and fixed overnight in 4% PFA. QPCR: Brains dissected into Trizol for mRNA extraction. Amount of mRNA was measured using a Nanodrop spectrophotometer. mRNA was converted into cDNA for later analysis. Statistical analysis: Two-way ANOVAs and subsequent graphs were constructed using Prism. These were used to analyze the two factor’s (background and dose) impact on the response variables (proportion of brain covered, circularity, roundness, and area), which were used to quantify melanophore pigment aggregation.

Results: 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

Muhammad Hassan, Alexis Crockett, Stephan Honodel, Lauren Holt, Dr. Michelle Olsen. School of Neuroscience, Virginia Polytechnic Institute and State University

Introduction: 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 language delay, 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, decrease in later volume, neurodevelopmental and morphological complexity, and increased neuronal packing. Additionally, previous studies have demonstrated that neurons...
in Rett syndrome, although there is increasing evidence that perinatal SSRI exposure may affect other behavioral measures. However, the long-term effects of early life SSRI exposure on adult behavior are not well understood.

Methods

We hypothesized that perinatal SSRI exposure would increase depression-like behavior (in the OFT and EPM). We found no significant differences in the time spent in the open arms of the EPM or in the amount of time spent in the center of the OFT in the experimental and control groups.

Conclusion

We hypothesized that perinatal citalopram exposure would increase depression-like behavior (in the OFT, Splash test, FST, and sucrose preference), but not anxiety-like behavior (in the EPM). We found no significant differences in anxiety behavior (in the OFT and EPM). However, we did find that there was more inhibition in SSRI-exposed males in the OFT, showing that SSRI-exposed males had increased anxiety-like behavior. This could suggest differences in the effect of early life SSRI exposure in males versus females. This may have important implications for the treatment of autism in males and females. As for the measures of depression,

The Behavioral Effects of Perinatal SSRI Exposure in Rats

Matthew Hyland, Matthew Glover, Chloë McCoy, and Sarah Clinton School of Neuroscience, Virginia Tech, Blacksburg, VA

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 in early childhood (Glover & Clinton, 2016). Selective serotonin reuptake inhibitors (SSRIs) have been the mainstay of treatment for depression in adults. However, the long-term effects of early life SSRI exposure on adult behavior are not well understood.

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

Introduction
Depression is a debilitating disorder that impacts approximately 300 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 (Krishnan et al., 2015b). 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 more resilient (Krishnan et al., 2015b). 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 versus 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 immobolize 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 one of the 3 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 2).

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 females 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 chart indicates the rate at which each test was the inconsistent test (figure 2).

References:
• Hodes et al., 2015a. Stressful women are known to experience 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.

Figure 1: Cross-Experiment SCVS Stress Susceptibility Scores

Figure 2: Prenatal citalopram exposure increased depression like behavior. (A) There was no change in % time spent grooming the bedding. (B) Nor was there a significant difference in the % time spent grooming in splash test, but males groomed significantly less than females. (C) There was significantly decreased sucrose intake in the SSRI group of both sexes in the sucrose preference test. (D) and increased immobility for the SSRI treated in the FST.
Altered cytochrome c oxidase activity in rats selectively bred to display anxiety and depression-like behavior

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Introduction:
Previous research has shown that differences in metabolic function have been associated with affective disorders like anxiety and depression1–3. 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 an anxious and depressive phenotype4. 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/IR model was used to provide a reliable method to produce anxiety and depression-like phenotypes in order to differentiate metabolic activity the two groups. The HR/IR 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 dot blots 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 MicroTec ScanMaker 9800XL in 16-bit grayscale without corrections. From the dot blot, the optical density of staining was correlated to micrograms of COX reaction product.

Results

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

Figure 1: COX activity in the subregions of the amygdala. In all tested regions of the amygdala (lateral (A), basolateral (B), basomedial (C), and central (D)) there was no significant differences found in COX activity between HR and LR rats.
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 the large multi-subunit protein structure called the proteasome. There are diverse ubiquitin tags that a protein can acquire, which varies the number of ubiquitin molecules bound to the target as well as the lysine (K) site at which they bind, and some of these ubiquitin modifications target a protein for functions other than protein degradation. However, while previous studies have focused on degradation-specific polyubiquitination, it is unknown how consolidation alters other ubiquitin modifications. Furthermore, it is unknown how UPS activity changes within the nuclear, cytoplasmic, and synaptic regions in response to learning.

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 then collected nuclear and cytoplasmic fractions using nonionic detergents and a crude synaptic fraction using a sucrose gradient. In response, we measured protein polyubiquitination levels in these samples with western blotting and proteasome activity using an in vitro assay.

Results
Following training, overall protein ubiquitination and 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) 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 during fear memory learning (Figure 2). Suggesting that the UPS may regulate memory consolidation via unique mechanisms other than protein degradation which varies across cellular compartments.

References
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 functions 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 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 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 is associated with a-hyperkinetic activity in the hypothalamic-pituitary-adrenal (HPA) axis (Zunszain, Anacker, vancomycin that was dissolved in antibiotic treatment in 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 same closed arm, the latency to enter the open arms, amount of time spent in open arms, closed arms, and depressed-like behaviors. In addition to the increased anxiety- and depression-like behavior, LR rats also show increased chronic stress susceptibility, and increased passive stress coping compared to HR rats. We hypothesized that HR/LR gut microbiota differences may be a contributing factor to their dissimilar behavioral phenotypes.

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.

In order to examine potential immune system differences in HR/LR control and antibiotic-treated rats, we used a MILLIPLEX MAP Rat Cytokine/Chemokine Magnetic Bead Panel to measure levels of 27 cytokines/chemokines.
in rat plasma. Samples were processed according to the manufacturer’s 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(1,26)=5.213, p=0.0306). 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 (R^2=0.1242, p=0.0479; R^2=0.1234, p=0.0487) (Fig. 1C-D; Eotaxin: R^2=0.1242, p=0.0487). RANTES: R^2=0.1234, p=0.0487).

Discussion: We hypothesized that antibiotic treatment would induce novel neuroendocrine changes 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 a better idea of how microbiota can affect emotional behavior.


Vascular Amyloid in an Alzheimer Mouse Model
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Alzheimer disease accounts for more than 50% of dementia cases worldwide. Traditionally, one of the pathological hallmarks of this disease is Amyloid beta (Aβ) plaques. Aβ is a 36-42 amino acid residue 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, improperly 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 blood parenchymal barrier exists for the purpose of keeping toxins separate from healthy brain parenchyma. We do not currently understand how exactly these vascular amyloid plaques cause blood brain barrier failure. However, we have found that areas of the vasculature laden with vascular amyloid do demonstrate a downregulation in expression of the tight junction proteins ZO1 and Claudin 5. These tight junction proteins are responsible for holding the endothelial cells of the vasculature together to seal the blood brain barrier. To demonstrate that this decreased expression of tight junction proteins was not just a failure of the antibodies to penetrate through the vascular amyloid, the tissue was also stained for vinculin, a component of the cytoskeleton found directly beside these tight junctions. There was no difference in vinculin labeling between areas with and without an amyloid burden, indicating that the amyloid is not preventing antibody penetration and that there is a true loss of tight junction protein expression.

Additionally, we studied whether these damaging vascular amyloid plaques display 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.

Long-term exposure to the antibiotic cefixime (C) is associated with increased plaque burden (A) and decreased blood brain barrier expression (B). Discoid arterioles (red lines) were quantified in the hippocampus of the (A) Control and (B) Antibiotic groups.

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).
The Effects of Inhibiting Estrogen Synthesis on Stress Susceptibility in Females

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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%. During adolescence, depression disorders peak. Specifically, studies have 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 differences. 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 behaviors the following morning, novelty suppressed feeding (NSF), forced swim test (FST). 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. 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 17B-estrodial 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/6 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 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, stress was a splash, novelty suppressed feeding (NSF), and forced swim test (FST). The NSF 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 behaviors the following morning, novelty suppressed feeding (NSF), forced swim test (FST). 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. 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 17B-estrodial 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.

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 shows a sex difference (p=0.0370) between the control vehicle and stress vehicle groups (Figure 1). This is similar to previous studies that use the SCVS paradigm and found females to be stress susceptible. There was no statistical difference between

Fig. 1 Vascular amyloidosis impairs the gliovascular unit in a mouse model of Alzheimer's disease.

Fig. 2 Most of the amyloid in the brain is found in plaques, not on the vasculature

References

Fig. 3 Amyloid was primarily found on arterioles and venules from 7-35 microns in diameter.

Fig. 4 There was not a significant difference in vascular amyloid accumulation between arterioles and venules.
the control and stress letrozole groups, showing that the stress effect was specific to the letrozole group, similar to the males in previous SCVS experiments. This data suggest that low levels of estrogen may decrease 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 same 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. Letrozole use increased body weight. The mice were weighted 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. The mice were weighted twice, marking the beginning of third instar. They remain in third instar for 2.5 to 3 days before entering their pupal stage where they will molt twice, marking the beginning of fourth instar. In their larval stage, they molt once again hatch as fully formed adults 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 Po-tential (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 chemoceptor, while TRPA1(B) is a warm thermoreceptor (Kang et al, 2011).

Methods:
We performed nine trials for each genotype at each time point. 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(A) mutant. Finally, we 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


References

Image 1
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 preference of the larvae by measuring the temperature of the agar and larval distribution on the agar.
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).

**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](image1.png)

**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](image2.png)

**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](image3.png)

**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.