School of Neuroscience
Summer Undergraduate Research Fellowship

COLLEGE OF SCIENCE
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VIRGINIA TECH

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 will be 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
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Erin Duricy
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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

School of Neuroscience Virginia Tech

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

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 Barnes[1,2], Malikah Ajose[1,2], Dr. Carmen Muñoz-Ballester[1], Dr. Stefanie Robel[1]

[1]Virginia Polytechnic Institute and State University, Blacksburg, VA

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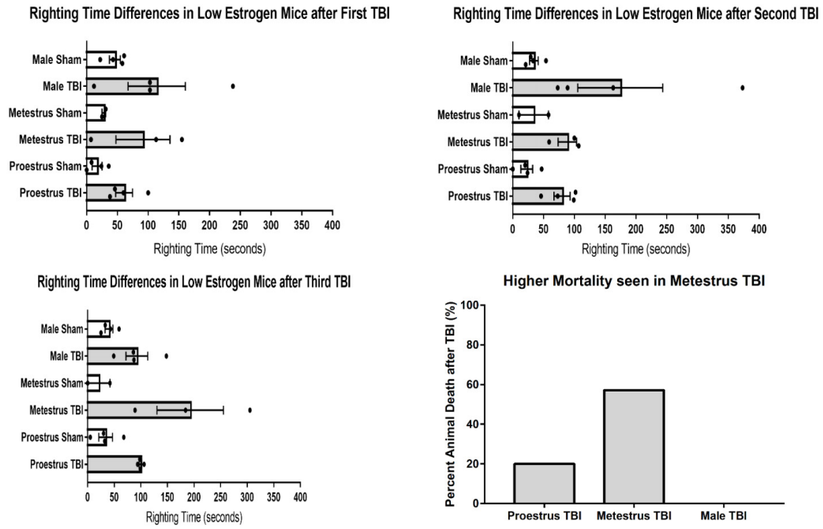
Introduction

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 potentially death. In an attempt to gain more insight on this worldwide epidemic, scientists have conducted several studies; however, most of this research only involves males. The few studies that include females show 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; however, 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 plasticity. While some aspects of cognition may recover from developmental insults, such as lead exposure, disruptions to critical periods of learning and neural organization can result in irreversible changes in brain structure and function. There is currently a gap in our understanding of the life-long consequences of exposure to levels of lead reported in cities such as Flint, MI, because effects on critical period vocal learning have not been rigorously characterized. 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) through 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 using light microscopy. We measured the areas and calculated the volumes of two brain regions involved in 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 divided the volumes of each brain region of interest by the telencephalon volume to control for possible overall differences in brain size. We tested the effect of treatment (lead or control) using a single general linear mixed model with brain region as as dependent variable and individual specified as a random factor to account for non-independence of samples.

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 no significant 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 songs in adulthood, reflecting compromised song learning early in 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

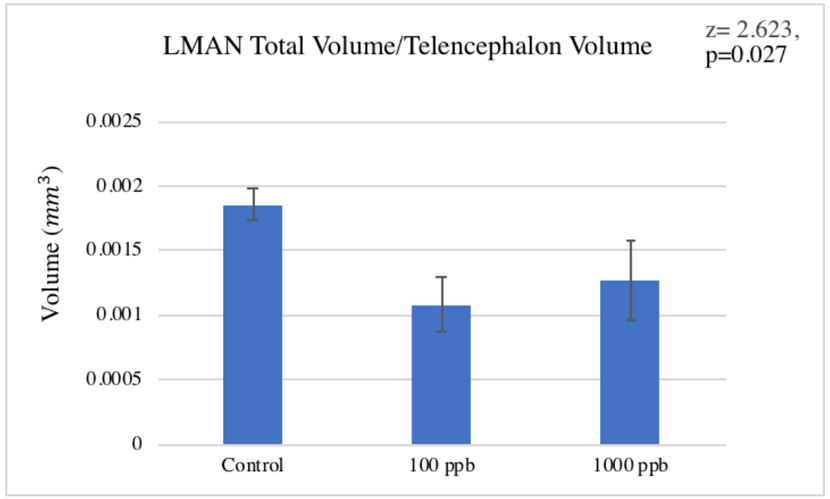


Figure 1. Mean volume and standard error of LMAN controlling for telencephalon volume from zebra finches provided with control (0 ppb lead), and lead-treated water (at 100 ppb or 1000 ppb) for the first 90 days of life. LMAN is a nucleus in the avian song learning pathway that is central to early vocal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

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 a the cellular level.

Importantly, our findings in songbirds are paralleled by fMRI 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. Devulapalli [1,2], Sabrina A. Orsi[1,2], Rithika Surineni[1,2] & 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 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 nonionic detergents and a crude synaptic fraction using a 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.

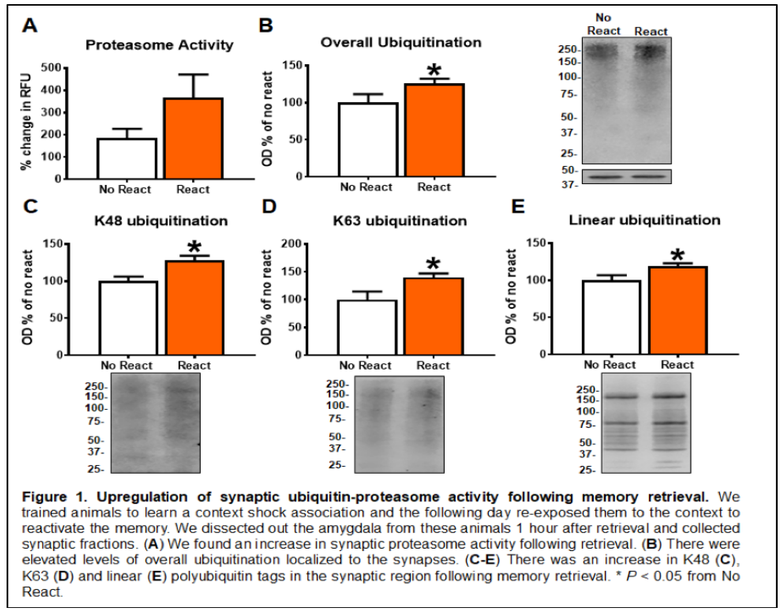

Figure 1. Upregulation of synaptic ubiquitin-proteasome activity following memory retrieval. We trained animals to learn a context shock association and the following day re-exposed them to the context to reactivate the memory. We dissected out the amygdala from these animals 1 hour after retrieval and collected synaptic fractions. (**A**) We found an increase in synaptic proteasome activity following retrieval. (**B**) There were elevated levels of overall ubiquitination localized to the synapses. (**C-E**) There was an increase in K48 (**C**), K63 (**D**) and linear (**E**) polyubiquitin tags in the synaptic region following memory retrieval. * $P < 0.05$ from No React.

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 therapeutic strategies for the treatment of various psychiatric disorders.

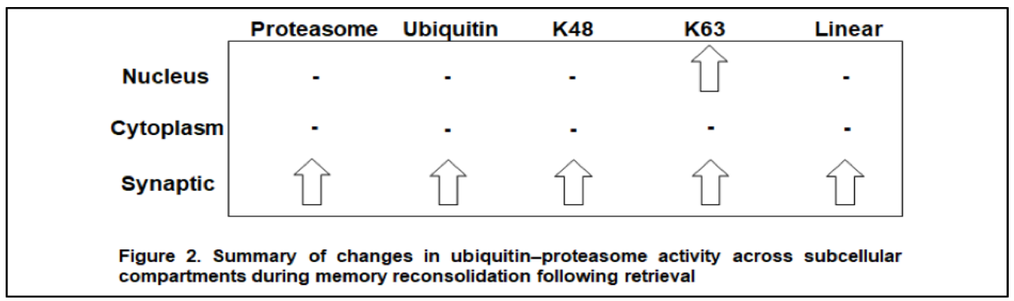

Figure 2. Summary of changes in ubiquitin–proteasome activity across subcellular compartments during memory reconsolidation following retrieval

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

Erin Duricy; Soad Elziny; Nadine Schuster; Makenzlie Taylor; Tina Taylo; Sarah Woolverton; Miguel Perez-Pouchoulen; J. Michael Bowers

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, 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 environmental factors (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:

Ultrasonic Vocalizations: The recording used the Ultrasound Microphone (Avisoft UltraSoundGate condenser microphone capsule CM16, Avisoft Bioacoustics, Berlin, Germany) to record the rat vocalizations in a sound attenuating chamber from Metris. The microphone was placed 30 cm above the rats. Vocalizations were recorded using Avisoft Recorder software (Version 5.1). Settings 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).

Western Blot: The 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 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 (60cm long X 30cm wide X 30cm high). Took place during dark phase of the light cycle and under red-light illumination. Testing began with the addition of a hormonally primed receptive female (10 ug estradiol benzoate in 0.1 ml sesame oil 2 days before testing and 500 ug 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)

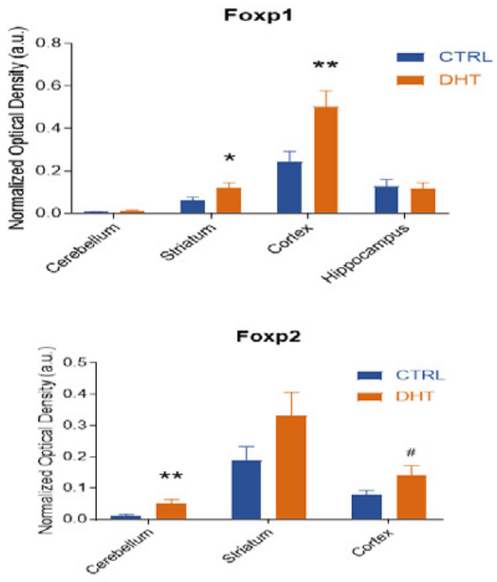

Figure 1

When the administration of sex hormones was delayed and administered for three consecutive days, from PN10-PN13, a different pattern was observed. For both transcription factors, no effect was observed for estradiol treatment. However, in contrast to the observed results with androgen treatment at PN4 increasing the protein levels of the two transcription factors, at this later time point androgens decreased protein levels (Figure 2). The brain regions significantly impacted were the cerebellum ($p < 0.01$), striatum ($p < 0.0001$), and hippocampus ($p < 0.03$) for Foxp1. Moreover, androgens were found to decrease Foxp2 protein levels in the striatum ($p<0.0001$) and cortex ($p<0.05$), but not the cerebellum ($p>0.05$).

We also tested vocal communication behavior during mating. We found males with previous sexual experience produced significantly higher number of vocal calls ($p<0.0001$) as compared to naïve males. Furthermore, the vocalizations were found to be significantly reduce after removal of the gonads. Gonadally intact males produced more vocalizations than after gonadectomy ($p<0.0001$). We then administered exogenous androgens to the gonadectomized males and found their vocalizations were significantly no different when compared to their pre-gonadectomy assessments ($p >0.05$, Figure 3).

Conclusions:

Androgens, but not estrogens, effects 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 analyzing mRNA and protein levels of Foxp1 and Foxp2 at PN4 and PN14 as well as after gonadectomy and hormone replacement in adulthood. We will also investigate the effects androgens and the Foxp genes 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.

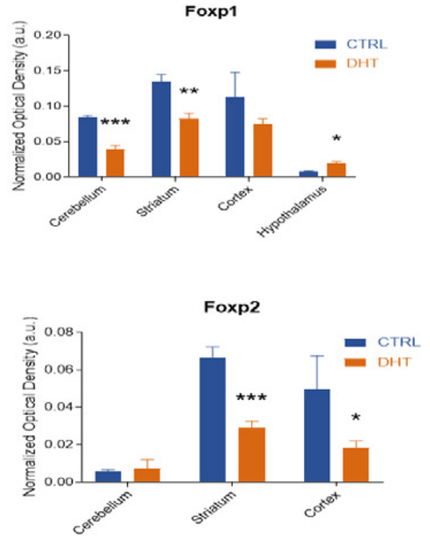

Figure 2

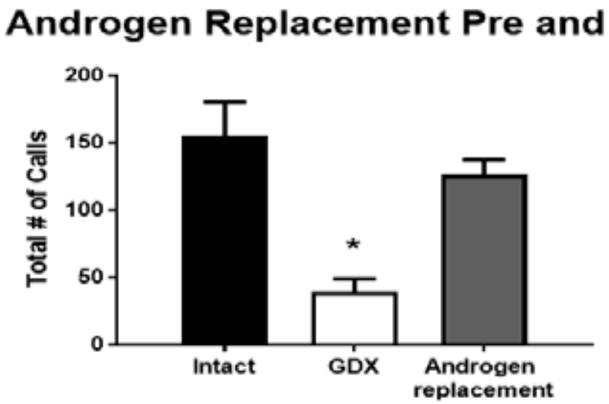

Figure 3

References:
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3. Phoenix CH, Goy RW, Gerall AA, Young WC. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology. 1959;65:369-82. Epub 1959/09/01. doi: 10.1210/endo-65-3-369. PubMed PMID: 14432658.
4. Samango-Sprouse CA, Sadeghin T, Mitchell FL, Dixon T, Stapleton E, Kingery M, Gropman AL. Positive effects of short course androgen therapy on the neurodevelopmental outcome in boys with 47,XXY syndrome at 36 and 72 months of age. Am J Med Genet A. 2013;161A(3):501-8. Epub 2013/01/25. doi: 10.1002/ajmg.a.35769. PubMed PMID: 23345253.
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6. Portfors CV. Types and functions of ultrasonic vocalizations in laboratory rats and mice. J Am Assoc Lab Anim Sci. 2007;46(1):28-34. PubMed PMID: 17203913.

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

Matthew Emanuel[1], Lara Dahora[2], Zahabiya Hussain[1], Alexa Figueroa Baiges[1], Christopher K Thompson[1,2,3,4]

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 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 contributing to the odor of bread, a better understanding of maltol's impact on brain and physical development is high priority. The current project assessed the effects of maltol on pigment aggregation in developing Xenopus laevis tadpoles. The effects of maltol were assessed by implementing dose-respons treatments and analyzing the effects on melanophore pigment aggregation in the dura under different light conditions. Confocal bright field microscopy was used to image the tadpoles for later analysis in imageJ. RNA extraction and qPCR were performed to assess maltol's impact on gene expression. These initial experiments were conducted to narrow down the 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 Steinbergs to make working strength of 100µM, 300µM, 600µM and 1mM. Groups of tadpoles were then kept in their corresponding solutions for up to four days.
Confocal Imaging and analysis: Fixed tadpoles were imaged using a Lecia SP8 confocal microscope; images were analyzed using ImageJ.
Sacrifice and tissue processing: 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 ANOVA's 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.

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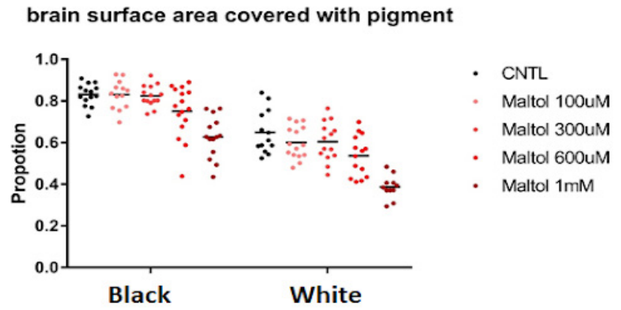
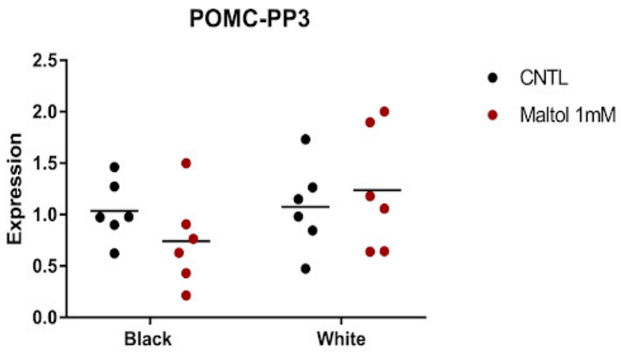


Conclusions:

- 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

Muhannah Hossain1, Alexis Crockett[1], Stephan Haraldsson[1], Leanne Holt1, Dr. Michelle Olsen1. [1]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 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, decreased brain volume, neuronal morphological complexity, and increased neuronal packing. Additionally, previous studies have demonstrated that neurons

The Behavioral Effects of Perinatal SSRI Exposure in Rats

Matthew Hyland, Matthew Glover, Chelsea 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 child bearing years (Glover & Clinton, 2016). Selective serotonin reuptake inhibitors (SSRIs) have been the mainstay treatment 10-20% of pregnant or postpartum women with major depression (Glover & Clinton, 2016). They are considered safe to use during and after pregnancy due to a relatively low number of birth abnormalities and pregnancy complications (Glover & Clinton, 2016).
SSRIs block the serotonin reuptake transporter of the presynaptic cell, thereby reducing reabsorption and transiently increasing synaptic serotonin levels. Serotonin affects many developmental processes in the brain. Thus, SSRI exposure can disrupt these processes, resulting in neurobiological and behavioral changes (Glover et al., 2015). 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 (Simpson et al., 2011, Rayen et al., 2014, Glover et al., 2015). Therefore, it is imperative to determine what 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 both male and female adult rats.

Methods:
One week before breeding, the female Sprague-Dawley rats in the experimental group began receiving the SSRI citalopram via their drinking water (10mg/kg/day), while control groups received normal water. This treatment was continued throughout pregnancy and until the pups were weaned on postnatal day (P) 21. After weaning, the pups were undistributed until adulthood (P60) and then began a behavioral test battery. The open field test (OFT) is conducted in a 100 × 100 × 50 cm Plexiglas box with no top. The rat is placed in one corner of the box and allowed to explore freely for 5 minutes. The locomotor activity and time spent in the center/periphery is measured. The elevated plus maze (EPM) consisted of a platform in the shape of a plus that was raised approximately 1 meter o¬ff the ground. Two opposite arms of the maze were open platforms and the other two were enclosed by walls. The rats were placed in the middle of the arms and the time they spent in each type of arm (closed or open) or in the middle of the two was recorded. The urine sniffing test (UST) is a test in which rats are placed in a test box with a few pieces of clean bedding in one of the corners. The rats are allowed to explore for 5 minutes and then the bedding is removed, and they are acclimated for 15 minutes. Bedding is then soaked in urine from the opposite sex and placed in the same corner of the box for 5 minutes; the time spent sniffing the diff¬erent bedding is recorded. The splash test is a test in which rats are squirted with a 30% sucrose solution on the middle of their back. For the 15 minutes of testing, the time that rats spend grooming themselves is scored. The forced swim test (FST) was performed in a Plexiglas cylinder of water. On FST day 1, rats were placed (one rat/cylinder) in the water for 15 minutes; 24 hours later the rats swam again and videotaped for 5 minutes. The sucrose preference test was conducted in the rats' home cages. In an animal's cage, one bottle with regular drinking water and one with a 0.5% sucrose solution was placed. The bottles were left in the cages for 12 hours and measured the next day.

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), but citalopram exposed males exhibited less locomotor activity (Fig. 1B; P = 0.0172) and more time grooming than male controls (Fig. 1C; P = 0.0061). There were also 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 (Fig. 1D; effect of sex P < 0.0001).
There was no effect of SSRI exposure in the time spent sniffing in the UST (Fig. 2A). Nor was there a significant difference in the time spent grooming in the splash test, although males groomed significantly less than females (Fig. 2B; effect of sex P < 0.0001). SSRI exposure did significantly decreased sucrose intake of both sexes during the sucrose preference test (Fig. 2C; P = 0.0031). We also found increased FST immobility in SSRI exposed males and females (Fig. 2D; P = 0.0033).

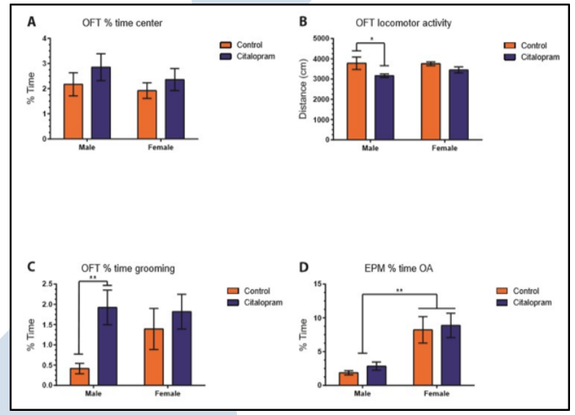


Figure 1: *Prenatal citalopram exposure effects anxiety in males. (A, B, C) There were no significant differences between groups in the % time rats spent in the center of the open field, however, male rats exposed to citalopram exhibited significantly less locomotor activity, and significantly more time 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.*

Conclusion:
We hypothesized that perinatal citalopram exposure would increase depression-like behavior (in the UST, Splash test, FST, and sucrose preference), but not anxiety-like behavior (in the OFT and EPM).
We found no significant differences in the time groups spent on the open arms of the EPM or in the amount of time they spent in the center of the OFT. 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 anxiety-like behavior in males versus females. This means that it may be important to consider how 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.

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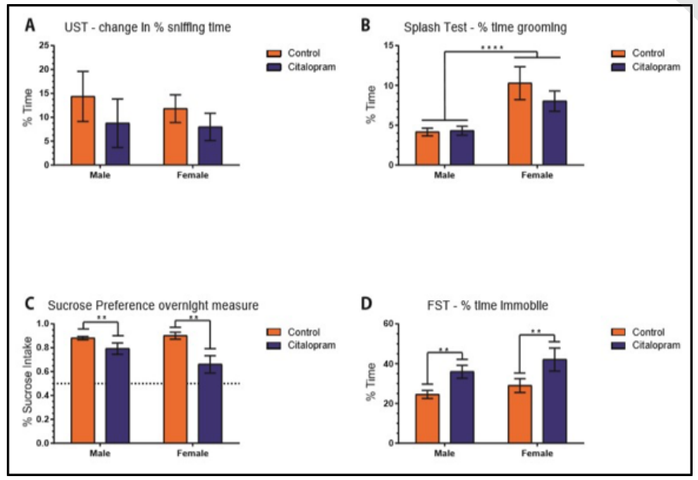

Figure 2: Prenatal citalopram exposure increased depression like behavior. *(A) There was no change in % time spent sniffing the bedding. (B) Nor was there a significant difference in the % time spent grooming in splash test, but males groomed significantly less than females. (C) There was significantly 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.*

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 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., 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 (Krishnan et al., 2007) . Previous research focusing on sex differences in stress susceptibility indicates that females become susceptible to six days of sub chronic 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 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 chart indicates 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 fell 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 subpopulations 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).

Alternative Method of Identifying Susceptible and Resilient Subpopulations among Females:

Females with consistent z-scores on all 3 tests in both the control and stress conditions were identified and (figure 3). The data indicate that in genetically identical female mice, individual differences exist between animals that contribute to differences in susceptibility or resilience, regardless of exposure to SCVS.

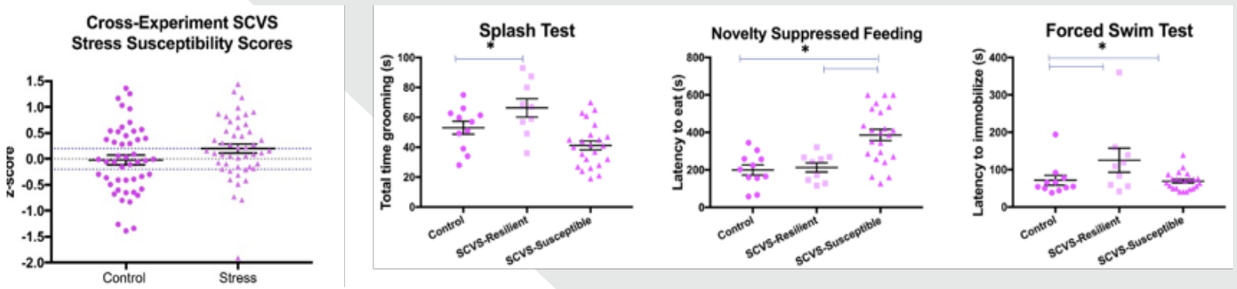

Figure 1

No females exposed to SCVS were resilient across all three tests in any of the 5 experiments examined. This suggests that SCVS may induce some form of susceptibility in all females in some form.

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

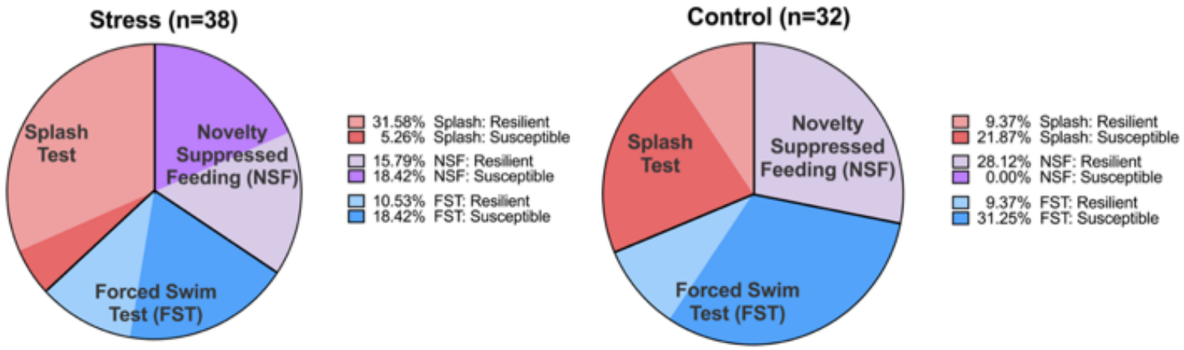

Figure 2

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

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.

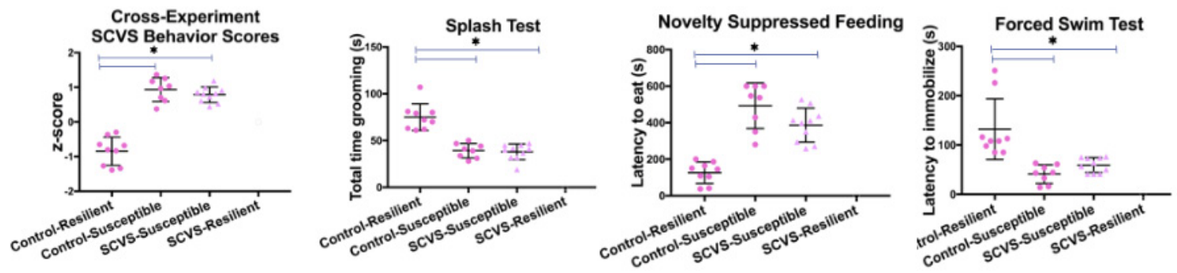

Figure 3

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

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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/LR 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/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 um. 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 a dot blot 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 (Fig. 1).
Measurement of COX activity within brain regions:
The slides were scanned in the same manner as the dot blot. Parallel sets of slides were cresyl violet-stained anatomical guides. Image analysis involved creating masks on the image of the COX-reacted slides. Two separate experimenters
used to a .01cm square voxel to measure COX activity levels in 5 locations within a region of interest. The measurements were

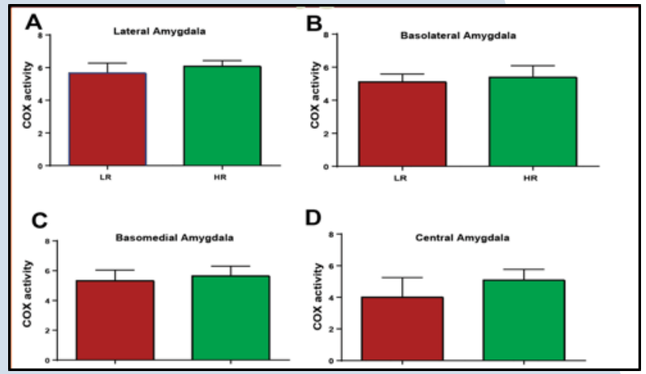

Figure 1: COX activity in the subregions of the amygdala. In all tested regions of the amygdala (lateral (A), basolateral (B), basomedial (C), and central (D)) there was no significant differences found in COX activity between HR and LR rats.

compared for consistency and the average between the two users was taken.

Statistical Comparisons:

All comparisons within each brain region were performed between behavioral phenotype (HR/LR) and utilized unpaired t-tests (p-value < 0.05).

Results

We evaluated the COX activity in two limbic regions: the amygdala (Fig. 1) and the hippocampus (Fig. 2). For even further specificity, we divided these regions into subregions and collected data from each subregion individually.

None of the four tested subregions of the amygdala showed significant difference between phenotypes (Fig. 1; $p > 0.05$). The dentate gyrus showed significantly increased COX activity in both the upper and lower blades in LR rats over HR rats (Fig. 2; $p < 0.05$).

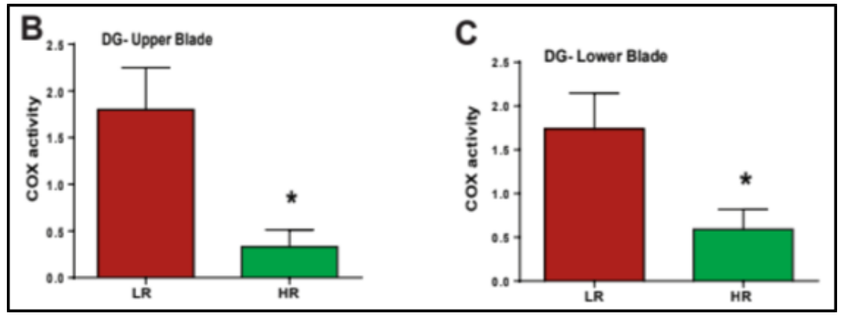

Figure 2: Dentate Gyrus measurements. The (B) upper blade and (C) lower blade of the dentate showed a significant increase in LR rats when compared to HRs ($p<.05$)

Conclusion

When we began our study, we hypothesized that LR rats would display an overall lower level of COX activity when compared to HRs, however, the results from the hippocampal data suggest otherwise. Both regions were selected for testing because of their role in regulating anxious and depressive behaviors. Due to the function of the amygdala in fear and anxiety, it was surprising to see that there was no difference in COX activity between HRs and LRs. This does make sense, as the amygdala aids in the body's ability to cope with of immediate stress situations rather than chronic stress. The dentate gyrus aides in reward feedback, memory, and fear conditioning, therefore possibly contributing more directly to long-term temperamental differences.

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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 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 degradationspecific protein polyubiquitination, it is unknown how consolidation alters other polyubiquitin tags that are not targeted by the proteasome. Furthermore, it is unknown how UPS activity changes within the nuclear, cytoplasmic, and synaptic regions in response to learning.

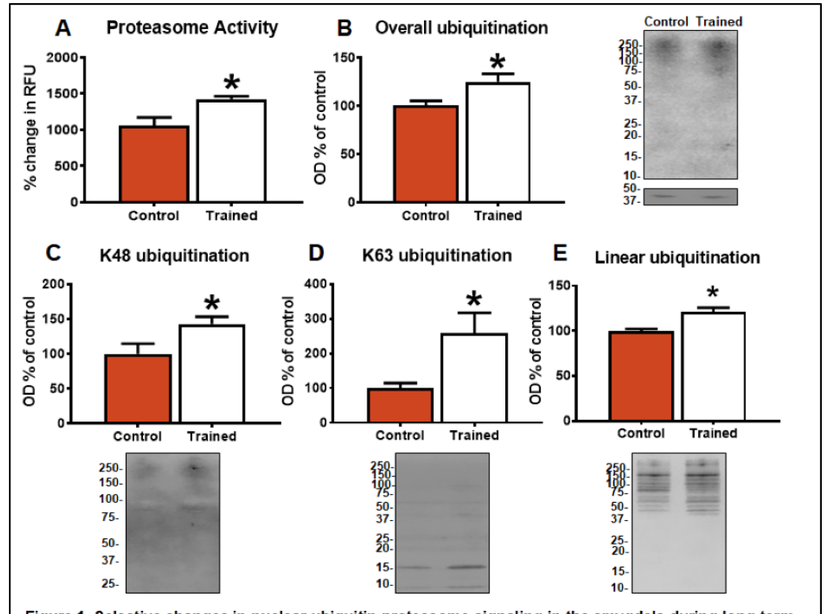

Figure 1. Selective changes in nuclear ubiquitin-proteasome signaling in the amygdala during long-term memory formation. We trained animals to associate a context with a footshock and 1 h later dissected out the amygdala and collected nuclear fractions. (**A-B**) Learning increased proteasome activity (**A**) and overall protein ubiquitination (**B**) in amygdala nuclei. (**C**) Degradation-dependent (K48) ubiquitination increased in nuclear fractions within the amygdala after training. (**D-E**) Degradation-independent K63 (**D**) and linear (**E**) ubiquitination increased after training. * $P < 0.05$ from Control

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. 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 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, M1) 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.

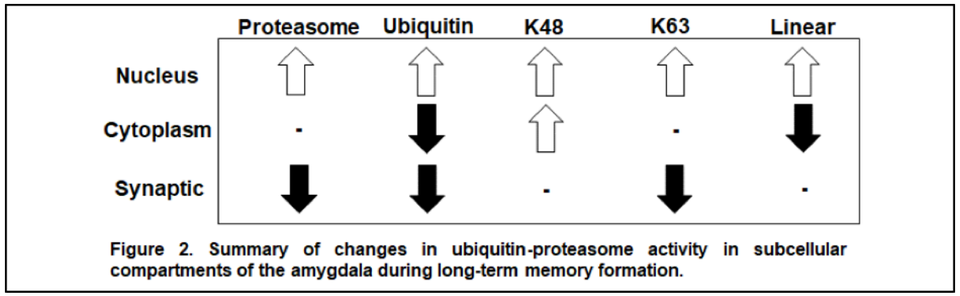

Figure 2. Summary of changes in ubiquitin-proteasome activity in subcellular compartments of the amygdala during long-term memory formation.

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 excreting 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 disorder are largely unknown. One theory proposes that the causal mechanisms of depression and other psychiatric disorders stems from perturbations in the brain-gut axis. Alterations to the microbiome have already been linked to many neurological and psychiatric disorders such as Alzheimer's Disease, Anxiety Disorders, Major Depression, and Parkinson's Disease (Dinan and Cryan 2012). Although gut microbiota cannot leave the gut, their metabolites can cross the protective intestinal epithelial layer and enter the bloodstream. These metabolites result in an accumulation of bacterial antigens that elicit an immune response. This results in an increase of pro-inflammatory cytokines and chemokines that are known to cross the blood brain barrier (BBB) and activate the hypothalamic pituitary adrenal (HPA) axis (Zunszain, Anacker, Cattaneo, Carvalho, & Pariante, 2011). Irritable bowel syndrome is highly comorbid with depression and anxiety; indicating acute changes in microbiota may influence emotional behavior (Rieder, Wisniewski, Alderman, & Campbell, 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. High Novelty Responder (HR) rats show high locomotor activity in a novel environment. On the other hand, Low Novelty Responder (LR) rats display very little activity in a novel environment and overall inhibited behaviors in line with standard rodent anxiety- and depression-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) were 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 the total distance traveled were 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 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 immobility measure has classically been considered an indicator of passive coping and depressive-like behavior.

Cytokine Assay

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 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.

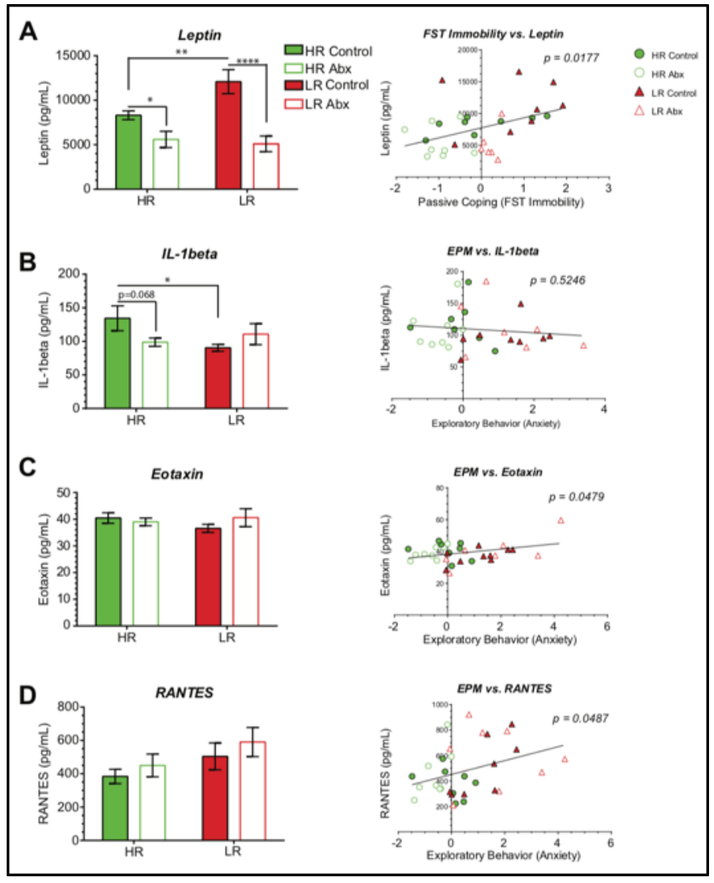

Figure 1: Antibiotic treatment reduced serum leptin in HR/LR, and leptin levels correlated with FST immobility (A). Control HRs had higher levels of circulating IL-1b compared to control LRs; however, IL-1b did not correlate with any behavioral measures (anxiety-like behavior in the EPM shown). (B). 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).

Although there was no significant effect of phenotype, there was a phenotype x treatment interaction on Leptin ($F(1,26)=5.213$, $p=0.0308$). 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 normalize 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 gut microbiota can affect emotional behavior.

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Vascular Amyloid in an Alzheimer Mouse Model

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Alzheimer disease accounts for ~80% of dementia cases worldwide. Traditionally, one of the pathological hallmarks of this disease is Amyloid beta (Aß) plaques. Aß 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 Aß oligomers, or plaques. In addition to plaques, incorrectly cleaved Aß 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 brain barrier exists for the purpose of keeping toxins separate from the 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 DIC imaging.

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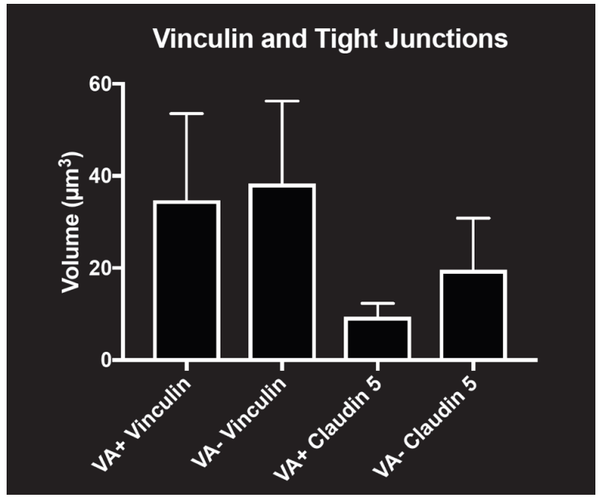

Fig. 1 There was no significant difference between the vinculin volume in vessels with and without vascular amyloid, indicating that antibody penetration is not affected by vascular amyloid buildup. There was also no significant difference in Claudin-5 between vessels with and without vascular amyloid. Therefore, we will be continuing these experiments with additional replicates as our sample size was too low to achieve sufficient statistical power.

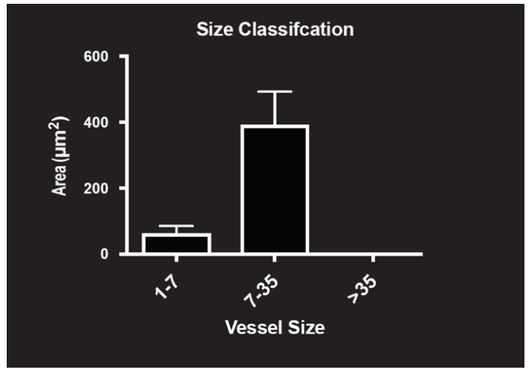

Fig. 2 Most of the amyloid in the brain is found in plaques, not on the vasculature

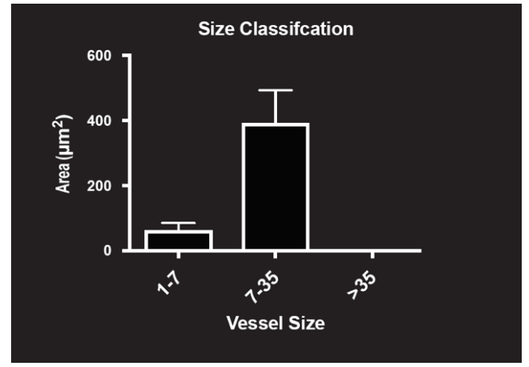

Fig. 3 Amyloid was primarily found on arterioles and venules from 7-35 microns in diameter.

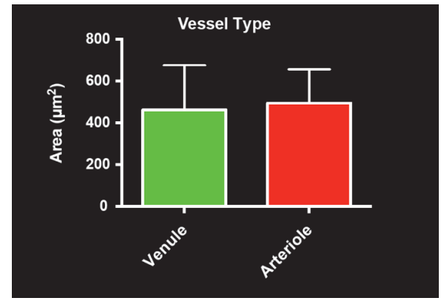

Fig. 4 There was not a significant difference in vascular amyloid accumulation between arterioles and venules.

The Effects of Inhibiting Estrogen Synthesis on Stress Susceptibility in Females

Katherine Vaughn, Jennifer R. Rainville, Georgia E. Hodes

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 adolescent 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 sex differences.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 behavioral tests: splash, novelty suppressed feeding (NSF), and 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.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 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/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 altering 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 behaviors. 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+ 1˚C) for 6 minutes. Each animal's latency to immobilize was measured.

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 ending 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 stress effect (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.4 There was no statistical difference between the control and stress letrozole groups, showing that the stress effect was lost in 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 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.[56]

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. Low levels of estrogen lead to atrophy of the uterus. A sample (n=5) of uteri were collected from each group of mice. There was a significant effect of letrozole. Interestingly, there was a trending stress effect found that stress in the absence of letrozole also decreased uterine weight. While was not significant with an n of 5, it possible that with more statistical power, there might be a stress effect, implying that stress affects estrogen levels.

There have been many studies examining estrogen at different life stages of women. Peaks of depression are recorded during puberty, postpartum, and perimenopause. All of these stages share a rapid change in estrogen levels, suggesting a relationship between changing estrogen levels and depression.7 Both postpartum depression and perimenopause depression can be reversed with the treatment of estrogen in some women. Additionally, there are lower levels of mood disruptions during the parts of menstrual cycle where estrogen is stable.7 Together, these studies support the concept that estrogen fluctuation has a causal role in depression in a subset of women.

Estrogen may also impact male depression. While there are only trace amounts of estrogen in the blood, men have elevated levels of estrogen in the brain. Multiple studies in rodents have evaluated the effects of interrupting the estrogen in the brain. Exposure to stress decreased aromatase activity in the brain of male rats only, suggesting that stress could be affecting estrogen synthesis in the brain leading to a sex difference in the behaivor.8 Recent studies in humans have examined the effects of increased estrogen levels cycling in the blood. Increased 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.

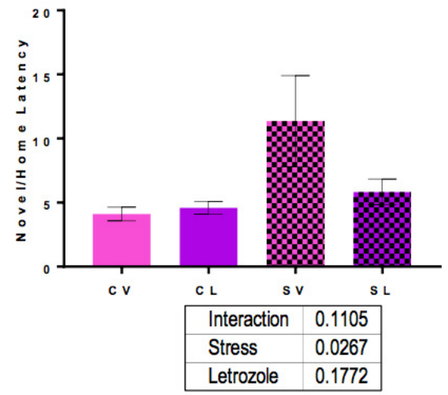

Figure 1

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Thermoreceptors in Drosophila Melanogaster Larvae

Jackson Wibourne, Jordan Tyrrell and Lina Ni

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 their 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 Ionotropic Receptors (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 chemoreceptor, 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 TrpA1ins mutant. Finally, we performed rescue experiment by expressing the wildtype 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 temperatur 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 by counting the number of larvae in each section of the thermogradient, dividing by the total number of larvae on the agar, and multi-plying by100. Larvae along the edge of the agar and off the agar were not included in the percent preference.

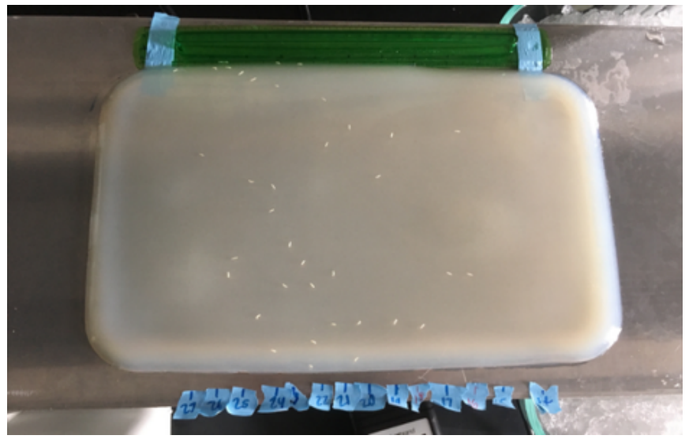

Figure 1 shows the 3% w/v agar used to establish a thermogradient using the hot plate (left side) and tray of ice (right side). It also shows the temperature prefer-ence of the larvae by measuring the temperature of the agar and larval distribution on the agar.

Results:

We observed that there was a dramatic shift in temperature preference during larval de-velopment. The early stage larvae at 48, 72, and 96 hours AEL prefer 24 ˚C, while the late 3rd in-star 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 con-sistent with both 72 and 120 hour AEL larvae (Figure 3 and Figure 4).

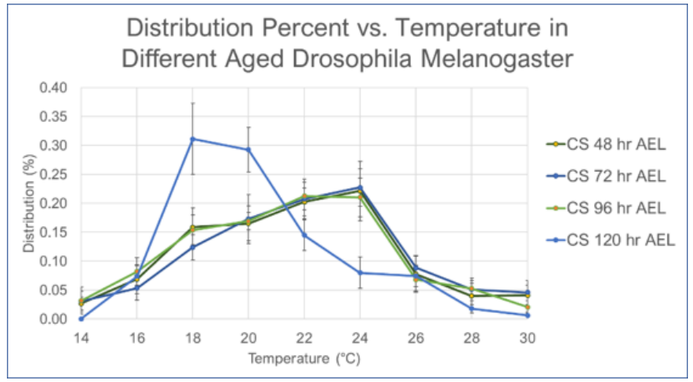

Figure 2 shows the temperature preference of 48, 72, 96, and 120 hour AEL larvae. The early stage larvae at 48, 72, and 96 hours AEL prefer 24 ˚C, while the late 3rd instar larvae at 120 AEL prefer 18 ˚C. CS: Canton S.

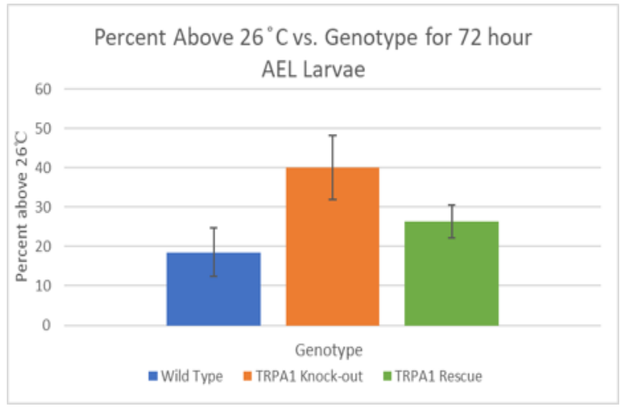

Figure 3 shows the percent preference of wild type, TrpA1 knock-out, and TrpA1 rescue larvae at 72 hour AEL. It shows that the increased distribution of TrpA1 knock-out when compared to a control. Such increasement was rescued by expressing the wildtype TrpA1.

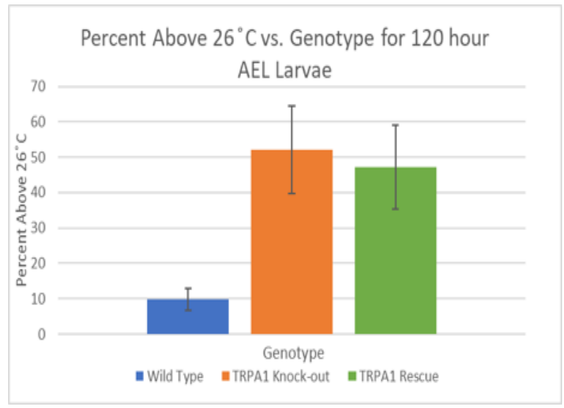

Figure 4 shows the percent preference of wild type, TrpA1 knock-out, and TrpA1 rescue larvae at 120 hour AEL. It shows that the increased distribution of TrpA1 knock-out when compared to a control. Such in-creasement was not rescued by expressing the wildtype TrpA1.

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 suggst that TRPA1 is the warmth receptor which is supported by Figures 3 and Figure 4.

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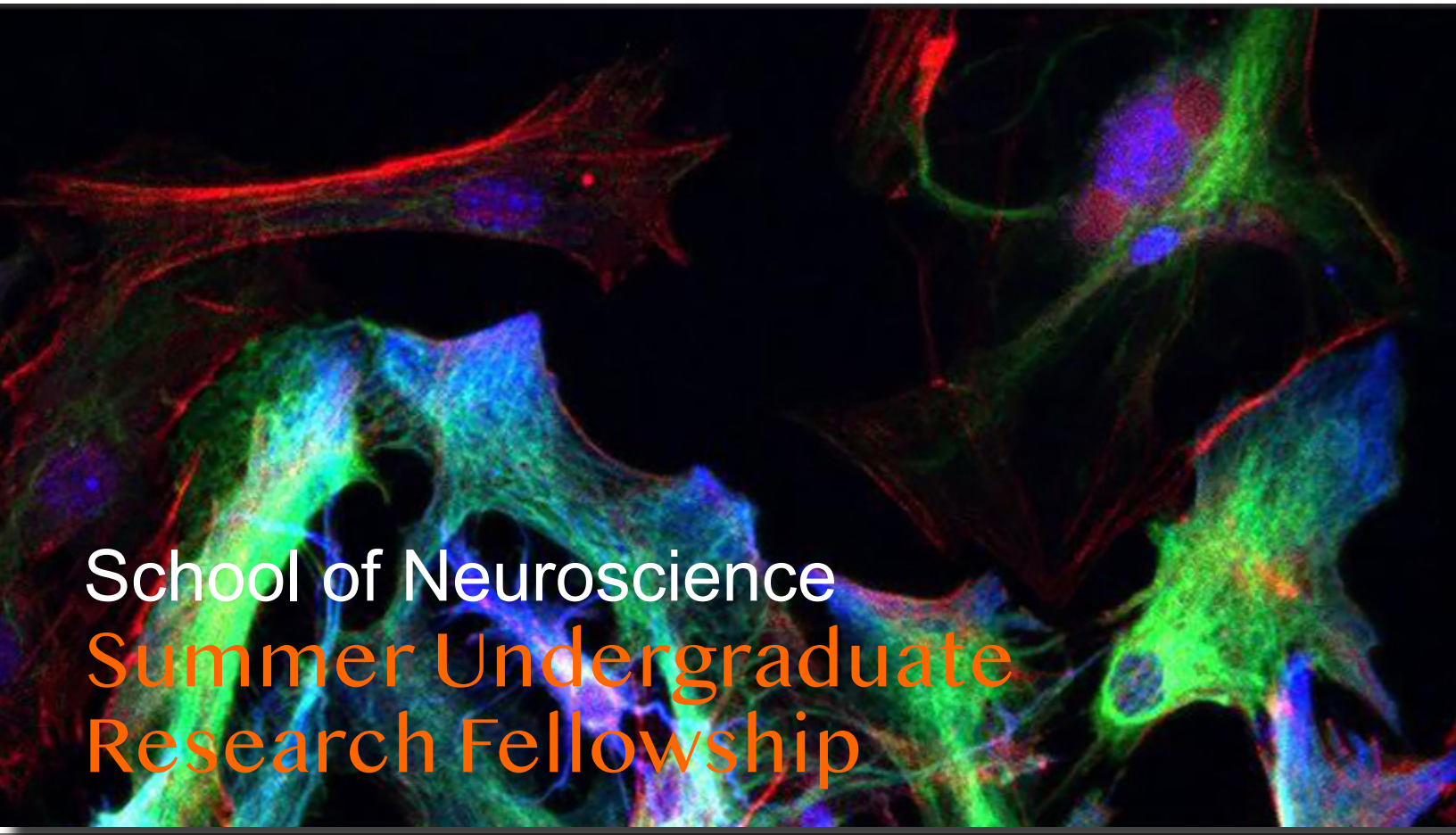

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