Fellowship Recipients

Dallece Curley    EngelNovitt Fellowship Recipient
Mentor: Dr. Stefanie Robel
Investigation of Astrocytic Reduction and Proliferation in Response to Mild TBI/Concussion

Alexa Figueroa Baiges   EngelNovitt Fellowship Recipient
Mentor: Dr. Chris Thompson
The flavor and fragrance additive maltol appears to act as a thyroid hormone agonist

Matthew Hyland   EngelNovitt Fellowship Recipient
Mentor: Dr. Sarah Clinton
The effects of SSRI Exposure on the Domains of Depression-Like Behavior in Rats

Ian Levine    EngelNovitt Fellowship Recipient
Mentor: Dr. Matt Buczynski
Validation of Locomotor Activity as a Model of Nicotine Exposure

Caroline McKenna   EngelNovitt Fellowship Recipient
Mentor: Dr. Andrea Bertke
H3K9me3 and H3K27me3 on the Latent Herpes Simplex Virus 1 ICP27 promoter in Sensory and Autonomic Neurons

Madison O’Donnell   EngelNovitt Fellowship Recipient
Mentor: Dr. Elizabeth Gilbert
Hypothalamic mechanisms of xenin-induced anorexia in Japanese quail

Amanda Patterson   EngelNovitt Fellowship Recipient
Mentor: Dr. Georgia Hodes
Sex and the immune system: Understanding the relationship between stress and cytokines

Abigail Weit    EngelNovitt Fellowship Recipient
Mentor: Dr. Michelle Olsen
Examination of GABA Transporter Expression Across Development

EngelNovitt Summer Research Program 2017

School of Neuroscience Virginia Tech
Investigation of Astrocytic Reduction and Proliferation 
in Response to Mild TBI/Concussion

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In the United States, a traumatic brain injury (TBI) occurs every 5 seconds, with 75% of these injuries being mild or concussive. Mild TBIs/Concussions can cause patients to suffer from long-term memory loss, concentration difficulties, and general cognitive deficits. Due to an absence of apparent lesions using various imaging modalities, the underlying cellular mechanisms of the progression of this disease have remained a mystery. Astrocytes, the most numerous glial cells in the brain, respond to brain injury with astrogliosis. This process aids in sealing off damaged from uninjured brain areas, but can come at the cost of housekeeping properties of astrocytes that ensure proper neuronal function. However, most research thus far has centered around more severe types of TBI with a clear site of injury, the experimental equivalent of gunshot wounds. Little is known, however, about the astrocytic response to mild TBI/Concussion. The specific aims of this study were to investigate possible astrocytic reduction/loss, as well as the proliferation response in sham (control) compared to mTBI (injured) mouse models.

Here, we used a mouse model of repeated mild TBI/Concussion to assess key features of astrogliosis, which includes astrocyte proliferation. Aldh1l1-eGFP//FVB/N mice, which label astrocytes with a transgenic green fluorescent protein, were injured using the Modified Mar-marou weight drop injury model. Tissue was fixed and collected at several time points after injury, and then immunohistochemistry was performed using antibodies against GFP, Ki67, and BrdU. GFP and similar astrocyte markers were used to assess astrocyte reduction/loss, while Ki67 and BrdU stained acutely proliferating cells and cumulatively proliferating cells respectively. BrdU, a DNA analog that binds during replication, was administered twice daily until sacrificed, followed by a pretreatment that denatured DNA in order to allow antibody binding and visualization. Quantification of astrocyte densities did not show areas of complete loss of astrocytes in mTBI mice, however we did find uncharacteristic reduction of GFP-reporter and proteins crucial for normal astrocyte functioning in these areas (p < 0.001, n=3). Astrocyte proliferation acutely stained by Ki67 showed negligible proliferation in mTBI mice (0.031% ki67+/GFP+, n=3) compared to shams, so the cumulative BrdU stain was used as a comparison. BrdU staining clearly indicated higher levels of proliferation in mTBI mice compared to shams, however differences did not reach significance due to variability and low sample size (n=3). The astrocytic reduction indicates that astrocytes respond very differently to repeated mild TBIs/Concussions than has previously been observed in other types of TBI, and that astrocyte dysfunction might contribute to the pathobiology and progression of this disease.
The flavor and fragrance additive maltol appears to act as a thyroid hormone agonist

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Background: Thyroid hormone is essential for normal brain and physical growth in almost all vertebrate animals during development. In Xenopus laevis tadpoles, it is one of the most important components to drive them through metamorphosis. It has been shown, however, that many products concerning the agricultural and consumer industries as well as industrial products may disrupt thyroid hormone signaling. In the Tox21 dataset, a collaborative effort between the NIH, the EPA and the FDA, the organic compound maltol was found to be a thyroid hormone agonist at relatively high concentrations. Maltol is used as a flavor enhancer and as an additive to fragrances because of its natural sweet smell. Everyday foods such as cocoa and coffee contain maltol and it is one of the main ingredients in contributing to the odor of bread.

Methods: The current project assessed the effects of Maltol on the development of the visual system in Xenopus laevis tadpoles, which are greatly sensitive to changes in thyroid hormone signaling and are therefore useful models for better understanding thyroid hormone disruption. Their development occurs externally, allowing for easier observation and manipulation of brain and physical development. The effects of maltol were assessed using several experiments. Effects on body length were analyzed by measuring tadpoles in increasing doses of maltol and compared to T4 on days 0, 2, 4, and 7. Then effects of maltol in combination with T4 on these same days were assessed. In vivo imaging was also performed, in which tadpoles were imaged every 24 hours and differentiation of neural progenitor cells into neurons was quantified for 3 days. Finally, cell proliferation in the optic tectum, telencephalon and pretectum in control, maltol concentrations (100um, 300um, 600um, and 1mM) and T4 groups was quantified and compared. Optic tectum morphology (width: length ratio) was also measured.

Results: Maltol seemed to have more synergistic than agonistic properties in the body length experiment, yet showed very similar results to the T4 group in the in vivo experiment. Brain morphology was also altered, and therefore width:length ratios in the optic tectum were measured in these same groups. Maltol also showed a dose-dependent increase in cell proliferation in the tectum, with the results of the highest concentration being comparable to those of the T4 group. As for brain morphology, the highest concentration of maltol (1mM) showed a significant difference from the control, to levels close to those of the T4 group.

Conclusion: Our studies suggest that maltol agonizes thyroid hormone signaling and may impact thyroid hormone sensitive molecular and cellular mechanisms important for brain development. The body length data suggests that maltol may act synergistically with T4 in order to induce metamorphic-like change. This suggests that maltol might actually act through retinoic acid X receptors, which heterodimerizes with TH receptors and is necessary for the full effect of TH signaling. Future experiments will explore the maltol-retinoic acid connection.
The Effects of SSRI Treatment on Multiple Domains of Depression-Like Behavior in Rats

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Background: In the United States, approximately 15 million adults are affected by a major depressive disorder every year. This disorder is characterized by a number of symptoms, including depressed mood or loss of interest or pleasure in daily activities for more than two weeks. Selective serotonin reuptake inhibitors (SSRIs) have been the mainstay treatment for these symptoms for the past 25 years. SSRIs block the serotonin reuptake transporter of the presynaptic cell, thereby reducing reabsorption and transiently increasing synaptic serotonin levels. SSRIs are considered safe and generally cause fewer side effects than many other antidepressants. Although SSRIs effectively treat many depressed patients, there is still a limited understanding of the etiology of depression as well as limited understanding of the exact mechanisms whereby SSRIs exert their effects. To better understand how SSRIs affect emotional behaviors relevant to a multifaceted disorder like depression, the present project uses a well validated rodent model organism to examine the effects of SSRI treatment on three behavioral domains commonly affected in the illness: anhedonia, self-neglect, and social withdrawal.

The purpose of the present experiments was to assess new tests that may better measure depression-like behavior in rodents. Adult male Sprague-Dawley rats were treated with the SSRI citalopram dissolved in drinking water [10 mg/kg] or normal water (control condition). After two weeks of citalopram (or control) treatment, rats underwent three behavioral tests to evaluate the effects of SSRIs on three behavioral domains commonly associated with depression in humans: 1) anxiety; 2) self-neglect; 3) sexual motivation, and reward-seeking behavior. Behavioral testing was conducted under low lighting (15 lux) from the hours of 09:00-12:00. All trials are recorded using Ethovision computer software (Noldus, Leesburg, VA). The EPM consisted of a platform in the shape of a plus that was raised approximately 1 meter off the ground. Two opposite arms of the maze were open platforms and the other two were enclosed by walls (pictured in Fig. 1A below). 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 splash test is a test in which rats are squirted with a 30% sucrose solution on the middle of their back. The rats are then allowed to roam around a test box for 15 minutes. The time that rats spend grooming themselves is then scored by a trained observer using computerized software (Observer, Noldus, Leesburg, VA). Finally, the female urine sniffing test (FUST) 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 roam for 5 minutes and then the bedding is removed and they are acclimated for 15 minutes. Bedding is then soaked in female urine and placed in the same corner of the box for 5 minutes; the time spent sniffing the different bedding is recorded. Data was analyzed using GraphPad 6 ( Prism, La Jolla, CA). Unpaired t-tests and repeated measures ANOVA were used when appropriate; significance was defined as p<0.05.

Results: We first sought to confirm former findings that SSRI treatment does not have a significant effect on anxiety-like behavior in the EPM. We found that there were no differences between SSRI treated rats (n = 10) and controls (n = 6) in the time spent in the open arms of the maze (Fig. 1B; p > 0.05), or how long it took them to begin exploring the open arms (Fig. 1C; p > 0.05). Due to previous studies in which it has been found that SSRIs caused decreased depression-like behavior (most notably in a test called the Forced Swim Test), we sought to determine whether citalopram treatment would also reduce depression-like behavior in two new tests being developed in our laboratory – the splash test of self-care behavior and FUST to evaluate anhedonic behavior. In the FUST, we found no effect of SSRI treatment on time spent sniffing either type of bedding (Fig. 2C; p > 0.05), nor in the number of times they frequented the bedding (Fig. 2D; p > 0.05). However, we did find a difference in the time animals spent investigating the female urine-soaked bedding versus control/bedding (main effect of the type of bedding, Fig. 2C; p = 0.0004). In the splash test, we found no significant difference between treatment groups in overall locomotor activity (distance they traveled in the test apparatus during the assessment; Fig. 3A; p > 0.05). There were also no group differences in latency to begin grooming (Fig. 3B; p > 0.05) or time spent grooming; although we did see a trend of SSRI-treated rats grooming more than controls (Fig. 3C; p = 0.06).

Conclusion: When we began our study, we hypothesized that SSRI treatment would not affect anxiety-like measures in the EPM, but that rats treated with citalopram would show improved depression-like behavior (such as greater self-care in the splash test and more reward-seeking behavior in the FUST). As expected, we found no group differences in overall locomotion or time spent in the open arms of the elevated plus maze. In the FUST – a test of anhedonia and sexual motivation – there were no effects of SSRI treatment on time sniffing female urine, which was unexpected. We did, however, see a main effect of the type of bedding, with male rats spending more time sniffing female urine soaked bedding compared to clean bedding; this suggests that the main mechanisms of the test are working as expected. Although we did not see significant effects of SSRI treatment, there may be technical aspects of the current paradigm that influenced our results. For instance, some other studies using this test allow experimental rats to acclimate to the test box for 40 minutes instead of just 15 minutes (as we did in our experiment). Other studies using FUST suggest that the female rats providing urine should be in estrous (a time when they are most fertile/sexually receptive), since this could enhance male rats’ sexual motivation and interest. Thus, future experiments will refine our FUST protocol to determine whether these factors affect the overall outcome of the study. In the Splash Test, we did...
not find group differences between the SSRI treated and control rats (i.e., no differences in time spent grooming). This was also unexpected, although our data showed a non-significant trend of control rats grooming less than SSRI-treated rats. It may be useful to refine our procedure for this test. For instance, we may put the sucrose solution on the rats’ upper neck as opposed to the middle of the back; this area moves more as a rat explores and might prompt more grooming. Furthermore, our sample sizes were somewhat small (n=10 per group), so we may have been underpowered to detect significant effects of SSRI treatment. Overall, this experiment helped us to make important first steps for establishing new depression-relevant behavior tests in our laboratory (i.e. the FUST and splash tests) and we will continue to refine our procedures to utilize these tests in future experiments.

Our results so far suggest that in rodents, SSRI treatment may be most effective in changing behavioral despair (as in the Forced Swim Test, which has been demonstrated in our lab and many others in previously published studies) and hedonic behavior (Sucrose Preference Test).
Nicotine addiction is a complex disorder that results from drug-induced changes in brain signaling after addiction-related behaviors including reward, dependence, and relapse. To uncover these drug-related molecular changes, we utilize mouse models of forced nicotine exposure to recapitulate components of addiction. We aim to establish a mouse model of nicotine exposure because of the extensive amount of genetic and pharmacological tools available for this species. Specifically, we utilize the locomotor activity model to model reward behavior. Past studies have reported that assorted doses yield results of higher activity, lower activity, or increased activity over time. To measure the acute effects of nicotine, we measured locomotor activity (distance, m) before and after drug exposure. Each mouse is placed in a plexiglass cage (35 x 22 cm) under red light for 60 minutes before (habituation) and after (drug exposure) nicotine injection. Video of session is digitally captured and analyzed using AnyMaze software to determine distance traveled (m), immobility time (sec), and immobility episodes (n) for each mouse. Nicotine locomotor activity was analyzed at four doses (0.05, 0.2, 0.4, 1.0 mg/kg, s.c.) on the 1st and 5th session of drug exposure. Each mouse was placed in a plexiglass cage (35 x 22 cm) under red light for 60 minutes before (habituation) and after (drug exposure) nicotine injection. Video of session is digitally captured and analyzed using AnyMaze software to determine distance traveled (m), immobility time (sec), and immobility episodes (n) for each mouse. Nicotine locomotor activity was analyzed at four doses (0.05, 0.2, 0.4, 1.0 mg/kg, s.c.) on the 1st and 5th session of drug exposure. Each mouse was placed in a plexiglass cage (35 x 22 cm) under red light for 60 minutes before (habituation) and after (drug exposure) nicotine injection. 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H3K9me3 and H3K27me3 on the Latent Herpes Simplex Virus 1 ICP27 promoter in Sensory and Autonomic Neurons

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Background: Herpes Simplex Virus-1 (HSV-1) is a major human pathogen, causing disease in more than 75% of the US population. During primary infection, HSV-1 establishes latency in sensory and autonomic neurons, from which it can reactivate to cause recurrent skin or ocular lesions throughout life. Although a variety of stimuli are known to reactivate the virus, the specific mechanism of reactivation within the neurons is unknown. We recently showed that cellular stress can remodel viral chromatin during HSV-1 reactivation (Cliffe), but stress hormones induce reactivation selectively in autonomic neurons, rather than sensory neurons (Bertke). Therefore, we hypothesized that different types of neurons maintain the latent HSV-1 genome in different chromatin conformations, allowing reactivation to occur more readily in autonomic neurons in response to stress.

Mouse infections: 6 week old female Swiss Webster mice (Envigo) were infected ocularly with 106 pfu HSV-1 in each eye and maintained for 28 days to allow the virus to establish latency. Sensory trigeminal (TG), sympathetic superior cervical (SCG) and parasympathetic ciliary ganglia (CG) were collected after euthanasia.

Chromatin immunoprecipitations (ChIP): ChIP assays were performed with antibodies against H3 (Abcam ab1791), H3K9me3 (Abcam ab8898), H3K27me3 (Milipore 07-449) and IgG control (Active Motif), followed by qPCR with primers/probe specific for the ICP27 promoter to quantify trimethyl deposition on histone H3 at the ICP27 promoter.

Future Directions

• Additional repetitions to enable statistical analyses of the data
• Additional qPCR studies to quantify H3K9me3 and H3K27me3 on other HSV-1 promoters, such as ICP0, ICP4, and VP16, which are important in the reactivation process
• Additional ChIP assays to identify chromatin marks associated with active open chromatin (H3K4me2/3)
• Comparisons of chromatin composition of HSV-1 and HSV-2 in sensory and autonomic neurons, since HSV-2 reactivates more frequently than HSV-1

Acknowledgments

This project was supported by the Engel Novitt undergraduate research fellowship and the VT-UA Collaborative Neuroscience Seed Grant. Special thanks to Angela Ives and Rebecca Powell-Doherty for their assistance in the lab.

Conclusions: H3K9me3 is associated with constitutively silenced chromatin, the ICP27 promoter lacks H3K9me3 in ciliary ganglia, suggesting that the HSV-1 genome is not as strongly silenced in these neurons. Although H3K9me3 can be remodeled during reactivation, its presence in sensory and sympathetic neurons can be a barrier to reactivation, while its absence in parasympathetic neurons could allow HSV-1 to re-activate more easily in response to reactivation stimuli.

Discussion

• H3K9me3 is associated with constitutively silenced chromatin
• H3K27me3 is associated with facultatively silenced chromatin, which is thought to be more reversible
• The ICP27 promoter lacks H3K9me3 in ciliary ganglia, suggesting that the HSV-1 genome is not as strongly silenced in these neurons
• Although H3K9me3 can be remodeled during reactivation, its presence in sensory and sympathetic neurons can be a barrier to reactivation, while its absence in parasympathetic neurons could allow HSV-1 to re-activate more easily in response to reactivation stimuli

Materials and Methods

Virus: HSV-1 (strain 17+)

Mouse infections: 6 week old female Swiss Webster mice (Envigo) were infected ocularly with 106 pfu HSV-1 in each eye and maintained for 28 days to allow the virus to establish latency. Sensory trigeminal (TG), sympathetic superior cervical (SCG) and parasympathetic ciliary ganglia (CG) were collected after euthanasia.

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In sensory trigeminal ganglia, silenced chromatin marks H3K9me3 and H3K27me3 were detected on the ICP27 promoter of the HSV-1 viral genome during latency.

In sympathetic superior cervical ganglia, H3K9me3 and H3K27me3 were also detected on the ICP27 promoter during latency.

In parasympathetic ciliary ganglia, the presence of H3K9me3 was very low, compared to the presence H3K27me3, at the ICP27 promoter.
Hypothalamic mechanisms of xenin-induced anorexia in Japanese quail

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Background: Xenin is a 25-residue amino acid peptide that was detected in the gastrointestinal tract of humans, Rhesus monkeys and dogs. Xenin has been isolated from the hypothalamus near satiety centers. It is best known as a factor that controls appetite. More specifically it has been demonstrated to act as an anorexigenic factor, meaning that it decreases food intake. When xenin was centrally injected into rats, food intake was decreased. The lab in which the research was conducted previously studied the effect of xenin in broiler chickens. It was found that when xenin was injected into the Ventromedial hypothalamus, food intake was decreased and the Quantity amount of c-Fos immuno-reactive cells was increased, indicating activity within the nuclei. It was this finding that led the lab to study the effects of xenin within a different avian species, Japanese quail were studied due to their more wild-type behavior. The Coh 500 broiler chickens that were previously studied have been selected to develop rapid muscle growth, not representing an accurate depiction of appetite. Therefore, quail were studied in order to test xenin in a more natural host. Since it was known that xenin decreases food intake, we sought to elucidate the molecular mechanisms by which xenin mediates its anorectic effect on appetite.

Methods and Results: In Experiment 1, 7 day-old quail were centrally (intracerebroventricularly; into the left lateral ventricle) injected with 0 (vehicle; artificial cerebrospinal fluid), 0.25, 0.5 or 1.0 nmol xenin and food and water intake were measured for 180 minutes. On a cumulative basis, quail that received 0.5 nmol xenin reduced food intake for 120 minutes. Water intake was not affected in any experiments. Experiment 2 measured the behavior of the quail to ensure that the neuropeptide did not have any competitive behaviors adverse side effects. Results yielded that exploratory pecks increased, as well as jumps. This data suggest that in fact the neuropeptide did not cause distress to the birds. In Experiment 3, the whole hypothalamus was isolated from xenin injected chicks at 1 hour post-injection. Next, total RNA isolation was performed and real time PCR performed to measure mRNA abundance of several appetite-associated factors. Quail injected with xenin expressed an increase in the opioid receptor delta and neuropeptide Y (NPY) mRNA expression level. Experiment 4 examined appetite-associated nuclei within the hypothalamus, these being the Ventromedial hypothalamus (VMH), Lateral hypothalamus (LH), Paraventricular nucleus (PVN), and Arcuate nucleus (ARC). Upon performing c-Fos immunohistochemistry, results displayed activation within the ARC, PVN, and VMH. This was quantified by the number of c-Fos immuno-reactive cells counted within the nuclei.

Conclusions: Central injection of xenin has been known to decrease food intake in rodent and avian species. However, until the current study, the molecular mechanisms by which xenin mediates its effects were poorly understood. Upon completing this study, a proposed pathway was identified due to the increased expression of CRF2 and UTS 2. The potent anorectic factor UTS 2 binds to CRF2, indicating that this interaction within the PVN may be the central mechanism exerting xenin’s anorexigenic effect within the quail model.
Men and women display different symptomologies during depression (Martin, Neighbors, & Griffith, 2013), but in pre-clinical trials attempting to find biomarkers or new treatments, females continue to be neglected (Prendergast, Onishi, & Zucker, 2014). Depression-like behavior of mice is quantifiable and can be used to distinguish differences in the stress response between sexes (Hodes et al., 2015; Laplant et al., 2010). This summer my project, under Dr. Georgia Hodes’ leadership, was focused on correlating stress susceptibility in mice with their individual cytokine profiles. Here we focus on the underlying peripheral biological influences to these behavioral differences, specifically cytokine types and quantities. Cytokines are signaling proteins secreted by cells of the immune system that allow communication with a number of different types of cells throughout the brain and body. The presence of cytokines can indicate with a number of different types of cells through a variety of behaviors. The presence of cytokines can indicate with a number of different types of cells through a variety of behaviors.

Introduction

Cytokines: Significant interactions between stress and sex

Stress Susceptibility vs. GM-CSF

Stress Susceptibility vs. IL-12(p40)

Stress Susceptibility vs. IL-10

Cytokines: Significant interactions between sex and stress

Cytokines: Main effect of sex

Sex and the immune system: Understanding the relationship between stress and cytokines

Figures

References

Stress and Behavior Timeline

References

Sex and the immune system: Understanding the relationship between stress and cytokines

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undergoing testing was then placed in the corner of the arena in a consistent manner. Behavior was measured in real time by an observer. A stopwatch was used to record the latency until the mouse initiated its first bite of food. A trial was ended at 10 minutes and the latency to eat was recorded as 600 seconds when animals did not eat within the allocated time. Following testing in the novel space animals were tested for latency to eat in the home cage. A new piece of kibble was placed in the center of the home cage. The trial ended when the animal took a bite of food or 10 minutes expired. Both latency times were recorded and analyzed. The final behavioral test was the force swim latency test. The stressed mice were placed in individual 4000 mL beakers filled to the 3000 mL mark with room temperature water (25°C ± 2°C) with coded identification cards attached to track their identities. The mice were placed in the beakers of water with body submerged. Each mouse’s test lasted for 6 minutes during which they were digitally recorded for subsequent analysis by an investigator blind to their conditions. A stopwatch was used to record latency till the mouse first bout of immobility. The stopwatch was also used to record the cumulative time the mouse spent immobile. Each behavioral response per individual was used to compile an overall stress susceptible score, indicating how stress susceptible the individual mouse was based on their times from the three behavioral tests. These individual stress susceptibility scores were correlated with cytokine levels. Susceptibility scores were correlated with cytokine levels.

The behavioral results are as followed. In the splash test, there was a significant main effect of stress and sex, overall measuring the stressed females grooming the least compared to all other groups. The less time spent grooming, the more indicative that the group is experiencing depression-like symptomology by lacking in hygienic care. In the novelty suppressed feeding test there was a significant main effect of stress and sex, stressed females indicated by the pink triangles. Focusing on the stress female group, there seems to be an anti-inflammatory response occurring to stress similar to the immune response to a parasitic worm or an allergy. Further research is needed to determine whether this is due to stress susceptibility or a sex differences in response to stress.

Conclusion: this experiment shows that there are both behavioral differences and immune response differences based on cytokine concentrations. It also supports that stressed females may be perceiving stress as an allergy and is responding as such. To understand if the stress like response is a result of a sex difference or a state of stress susceptibility versus a state of stress resilience, the next step of this project is to extend the term of the subchronic variable stress paradigm to a long-term test. Past research has shown with 28 days of this same SCVS model, the stressed males are no longer stress resilient. If we eliminate stress resiliency as a factor is this test, then we can determine if the overall stress response type is due to a sex difference or a stress susceptibility difference.

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**Examination of GABA Transporter Expression Across Development**

Abbie Weit, Helen Vanderpool, Leanne Holt, Michelle Olsen

1School of Neuroscience, Virginia Polytechnic and State University, Blacksburg, VA
2EngelNovitt Undergraduate Research Fellowship Program

Background: Astrocytes are one of the most abundant cell types in the brain. These glial cells envelop over 90% of glutamatergic synapses and decades of research indicate glutamate uptake via astrocytic glutamate transporters is critical for the termination of glutamatergic synaptic transmission. In contrast, little is known regarding a role for astrocytes at GABAergic synapses. Preliminary data we have generated in the laboratory by RNA sequencing revealed GABA transporters (GAT) are found in abundance in mature cortical astrocytes, and indeed appear enriched in astrocytes relative to other CNS cell populations. These transporters play an important role in the removal of GABA from extracellular space to prevent excess neuronal inhibition. The function and spatial distribution of GAT1 and GAT3 has been previously examined; however, their developmental expression has not been determined. This project examined RNA and protein expression of GAT1 and GAT3 across cortical development in both males and females. Our preliminary studies reveal interesting developmental and sex-specific trends in cortical GAT expression which may contribute to early cortical development and maturation. These studies lay the groundwork for future experiments in the Olsen lab which will examine GAT expression in isolated astrocyte and neuron populations.

Methods: In the current study we utilized quantitative PCR and Western blot to examine gene and protein expression levels of GAT1 and GAT3 in the cortex. We evaluated gene and protein expression at multiple time points during early postnatal development.

Results: Our results indicate that mRNA for the GAT1 transporters reaches a peak at postnatal day 7 in male and female mice and remains stable throughout development. Whereas GAT3 expression peaked at postnatal day seven and then decreased to approximately 30% of its peak, where it remained.

Conclusions: Our preliminary studies reveal interesting developmental and sex-specific trends in cortical GAT expression which development and maturation. These studies lay the Olsen lab which will examine GAT expression in isolated astrocyte and neuron populations.
FOXP2 is a member of a large transcription factor family and is one of the 5% most-conserved proteins with only two amino acids separating humans from chimpanzees. An identified mutation of FOXP2, (R553H), has been found in patients that is consistent with speech and cognitive abnormalities. The forkhead domain of the FOXP protein is important for nuclear localization and DNA binding. A novel truncated splice variant of FOXP2, first reported by Bruce and Margolis (2002; Hum Genet 2002), prevents the nuclear localization of FOXP2 because it lacks the C-terminal region coding for the forkhead domain. This truncated variant causes the FOXP2 protein to aggregate in the cytoplasm similar to the R553H mutation.

We have found an analogous truncated variant in the rat using endogenous qRT-PCR on neonatal brains. AAV-mediated truncated FOXP2 injections were administered to 28 neonatal pups and vocalisation recordings were compared for communication differences between sexes and injected versus control animals. Alternations in a downstream signaling target of FOXP2, CNTNAP2, were confirmed with Western Blot analysis in fore brain regions, cortex, hippocampus, striatum, and cerebellum. Our study provides sufficient evidence that truncated FOXP2 results in altered vocal communication. This study aims at providing a model of the coexpression of upregulated truncated FOXP2 expression in the developing autistic brain.

Introduction

FOXP2 is a member of a large transcription factor family and is one of the 5% most-conserved proteins with only two amino acids separating humans from chimpanzees. An identified mutation of FOXP2, (R553H), has been found in patients that is consistent with speech and cognitive abnormalities. The forkhead domain of the FOXP protein is important for nuclear localization and DNA binding. A novel truncated splice variant of FOXP2, first reported by Bruce and Margolis (2002; Hum Genet 2002), prevents the nuclear localization of FOXP2 because it lacks the C-terminal region coding for the forkhead domain. This truncated variant causes the FOXP2 protein to aggregate in the cytoplasm similar to the R553H mutation.

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AAV-Mediated Truncated FOXP2 Splice Variant Injections in Developing Rat Brain Alters Vocalization Complexity and Social Interactions

By: Dawn Wright, Makenzlie Taylor, Tina Taylor, Clint Roby, Kareem Omeish, J. Michael Bowers
School of Neuroscience, Virginia Polytechnic Institute & State University, Blacksburg, VA

FOXP2 is a member of a large transcription factor family and is one of the 5% most-conserved proteins with only two amino acids separating humans from chimpanzees. An identified mutation of FOXP2, (R553H), has been found in patients that is consistent with speech and cognitive abnormalities. The forkhead domain of the FOXP protein is important for nuclear localization and DNA binding. A novel truncated splice variant of FOXP2, first reported by Bruce and Margolis (2002; Hum Genet 2002), prevents the nuclear localization of FOXP2 because it lacks the C-terminal region coding for the forkhead domain. This truncated variant causes the FOXP2 protein to aggregate in the cytoplasm similar to the R553H mutation.

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Methods

PCR amplification, cloning, and sequencing of the FOXP2 gene and truncated splice variant created plasmids used for cortical injections. Western blot imaging techniques enabled quantification of signaling partners in virus-injected and control groups. Blots were later analyzed for sex-differences within experimental groups.

Conclusions

Future studies will include quantification of FOXP2 signaling partners with Western Blot analysis and investigation of sex differences in FOXP2 expression levels after treatment with virus. Additionally, we will be investigating the molecular signaling patterns that contribute to lingual development in neonatal brains that contribute to cortical maturation. We would like to thank the EngelNovit Undergraduate Research Fellowship for providing me with the opportunity to immerse myself in such a gratifying research project. I would also like to express my gratitude to Dr. Mike Bowers and the rest of the Bowers’ Lab for their contributions and support.
Background

Gliovascular Unit

> Plays an essential role in sustaining the brain’s physiological activity
> Glia arise from these cells and make up a majority of malignant brain tumors
> Gliovascular system assures necessary blood supply to the active brain regions

3D Bioprinting

> Allows for cell function and viability to be preserved within a precise construct
> Reproducibility of the neural model is a unique benefit with this approach

Vascularization of Models

> Huge 3D printing challenge
> Necessary for long-term cell culture of thick tissues
> Helps to understand mass transport and reproducing pathophysiological trajectories associated with the blood-brain barrier

Research Questions

(i) Can we leverage 3D printing to create a model of the gliovascular unit?
(ii) How can we apply 3D printing to understand the migration of glioma cells due to biochemical cues?

Glia Migration Chamber

The migration chamber is a 2D model of the gliovascular unit. First, a CAD model of the channel was designed and then translated into code to be printed with SI595 RTV Silicone Clear using a 27 GA tapered tip. The printed chambers were placed aside to cure over a short time period.

Results/Conclusions: We were able to successfully print gliovascular models with an open channel to be used for fluidic studies. The silicone migration chambers were successfully created and optimized for use in time-lapse migration studies. Future directions for this study include integration of the 3D printed gliovascular unit with fluid handling components and application of migration chambers in different biochemical settings to understand the effects of cue concentration magnitude and profile on migration rates.
Anorexigenic mechanisms of substance P in alternative vertebrate models
Christopher Buenaventura, Elizabeth R. Gilbert and Mark A. Cline
Neuroendocrinology Laboratory, Virginia Polytechnic Institute and State University

Background: Substance P (SP) is a member of the neurokinin (NK) family, a group of neuropeptides that are distributed throughout the peripheral and central nervous systems. Substance P is involved in multiple physiological processes, including emesis, pain perception and transmission, and food intake, effects of which are mediated via NK receptors. Central administration of SP elicited anorexigenic effects in rats and our group recently demonstrated a similar effect in domestic broiler chicks (Gallus gallus). The central mechanisms underlying the anorexigenic effects of SP are still unclear and elucidating such pathways may provide information that could facilitate a novel strategy to treat eating disorders. The objective of this research was to evaluate the anorexigenic effect of SP in Japanese quail (Coturnix japonica), an avian species that has not undergone intense artificial selection for growth-related traits but adapts well to a cage environment, and thus provides evolutionary perspective.

Methods and Results: In Experiment 1, 7 day-old quail were centrally (intracerebroventricularly; into the left lateral ventricle) injected with 0 (vehicle; artificial cerebrospinal fluid), 0.25, 0.5 or 1.0 nmol SP and food and water intake were quantified for 180 minutes. On a cumulative basis, quail that received 0.5 and 1.0 nmol SP reduced food intake during the entire observation period (50% reduction compared to controls at 30 minutes post-injection). Water intake was not affected. In Experiment 2, whole hypothalamus was isolated from SP-injected chicks at 1 hour post-injection, total RNA isolated, and real time PCR performed to measure mRNA abundance of several appetite-associated factors. Quail injected with SP expressed less agouti-related peptide (AgRP) mRNA than vehicle-injected chicks. In Experiment 3, a comprehensive behavior analysis was performed. Quail that were injected with SP displayed fewer feeding pecks and reduced locomotion compared to vehicle-treated birds during the first 30 minutes post-injection. In Experiment 4, c-Fos immunoreactivity (an indicator of neuronal activity) was quantified in appetite-associated hypothalamic nuclei. There were fewer c-Fos immunoreactive cells in the lateral hypothalamus (LH) of SP-injected chicks than vehicle-injected chicks at 1 hour post-injection. In Experiment 5, the LH was collected for total RNA isolation and gene expression analysis via real time PCR. Quail injected with SP expressed less agouti-related peptide (AgRP) mRNA than vehicle-injected birds in the LH at 1 hour post-injection.

Conclusions: Central injection of SP was associated with decreased food intake in Japanese quail, similar to rodents and chickens. Molecular mechanisms appear to differ between the two avian models. In chickens, we found that SP injection was associated with increased c-Fos immunoreactivity and urotensin 2 mRNA in the paraventricular nucleus of the hypothalamus, whereas in quail there was a reduction in c-Fos and AgRP mRNA in the LH. While AgRP is expressed in several hypothalamic nuclei, especially the arcuate nucleus, the whole hypothalamus and LH gene expression results suggest that the decrease in AgRP in SP-injected chicks is of LH origin. Future research will focus on understanding the role of the LH and AgRP in SP-mediated feeding behavior via microinjection and receptor blockade studies.

Works Cited

36 Month EEG Predicts Reading Achievement at Age Six through Executive Function

**Katherine Vlahcevic, Alleyne Broomell, and Martha Ann Bell**
Virginia Polytechnic Institute & State University, Blacksburg VA

**Introduction**

Electroencephalogram (EEG) is the measure of electrical activity in the brain. (Birn, 2016). EEG is used to measure cortical maturation and is associated with an increase in executive function skills (Almas, 2012). Executive function (EF) is a higher cognitive processes that controls goal-directed behaviors. EF skills include working memory, inhibitory control, cognitive flexibility and more. EF is proving to be developmental in children, serving as predictors and contributors to children’s word and non-word reading skills (Cartwright, 2012).

At 3 years old, EF tasks improve from understanding basic sight words to major phonological and comprehension development. Children at 6 years old begin to identify words by sounding them out and even begin to read on their own (Carnine, 2014).

Developing basic reading skills is essential because they provide basic building blocks of education and contribute to the future success in school. (Dickinson & Porche, 2011). Developing basic reading skills is essential because they provide basic building blocks of education and contribute to the future success in school. (Dickinson & Porche, 2011). We hypothesized that EEG at 3 years old would predict 6-year-old executive function which would then predict 6 year old reading achievement in a typically developing sample.

**Methods**

At 36 months the baseline EEG was recorded with a 16 channel cap while the child watched a one-minute clip of turtles swimming from Finding Nemo. At 6 years old the children completed the reading achievement tests and the EF tasks. To test for reading achievement the children completed Woodcock-Johnson Passage Comprehension and Woodcock-Johnson Reading Fluency. These are standardized measures of academic achievement. For the EF battery three tasks were completed. The first task was the Dimension Change Card Sort (DCCS), a card sorting task that measures cognitive flexibility. Children sorted a set of cards by either color or shape then were instructed to sort by one dimension if the card had a border and the other dimension if the card did not have a border. Proportion correct on the borders condition was the variable of interest. The Number Stroop is an age appropriate measure of inhibitory control. The child was given a set of numbers, 44 and asked to number the number of digits on the screen, not the numbers themselves. Response time or how long it took them to answer was the variable of interest. The Backwards Digit Span task asked children to repeat a string of numbers backwards as a measure of working memory, with the number of digits increasing until the child could no longer perform the task. The highest correct span was the variable of interest.

**Results**

EF was measured through three tasks, cognitive flexibility, inhibitory control and working memory, all of which loaded onto a single EF factor, $p < .001$. The tests for reading achievement were Woodcock-Johnson Passage Comprehension Test and Woodcock-Johnson Reading Fluency, which loaded onto a reading achievement factor, $p < .001$. Our structural equation models show that EEG at 36 months predicts executive function at 6 years, $p < .001$. Subsequently, concurrent EF predicts reading achievement at 6 years, $p < .001$. The EF statistics for our model are mixed, with a chi-square value of 13.976, $df = 8$, $p = .043$, suggesting the model is not a good fit for the data and we should re-evaluate the model. The significant chi-square value may be due to the large sample size and taken in conjunction with the other goodness-of-fit statistics does not warrant a rejection of the model.

**Discussion**

If children who are at risk for reading problems receive EF interventions, then this may increase their school readiness. EF skills start to develop at 3 years old (Cartwright, 2012). These findings could help children from a young age who are at risk for developing issues with reading obtain professional guidance. If children who are at risk for reading problems receive EF interventions, this may increase their school readiness.

**References**


**36 Month EEG Predicts Reading Achievement at Age Six through Executive Function**

Katherine Vlahcevic, Alleyne Broomell, and Martha Ann Bell
Virginia Tech

**Results cont.**

**Discussion**

Our findings show that EEG, a measure of cortical maturity, at 36 months is able to predict EF at 6 years old, which in turn predicts reading achievement. Major executive function skills start to develop at 3 years old (Cartwright, 2012). These findings could help children from a young age who are at risk for developing issues with reading obtain professional guidance. If children who are at risk for reading problems receive EF interventions, then this may increase their school readiness.

Future directions could include investigating an indirect effect between EEG at 36 months old and reading achievement at 6 years old. Additionally, future work could be to see if cortical maturity at even younger ages predicts EF and reading with the same pattern demonstrated here.

The fit statistics for our model are mixed, with a chi-square value of 13.976, $df = 8$, $p = .043$, suggesting the model is not a good fit for the data and we should reject the null. However, the Root Mean Square Error of Approximation (RMSEA) was .057, suggesting a statistically acceptable fit and the Comparative Fit Index (CFI) value for the model was .973, which indicates the model to be a good fit for the data. The significant chi-square value may be due to the large sample size and taken in conjunction with the other goodness-of-fit statistics does not warrant a rejection of the model.

**References**

**EF Battery**

- Woodcock-Johnson Passage Comprehension Test and Woodcock-Johnson Reading Fluency
- EF Battery
- Dimension Change Card Sort (DCCS)
- Card sorting task that measures cognitive flexibility.
- Number Stroop
- An age appropriate measure of inhibitory control.
- Backwards Digit Span
- Asked children to repeat a string of numbers backwards as a measure of working memory. The highest correct span was the variable of interest.

**Model 1.** Executive Function Model

<table>
<thead>
<tr>
<th>EF 3Yrs</th>
<th>EF 6Yrs</th>
<th>Reading</th>
<th>Passage Comprehension</th>
</tr>
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<tbody>
<tr>
<td>$\beta$</td>
<td>$B$</td>
<td>SE</td>
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<td>1.000</td>
<td>.051</td>
</tr>
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</table>

Table 1: Table of Beta and $R^2$ values

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Extracellular Matrix Degradation Causes Reactive Astrogliosis

Kaliroi Engel, Bhanu P. Tewari, and Harald Sontheimer
Virginia Tech School of Neuroscience, Virginia Tech Carilion Research Institute
Undergraduate Research Fellowship Recipient, School of Neuroscience at Virginia Tech

Background: Extracellular matrix has been known to influence the neuronal activity and synaptic plasticity. In different regions of the brain, extracellular matrix forms lattice-like structures known as perineuronal nets (PNNs). The PNNs are specialized assemblies of chondroitin sulfate proteoglycans (CSPGs), tenascins-R, hyaluronan and link proteins that surround fast spiking parvalbumin interneurons in the cerebral cortex. PNNs ensheathe cell soma, axon and proximal dendrites, and lattice holes are the only access points for the interaction with astrocytes. Due to this space constrain, astrocytes may have a unique physiological relationship with the PNN surround ed neurons. The spatial proximity of astrocytic processes and neurons is critical for the smooth ongoing of astrocytic housekeeping functions such as extracellular K+ buffering and glutamate clearance. We hypothesized that to balance this space constrain astrocytic processes in contact with the PNN holes might possess specialized activity domains. These domains might have differential expression of astrocytic proteins involved in neuron glia information exchange and housekeeping functions.

Methods: Adult Bl6 (Black 6) mice were intracranially injected with Chondroitinase ABC (ChABC) enzyme, which degrades the chondroitin sulfate chains in PNNs and interstitial matrix. Animals were transcardially perfused with paraformaldehyde for whole body fixation and brains were dissected out. Brains were sectioned with a vibratome to obtain 50 micrometer thin sections and stored in phosphate buffered saline for storage until usage. Sections were immunostained with primary antibody for PNN marker WFA (wisteria Floribunda Agglutinin), 2B6 (an antigen stub which is exposed after CSPG degradation), and GFAP (glial fibrillary acidic protein) to stain the astrocytes. Appropriate fluorophore tagged secondary antibodies were used to detect the antigen bound primary antibodies. On staining completion section were mounted on slides and images were taken with the Nikon A1 confocal microscope.

Results: First we confirmed the PNN/CSPG degradation by WFA staining. We observed that ChABC injection causes widespread ECM/PNN degradation exhibited as negligible WFA staining and disintegrated PNNs. Contrary to the injected hemisphere, the contralateral half as well as the PBS injected brain exhibited normal WFA staining and intact PNNs. Secondly, the 2B6 staining in the injected area confirmed ECM degradation by ChABC. Chondroitin sulfate cleavage by ChABC exposes 2B6 antigen which can be stained with Anti-2B6 Ig. The combined staining of 2B6 and WFA further proved PNN degradation due to ChABC, showing an inverse relationship between the 2B6 and WFA stains. Most importantly, we observed a global reactive astrogliosis in the ChABC injected hemisphere as evident by increased GFAP positive cells. Spatially, the ChABC injection causes reactive astrogliosis in almost the complete hemisphere compared to the contralateral half. In the sham animal we observed that reactive astrogliosis, which was confined to the incision site. This suggests that reactive astrogliosis in ChABC injected animals was a consequence ECM degradation rather than the incision.

Conclusion: In conclusion, we observed that ChABC injection in mice brain can lead to widespread PNN disintegration and reactive astrogliosis, which may have pathological consequences.

Acknowledgements: Lata Chauhan, Dipan Patel, Ian Kimbrough and Paul Youmans

Extracellular Matrix Degradation Causes Reactive Astrogliosis

Kaliroi Engel *1, Bhanu P. Tewari PhD 2, and Harald Sontheimer PhD 1, 2
1. Virginia Tech School of Neuroscience, 2. Virginia Tech Carilion Research Institute
Undergraduate Research Fellowship Recipient, School of Neuroscience at Virginia Tech
Dysfunctions of the Gliovascular Unit in Alzheimer Disease

Emily Barritt, Ian F. Kimbrough, William A. Mills III, Lata Chaunsali, Chris Liao, Harald Sontheimer
Virginia Polytechnic Institute, Virginia Tech School of Neuroscience, VT Carillion Research Center, Center for Glial Biology

The gliovascular unit (GVU), consisting of astrocytes and its processes, associated neurons, and microvessels blood vessels functions to maintain homeostasis. In this unit, astrocytes function to regulate local blood flow. In addition to this role, astrocytes also serve to maintain tight junction proteins, which make up the blood brain barrier (BBB)—the brain’s main line of defense against blood borne toxins. These roles are carried out by the release of vasoactive factors, which signal to the associate blood vessels. In a recent publication, our lab showed that an invading glioma tumor cell has the ability to invade the intimate space along the vasculature and displace the astrocytic end foot. The end foot, displaced by the tumor cell, was no longer able to signal the vasculature. Subsequently, breeches of the blood brain barrier, the brain’s main line of defense, occurred. Associated down-regulations of tight junction proteins occurred as well.

Alzheimer’s disease shows pathology that mirrors that of glioma. The larger amyloid beta aggregates, referred to as vascular amyloid, deposit along the vasculature in a similar location as the tumor cells. This similar placement lead to questions about how the GVU is affected in Alzheimer’s disease (AD).

Using the hAPPJ20 mice model of AD which express the mutated human APP gene in neurons we employed a combination of ex vivo and in vivo imaging approaches to assess GVU function in control and hAPPJ20 mice. We showed that amyloid deposits associated with the endothelial vessel wall separated astrocyte end feet from blood vessels. Multi-photon in vivo imaging through a cranial window showed that, as in the glioma model, large breeches of the BBB as well as associated down regulation of tight junction proteins occurred in areas with heavy vascular amyloid burden. Using a label free optical activation technique, we were able to show that when vascular Us express the mutated human APP gene in neurons we employed a combination of ex vivo and in vivo imaging approaches to assess GVU function in control and hAPPJ20 mice. We showed that amyloid deposits associated with the endothelial vessel wall separated astrocyte end feet from blood amyloid was present, stimulation of astrocytes or vascular smooth muscle cells produced only poor vascular responses. Strikingly, vessel parts that were unaffected in hAPPJ20 mice did respond to the same extent as vessels from age-matched control animals. This led us to conclude that while astrocytes can still release vasoactive substances, amyloid deposits render blood vessels rigid and reduce the dynamic range of affected vessel areas. One interpretation of these results, is the aggregation of vascular amyloid is physically separating the endfeet from the vessel wall. Alternatively, another mechanism may exist that precedes plaque deposition that could in part account for the endfoot displacement and the reduction in cerebral blood flow in AD patients.

Current studies aim to further tease out the potential mechanism by which amyloid beta is effecting the GVU; specifically looking at the role of the soluble oligomeric form of amyloid beta. Our lab is employing several new tools and techniques to expand our abilities to investigate and gather quantifiable data to answer several of the remaining questions about the pathology that leads to Alzheimer’s disease.

We are continuing long term imaging studies of hAPPJ20+ and age matched control animals to gather more data to further understand the changes to the vasculature and the BBB with the progression of the Alzheimer’s disease model. In addition to the BBB long-term studies, we have begun several experiments to test the effects of soluble amyloid beta on astrocytes.
The data shows a significant dose dependent change in the area of the tectum; the lowest concentrations of lead showed an increase in area in the developing tadpole brain. At even the lowest concentration of lead (10 ppb), there was an increase in cell death and swelling of the brain. This is alarming because this concentration is below the EPA safety standard of 15 ppb in drinking water in the U.S., and may be cause to reexamine the standards for safe drinking water in the United States.

Conclusions: Increased lead concentration was associated with higher quantity and density of cell-death markers H2AX and Caspase-3. Lead also induced changes in tectum area. Lower lead concentrations increased area relative to control. There was also a significant difference in cells positively marked for both H2AX and caspase-3 even at the lowest concentration of lead, 10 ppb. Density was then considered in order to account for the changes in tectum area. This showed significant increases in positively marked cells at the highest lead concentrations. We also observed that lead decreased expression of transthyretin in the liver in a dose-dependent manner.

Methods: Xenopus laevis served as our model because their external development allows observation of the early stages of brain development that occur in utero in many mammalian systems. This model is also useful because they are acutely sensitive to changes in thyroid hormone which is critical to metamorphosis and whose signaling is thought to be disrupted by lead toxicity.

We worked with albino tadpoles NF stage 46-47 (7-10 days old). The tadpoles were placed into one of eight groups: a control bath or seven concentrations of lead (II) acetate trihydrate (10 ppb, 50 ppb, 100 ppb, 500 ppb, 1,000 ppb, 2,000, 10,000, and 100,000 ppb) for four days. These concentrations were made with two different stock solutions of 26.3 M and 0.263 M in water and then diluted into 1X Steinburg’s solution. Groups of tadpoles were reared in 200ml of solution for four days. Tadpoles were killed on Day 4 with an overdose of MS222 and fixed overnight in 4% paraformaldehyde. Standard immunohistochemistry methods were used to stain whole-mount brains for H2AX (Millipore-Sigma, 1:100), Caspase-3 (AbCam, 1:400), and Sytox-O-Life Sciences, 1:500), all markers for cell toxicity. PFA-fixed brains were then imaged on a Leica SP8 confocal microscope, and images were analyzed with ImageJ.
Sulfasalazine as a Treatment for Acquired Epilepsy

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Background: Epilepsy affects approximately 2.2 million Americans and 1-in-26 people will develop epilepsy within their lifetime. Current therapeutics, targeting neuronal networks, are not effective in 1-in-3 epileptics. A recent study revealed that glutamate release by primary brain tumors induce epilepsy in mice implanted with human glioma cells. System xc (xCT), a glutamate/cystine antiporter, was identified as a major contributor to elevated glutamate levels, resulting in tumor associated epilepsy. This glutamatergic hyperexcitability was shown to be susceptible to inhibition of xCT via sulfasalazine (SAS), an FDA approved drug. Neuronal death, inflammation and gliosis have been suggested to play a role in acquired epileptogenesis, however, no clear consensus has been reached. This project aims to elucidate the role of xCT in acquired epileptogenesis and to test whether SAS can be used as a broad acting anti-epileptic drug.

Methods: In order to study the effects of SAS, we used the kainic acid (KA) and beta-1 integrin knock-out (KO) models of epilepsy. The KA model is a well characterized, chemically induced model of human temporal lobe epilepsy. The beta-1 KO model is a neuroinflammation model characterized by widespread gliosis leading to spontaneous seizures. One round of experiments lasted approximately 5 weeks consisting of EEG implantation, 24/7 video-EEG recording, SAS treatment and tissue analysis. SAS treatment consisted of two injections daily for 1 week. To study the effects of seizure induction and SAS treatment on xCT expression, we conducted qPCR and western blot experiments to look at mRNA and protein expression.

Results: Our preliminary findings showed that KA induced seizures and microglial activation in the hippocampus. The EEG recordings of KA-treated animals showed abnormal activity, which was restored by SAS treatment. In addition, treatment with SAS decreased hippocampal xCT protein levels compared to controls. Our KA model allowed us to study the short-term effects of KA seizure induction on xCT expression, however, we found that the chronic seizure frequency was too low to study the effects of SAS long-term. For long-term effects of SAS on seizure activity we conducted EEG recordings from our beta-1 KO mice. Beta-1 integrin KO mice exhibited spontaneous seizures at a frequency of ~1 seizure/day and SAS treatment reduced the seizure activity.

Conclusions: These data suggest that xCT may play a role in the pathology of acquired epilepsy and that treatment with SAS may work as a broadly acting anti-epileptic drug. Future experiments aim to validate our preliminary data on xCT expression and the effects of SAS treatment on seizure frequency.
Alterations in the Expression of Connexins in the Peritumoral Cortex of Pediatric and Adult Glioma Models

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Background: Glioblastoma multiforme is a grade IV primary brain cancer with extensive migratory and infiltrative properties. Growing evidence suggests that gap junction proteins, called connexins, might be markers of glioma progression. In particular, the expression of the connexin 43 subtype was shown to be inversely correlated with tumor grading, and changes in connexin expression occurs in some seizure models. Using an adult glioma model, we previously showed that regions surrounding the tumor mass, peritumoral cortex, exhibited marked hyperexcitability, however it is unknown whether changes in connexin expression in the peritumoral cortex contributes to peritumoral hyperexcitability. Additionally, most studies on connexins are in the context of glioma in adult tissue. The goal of this study is to examine peritumoral neuronal activity in a pediatric glioma model and determine whether changes in connexin expression is associated with changes in neuronal activity.

Methods: We developed a pediatric and adult glioma model where pediatric patient-derived glioma cells were intracranially injected into the cortex of postnatal day 2 and 3 (p2-3) mouse pups and adult glioma cells were implanted into adult mice (p60-p80). After 7 days, the brains were harvested and whole-cell patch clamp recordings of layer 2/3 pyramidal cells were conducted in the peritumoral cortex. Confocal microscopy was used to evaluate the expression of connexin 43 and connexin 30 in peritumoral cortex and compared to distant cortex. The sample size for this procedure section was performed from 3 animals. We used Western Blot to compare connexin 43 protein levels in adult and pediatric glioma cells.

Results: Our results showed for the first time that peritumoral neurons from a pediatric glioma model displayed spontaneous epileptiform activity and alteration in neuronal firing properties. The action potential threshold for peritumoral neurons in pups was significantly reduced compared to neurons from sham animals. We also found that peritumoral expression of connexin 43 was unchanged in the pups, but was decrease at the tumor border compared to distant regions. In addition, the mean action potential threshold in pediatric peritumoral neurons was lower than that of adult peritumoral neurons. We also found that peritumoral expression of connexin 43 was unchanged in the pups, but was decreased in pediatric peritumoral cortex. Western Blot was used to compare connexin 43 protein levels in pediatric and adult glioma cells.

Conclusions: These findings suggest that changes in the peritumoral expression of connexin 43 and connexin 30 are associated with hyperexcitability in the adult glioma model but not the pediatric glioma model. This suggests that connexins may have distinctive interactions with the adult and pediatric brain environment and therefore age should be accounted for in future studies.
Characterizing stress induced sex differences in pre- and post-synaptic plasticity in the Hippocampus and Nucleus Accumbens

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Background: depression and anxiety disorders are more prevalent in females; however, the majority of research in animal models has focused predominantly on males. Major depressive disorder (MDD) is known to affect the hippocampus and nucleus accumbens. Numerous studies have shown that MDD shrinks hippocampal volume (Malykhin & Coupland, 2015). Other studies have shown decreased activation of the nucleus accumbens (NAc), which plays a central role in the reward system of the brain (Pizzagalli et al., 2010). However, little is known about how plasticity in these brain regions may differ in males and females. Previous studies have used immunofluorescent staining of vesicular glutamate transporter 1 (VGLUT1) and post-synaptic density 95 (PSD95) as markers for plasticity in pre-synaptic and post-synaptic regions. Our early research has indicated a significant decrease of pre-synaptic VGLUT1 in the glutamatergic neurons of the nucleus accumbens (NAc) with no detectable changes in post-synaptic density, indicating possible circuit specific reorganization in only female mice that underwent 6 days of variable stress (Brancato et al., 2017). In this model, female mice are susceptible to 6 days of stress and males are resilient. Therefore, we do not know if the female pre-synaptic plasticity effects are due to stress susceptibility resilience or are sex specific. We can test this by extending stress out to 28 days where both males and females show behavioral stress susceptibility. The studies presented here expand on this research by exposing mice to 6 days of sub-chronic variable stress (SCVS) and 28 days of long-term variable stress (LTVS) to measure the effects on glutamatergic neuroplasticity in the nucleus accumbens (NAc) and hippocampus of both males and females.

Methods: We performed alternating stressors of foot shock, tail suspension, and restraint tube on a 6-day sub-chronic variable stress (SCVS) model and a 28-day long-term variable stress (LTVS) model. Each study consisted of 40 mice (n= 10 per group/sex). 24 hours after the last stressors, animals were deeply anesthetized and perfused with saline and 4% paraformaldehyde. Brains were stored, for two days in PFA then 3 days of increasing concentrations of sucrose. We sliced the tissue in to 40-micron sections on a freezing microtome and collected every 6th NAc and hippocampal slice. Immunohistochemistry was optimized to detect the presence of VGLUT1 and PSD95, and most of the time was spent performing immunohistochemistry alterations to optimize the staining. After extensive optimization we ended up switching from a free-floating to a slide mounted staining procedure. Sections were slide mounted, allowed to dry over night and then washed in PBS (1x). Sections were blocked in 3% donkey serum, washed again in PBS (1x) and then incubated overnight in primary (PSD95 from abcam, #ab21993, at 1:5000), and VGLUT1 from milipore, #AB5905, at 1:10000. Slides were washed again and incubated in secondary antibody by Jackson ImmunoResearch, Cy2 Anti-Goat 705-225-147; Cy5 Donkey Anti-Guinea Pig 706-175-148 for 2 hours at room temperature. After subsequent washing, sections were coveredslipped with Vectashield with Dapi (by Vector-lab) and imaged on a confocal microscope (Nikon A1).

Results: During the initial runs of the free-floating protocol the sections became too fragile to mount, so a new protocol was created. The sections were slide mounted before staining to create a successful immunohistochemistry procedure. However, excess auto-fluorescence detected in unstained controls has caused us to test further concentrations and optimization of secondary antibodies.

Summary: We created a brain bank of 40 SCVS and 40 LTVS mice, which will allow us to examine a number of immunohistochemical markers in both the Hippocampus and Nucleus Accumbens. While much of our time has consisted of optimizing our IHC protocol, we will use this new protocol in the fall to continue this project. Our protocol will allow us to successfully and efficiently stain for VGLUT1 and PSD95 to measure pre- and post-synaptic plasticity, respectively. We will examine the amount of fluorescence, number of puncta, and co-localization of markers in both short-term stress conditions and long-term stress conditions, expanding on our previous research. Our intention is to further explore the sex differences in the complex framework of glutamatergic dysfunctions in susceptibility to stress and depression.

References

Figure 1. Timeline of SCVS and LTVS Stress Models for Immunohistochemistry

Figure 2. Immunohistochemistry for Dapi for cell bodies (Blue) pre-synaptic marker VGLUT1 (red) and post synaptic marker PSD 95(green) in hippocampus
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