SEX DIFFERENCES IN LONG-TERM REOVERY FROM TRAUMATIC BRAIN INJURY

An Undergraduate Research Scholars Thesis

by

GIANMARCO A. CALDERARA

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ABSTRACT

Sex Differences in Long-Term Recovery from Traumatic Brain Injury

Gianmarco A. Calderara
Department of Biomedical Sciences
Texas A&M University

Research Advisor: Dr. D. Samba Reddy
Department of Neuroscience & Experimental Therapeutics
Texas A&M University

Traumatic brain injury (TBI) is a widespread neurological condition suffered by millions of individuals worldwide, including over 2.8 million cases of TBI per year in the U.S. alone. Most frequently induced as a consequence of contact sports, vehicular accidents, and military injuries, TBI can induce post-traumatic epilepsy (PTE), a condition characterized by recurrent, unprovoked seizures as a result of sustained trauma to the brain. Despite the ubiquity of PTE, much of its underlying mechanism and pathophysiology remain unclear. Among other pre-injury factors, biological sex, which has been thought adequately as a critical factor, may have significant impacts on patient outcomes. Further, with more women participating in sports and deploying in military operations than in previous decades, there is an increased demand for understanding the molecular and physiological basis for sex-related variations in recovery from TBI and PTE. In this study, we studied TBI in a female mouse cohort in order to explore the sex-dependent discrepancies associated with PTE. TBI was induced in the subjects before implanting deep-hippocampal electrodes, allowing us to monitor brain activity through EEG analysis. Following the procedures, behavioral testing was performed at various timepoints over a 4-month period before the subjects were sacrificed and their brains removed. We then performed

brain histology to determine the extent of neurodegeneration exhibited by the animals. These results were then compared to a previously studied male cohort that underwent an identical protocol. The data shows marked sex differences in functional recovery outcomes of TBI in males and females.

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NOMENCLATURE

AED	Antiepileptic Drug
AP	Allopregnanolone
EEG	Electroencephalography
EPM	Elevated Plus Maze
LOC	Loss of Consciousness
PTE	Post-Traumatic Epilepsy
PTS	Post-Traumatic Seizures
SE	Status Epilepticus
TBI	Traumatic Brain Injury

CHAPTER I

INTRODUCTION

Epilepsy

Epilepsy is a neurological condition characterized by a persistent tendency to have recurrent, unprovoked seizures. These recurrent seizures often occur as a result of excessive electrical discharges of cortical neurons in the brain and can present in a variety of different forms and severities (Cross, 2004). Symptoms of epileptic seizures can vary greatly, ranging from sudden changes in affect to involuntary jerking movements of the extremities. Individuals are said to have epilepsy after displaying a pathologic and enduring propensity to have unprovoked seizures (Falco-Walter, 2017).

There are approximately 65 million people suffering from epilepsy in the world today, representing a significant portion of the global disease burden (Devinsky et al, 2018). Additionally, there are approximately 3 million people in the United States that have some form of epilepsy, representing nearly 1% of the entire U.S. population (Tian, 2018). The vast majority of epileptic seizures are non-fatal. However, epileptic patients frequently experience a myriad of non-biological burdens in addition to the physiological symptoms of the disease. Research has demonstrated that these men and women are more susceptible to acquiring psychiatric disorders such as anxiety and depression (Hill, 2007). This presumably occurs as a consequence of several different factors. For example, it is not uncommon for affected individuals to be subjected to significant economic burden and hardship. Epileptic patients routinely encounter high medical bills in the form of prescriptions and hospital stays. The cost of receiving treatment is not insignificant in itself, but these patients also frequently experience difficulty finding and keeping

jobs. Unable to safely perform certain tasks in the workforce, those suffering from epilepsy are often limited in the tasks they can complete, making them far less competitive as potential workers. This has led to higher rates of unemployment in epileptic populations, placing additional strain on patients' families (Fitch, 2019). Beyond this, patients suffering from epilepsy often report high rates of perceived social stigma which can foster social isolation, increase anti-social behavior, and reinforce economic hardship.

The development of epilepsy (epileptogenesis) can occur through a variety of different mechanisms, adding an additional challenge for neuroscience researchers. Some individuals, for example, are genetically predisposed to having seizures and can begin showing signs of epilepsy at young ages. For others, epileptogenesis can occur through traumatic brain injury, infection, or exposure to chemical nerve agents such as sarin gas and other organophosphates (Reddy, 2016; Pardo et al., 2014). The outcomes of the disease can also vary significantly, with the vast majority of seizures resulting in no long-term consequences. However, a subgroup of epileptic disorders can lead to serious impairment and deficits. Status epilepticus (SE) is one such condition and is considered to be a neurological emergency. Defined as a prolonged seizure lasting 30 minutes or longer, SE can give rise to significant brain damage or even death (Seinfeld et al., 2016).

The options for epilepsy treatment are limited, although researchers are continuing to develop better pharmaceutical alternatives for patients. Antiepileptic drugs (AEDs) such as benzodiazepines and barbiturates are perhaps the most common strategy for controlling epileptic seizures. These drugs act at specific receptors within the brain and produce a calming effect by inducing neuronal electrical inhibition (Griffin III et al., 2013). Unfortunately, treatment with current AEDs is accompanied by several limitations including adverse side effects, tolerance

development, and multi-drug interactions (Patsalos et al., 2008). Of the individuals that develop epilepsy, approximately 20-40% of them will become refractory to current anti-epileptic drugs (AEDs) and are thus unresponsive to pharmaceutical treatments (French, 2007). This trend has led researchers to developing novel anti-epileptic drugs. Synthetic neurosteroids are one such class of drugs which has demonstrated considerable anticonvulsant properties and has subsequently become a frontier of anti-epilepsy drug research.

Traumatic Brain Injury

Traumatic brain injury (TBI) is among the most significant contributors to the prevalence of epilepsy with an estimated 69 million worldwide cases of TBI each year (Dewan et al., 2019). The majority of TBIs are non-fatal and tend to result in few serious consequences. However, it is estimated that between 3.2 million and 5.3 million people are living with a TBI-related disability in the United States, with those between the ages of 0-4, 15-19, and 75+ being the most at-risk for sustaining a TBI (Selassie et al., 2008; Faul et al., 2010). Most frequently caused by falls, motor vehicle accidents, sports injuries, and military operations, TBI is often accompanied by significant cognitive and neural deficits. These deficits can have a profound impact on a patient's self-image and quality of life, adversely affecting their interpersonal relationships, emotional health, and occupational functioning (Rabinowitz and Levin, 2014). Thus, TBI not only has deleterious biological consequences but can also negatively impact patients socially, psychologically, and economically.

TBIs can vary substantially in severity and are characterized as either mild, moderate, or severe depending on the patient's clinical presentation. Symptoms of TBI frequently resolve themselves and are not accompanied by significant long-term deficits. Yet, the likelihood that a patient will experience persistent symptoms increases with the severity of the sustained TBI, and

these symptoms can frequently lead to chronic disability (Frey, 2003). Mild TBIs are those in which patients experience a loss of consciousness (LOC) for less than 30 minutes and do not sustain any associated skull fractures. Moderate TBIs are seen in patients who experience LOC for greater than 30 minutes as well as an associated skull fracture or other similar injury. Finally, patients are said to have sustained a severe TBI after experiencing more than 24 hours of LOC or presenting with a documented brain contusion or intracerebral hematoma (Annegers et al., 1998). However, the literature does not indicate a currently accepted universal classification for TBI severity, and the classifications noted above are generally accepted amongst researchers and physicians.

Post-Traumatic Epilepsy

Among a vast number of other negative outcomes, TBI may result in the acquisition of post-traumatic epilepsy (PTE), a symptomatic form of epilepsy that develops in previously non-epileptic individuals (Agrawal, 2005). In order for an individual to be diagnosed with PTE their post-traumatic seizures (PTS) must continue to persist at least 1 week after the initial injury. Seizures within the first week of injury are an acute result of trauma and are considered provoked (Verellen and Cavazos, 2010). Seizures occurring in this timeframe are said to be early PTS while seizures occurring outside this timeframe are said to be late PTS.

The likelihood that an individual will develop PTE is generally correlated with the severity of the sustained TBI with post-traumatic seizures typically occurring within the first year following the injury (Annegers et al., 1998; Ferguson et al., 2010). However, the underlying molecular mechanisms of the development of epilepsy as a result of TBI are not fully understood (Reddy, 2013). There is evidence indicating that certain AEDs including phenytoin, phenobarbital, and carbamazepine are effective countermeasures for treating early PTS.

However, such drugs are ineffective for limiting PTS or PTE (Verellen and Cavazos, 2010; Schierhout and Roberts, 1998; Temkin, 2001). Post-injury treatment and intervention can have a profound influence on health outcomes following TBIs. Other factors such as age and biological sex can also affect recovery, although there is still much to learn about these processes.

Neurosteroids

The anticonvulsant properties of endogenous neurosteroids such as allopregnanolone (AP) and pregnanolone have been gaining recognition amongst researchers for the past several decades (Selye, 1941; Selye and Masson, 1942). These compounds are synthesized in the brain through reductions of cholesterol and other steroid precursors and offer inhibition in the brain by acting as positive allosteric activators of both synaptic and extrasynaptic $GABA_A$ receptors (Reddy, 2010; Reddy, 2016). Indeed, synthetic analogs of endogenous neurosteroids have been produced in the hopes of minimizing the detrimental side effects associated with traditional AEDs while still maintaining their anticonvulsant properties. For example, ganaxolone (GX), a synthetic analog of AP, has shown promising results in clinical trials for the treatment of epilepsy (Reddy and Woodward, 2004).

When acting at synaptic receptors, neurosteroids produce inhibitory post synaptic currents (IPSC), a transient form of inhibition referred to as 'phasic' inhibition. In this case, neurosteroids activate synaptic membrane receptors, and phasic inhibition is produced as a result of the presence of GABA within the synapse (Farrant and Nusser, 2005). This inhibition is only present when GABA is found within the synapse, accounting for its transient nature. In contrast, the concentration of GABA just outside the synaptic cleft is far more constant. When neurosteroids potentiate extrasynaptic $GABA_A$ receptors, a less transient form of inhibition is

produced. This is referred to as tonic inhibition and is known to have a profound impact on neuronal excitability and seizure thresholds (Farrant and Nusser, 2005).

The actions of neurosteroids on excitability are the basis for this study. The anticonvulsant and antiseizure properties of progesterone metabolites have been documented for quite some time. Progesterone is primarily produced in the ovaries and is crucial for the endometrial transition from the proliferative to the secretory stage as well as to the maintenance of pregnancy, and its levels are thus significantly higher in females than in males (Taraborrelli, 2015). Progesterone is converted into various neurosteroids in the brain, accounting for its positive influence on seizure resistance (Reddy, 2004; Reddy, 2012). Allopregnanolone (AP) is one such metabolite that is known to have broad anticonvulsant properties in the brain (Dhir and Chopra, 2015). Thus, increased AP levels in females compared to males may provide better relative long-term outcomes from TBI and protection from PTE. However, these sex differences have largely been overlooked in the past. Therefore, in this study, we sought to explore the discrepancies in functional recovery in male and female cohorts from TBI.

CHAPTER II

METHODS

Animals and Experimental Groups

This study used a cohort (n=12) of wildtype female mice. The animals were initially housed in group cages kept in an environmentally controlled animal facility for several weeks prior to administering treatments. This was done to ensure proper acclimation to the environment they would be housed in for the duration of the experiment. The facility maintained a 12h light/dark cycle throughout the study, giving animals open access to food and water ad libitum. Once acclimated, the animals were then subjected to the experimental procedures and subsequently single-housed for the remainder of the study. All animals were cared for in compliance with the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all procedures were performed according to a protocol approved by the university's Institutional Animal Care and Use Committee.

Surgery and Recovery

Animal weights were recorded on day 0 immediately prior to performing any experimental procedures. A standard mixture of ketamine and xylazine injection was administered to anesthetize mice prior to surgery. The subjects were then mounted on a stereotaxic frame and received a 4.5 mm craniotomy over the left hippocampus to expose the underlying neural tissue. The animals then received a controlled cortico-hippocampal injury at a velocity of 4.0 m/s using a 3mm diameter rounded-tip impactor, resulting in a TBI injury with a depth of 2 mm. Deep-hippocampal electrodes in the contralateral hippocampus were implanted for EEG recording and secured to the skull using dental acrylic and small anchor screws; the

electrodes were placed 2.9 mm posterior, 3.0 mm lateral, and 3.0 mm below dura (Franklin and Paxinos, 1997). Animals were allotted 7-10 days for recovery before being single-housed in the controlled animal facility for constant EEG-video recording. Animals continued to receive access to food and water ad libitum and were able to move throughout their cages unhindered. The mice were checked daily to ensure they were receiving proper care as well as to ensure proper functioning of the EEG equipment.

EEG-recording and Seizure Analysis

Following the recovery period, animals were single-housed in the vivarium and connected to an EEG-recording system as well as constant video-EEG monitoring. Brain activity was recorded using AscopeSoftware, and seizures were identified using a customized Matlab analysis software that detected electrical signals with an amplitude and frequency more than double their respective normal for a duration between 8 and 75 seconds. Seizures that presented within the first two weeks of the study following animal surgeries were omitted from the analysis, as the events were likely to be related to immediate trauma and therefore not a result of epileptogenesis.

Tissue Collection and Immunohistochemistry

The animals were anesthetized with a ketamine-xylazine mixture and transcardially perfused at 4 months post-TBI with a 4% paraformaldehyde solution (Fischer Scientific) in a sodium phosphate buffer with a pH of 7.4. Each animal's brain was then removed and subsequently fixed in a 4% paraformaldehyde solution for 16 h at 4° Celsius and later treated with a sodium phosphate buffer for 24h. The removed brains were then placed in 10%, 20%, and 30% sucrose solutions for 72h each. The tissue was then frozen with O.C.T compound (Sakura Fintex, CA, USA) on dry ice, and 30μM sections were cut coronally through the mouse forebrain

from bregma -1.15mm to -3.75 mm (Paxinos and Watson, 2007). The sections were then processed with glial fibrillary acid protein (GFAP), Nissl bodies (CV), ionized calcium binding adaptor molecule-1 (IBA-1), doublecortin (DCX), parvalbumin (PV), and neuronal nuclei antigen (NeuN)-immunoreactivity.

Stereology

Stereological analysis was to be performed following the perfusions according to an established neurostereology protocol (Golub et al., 2015). The stereological equipment includes an Olympus BX53 microscope fixed with a DP73 digital color camera (Model: DP73-1-51-, Olympus, Tokyo, Japan), a motorized stage (Model: H101ANNI, Prior Scientific, Rockland, MA, USA), and a universal microscope automation controller with encoder (Model: 500-H3XYZEF, ProScan III, Prior Scientific, Rockland, MA, USA). Individual brain slices were to be visualized under the microscope using a 1.25x lens objective. The regions within the hippocampus were defined using the newCAST software (Version: VIS4.6.1.630, Visiopharm, Denmark); these regions include the CA1, CA2, CA3, DG, and DH. Finally, the volume and cell density of each hippocampal region were to be calculated using the 10x and 60x objective respectively. Unfortunately, due to the unforeseen events surrounding the COVID-19 virus in Spring 2020, stereological counts were unable to be completed before submission of this undergraduate thesis.

Health Score

A health scale evaluation was utilized throughout the first month of the study to assess the relative recoveries of the animals. Scores were given based off of observed behaviors and appearances. Higher scores were indicative of better recovery, with a maximum score of 16 typically being the score assigned to sham animals. The scoring criteria were as follows:

alertness and normal activity (0-1), grooming behavior (0-1), skin condition (0-3), cleanliness and health of eyes (0-2 for each eye), lack of kyphosis (0-2), weight support (0-3), and posture while sitting or walking (0-2).

Beam Walk

Deficits in fine motor control and balance were assessed by using an elevated balance beam. Animals were placed on a wooden beam situated approximately 60 centimeters above the ground. The surface measured 3 cm in width, and the animals were allotted 60 seconds to cross from one side of the beam to the other. The beam measured 1 meter in length, and performance was determined by the percentage of successful completions within the 60 second timeframe.

Rotarod

A rotarod apparatus was utilized as an additional assessment of fine motor control and balance (IITC Life Science Instrument). The animals were placed in the rotarod device for 60 sec, and the beam rotated at a rate of 5 rpm. Performance was measured by the amount of time each animal was able to stay on top of the apparatus, and values represent an average of 3 tests per timepoint. Healthy animals are typically able to maintain positioning on the rotating beam with relative ease for the full 60 second trials. Thus, when animals fall from the apparatus before the full completion of the trial, it is interpreted as a deficit in balance and fine motor control.

Elevated Plus Maze

The elevated plus maze (EPM) is an evaluation tool used to test anxiety-like behaviors in animals. Mice have a general tendency to explore novel spaces and will do so if unhindered. Additionally, mice have an aversion to open areas and heights. This test utilizes the conflict present in these animals between exploration and aversion by placing them in a scenario that offers all aspects together (Lister, 1987; Kulkarni and Reddy, 1996). The EPM was composed of

two closed arms measuring 16x10cm and two open arms measuring 16x10cm which stood 25cm above the ground. The animals were given 5 minutes to freely explore the maze after being placed in the center, facing one of the 2 open arms. All equipment was wiped down using an isopropyl alcohol solution between each trial. An entry was recorded when the animal's head and two front paws entered into one of the arms. The percent of open arm entries was determined by dividing the number of entries into the open arms divided by the total number of arm entries. Additionally, we recorded the relative amount of time that the animals spent on the open arms versus the closed arms. Anxiogenic behavior was indicated by a decreased number of open arm entries in addition to a decreased percent of time spent on the open arms.

CHAPTER III

RESULTS

Health Scores

The results of the health score analysis show significantly different outcomes for TBI male and female groups from the onset of injury to just before the 30d timepoint. The female animals showed greater resilience and acute recovery following the surgery procedure. This data was actively referenced in the lab as a means of determining which animals were displaying a failure to thrive. Scores were determined based on a variety of factors including alertness and normal activity (0-1), grooming behavior (0-1), skin condition (0-3), cleanliness and health of eyes (0-2 for each eye), lack of kyphosis (0-2), weight support (0-3), and posture while sitting or walking (0-2). Higher scores are indicative of better recovery, and figure 1 demonstrates that the female cohort was better equipped to endure the acute effects of TBI. This is consistent with the literature concerning sex differences in short-term recovery from traumatic brain injury.

Previous studies performed in our lab have demonstrated that animal health does not deteriorate significantly in the sham, control, or experimental groups after the 30d timepoint. In this study, animals were all showing signs of stable recovery at 30d post-injury and were no longer considered to be at-risk of fatality. This is the primary reason the 30d timepoint was chosen as the final health score assessment, as data beyond this timepoint would have been unnecessary. The results would likely have shown nearly identical outcomes for all three groups, as suggested by the trend in figure 1.

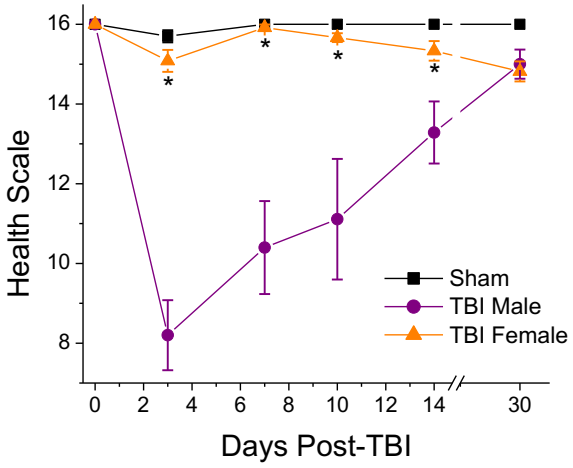

Figure 1. Animal Health Scores

Beam Walk

Next we began looking at functional recovery from TBI. We did this by utilizing a beam walk as a measurement of fine motor control and balance. The test involves having the animals cross a narrow wooden beam in 60s or less, and each trial was marked as successful if the animals were able to cross the apparatus in the allotted timeframe without falling off. To incentives movement, a bright lamp was used as an aversion stimulus at one end of the beam. Values were given as a percentage of the animals that were able to successfully complete the task within each respective group.

Our data shows significantly different outcomes between male and female TBI groups from 7d to 120d post-injury. We did not perform any trials prior to the 7d timepoint, as the animals were still in the acute recovery phase and were showing signs of significant incoordination. Throughout the duration of the study, the female animals showed better recovery in motor control and balance compared to male counterparts. This trend continued all the way to the 120d timepoint, and figure 2 highlights the increased resilience from TBI in female individuals.

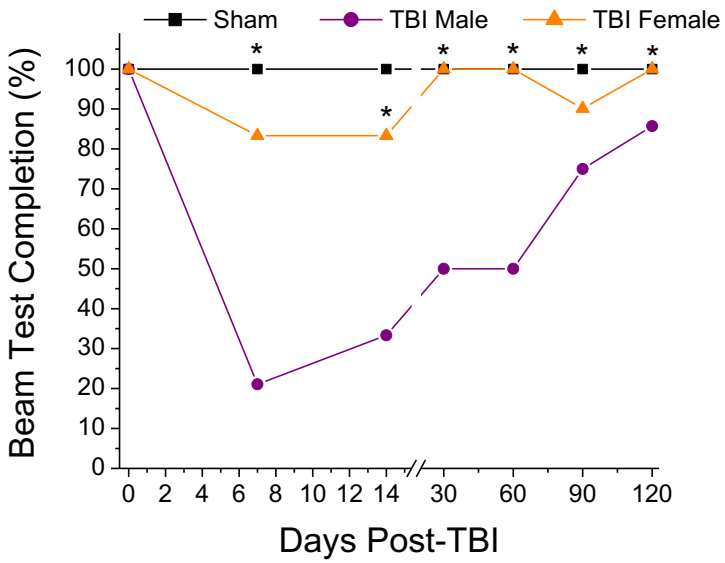

Figure 2. Beam Walk Test

Rotarod

The rotarod test can measure motor control and balance and was used to confirm the results of the beam walk test. This test involves placing the animals on a narrow beam that rotates at a rate of 5rpm. The length of time that each animal was able to stay on top of the rotarod device before falling off was recorded, and the scores illustrated in figure 3 depict the average amounts of time recorded for each experimental group. Our results indicate a statistically significant difference in rotarod performance at each timepoint after the initial injury. The female group was able to perform the task better than the male cohort throughout the entirety of the study. Further, the female TBI cohort showed significantly improved recovery in comparison to itself at the end of the study, with their average latency to fall increasing from approximately 25s to 55s. The same cannot be said of the male TBI cohort as their recovery was far less ideal, increasing from an initial average of 10s to just under 20s at the conclusion of the

study. This is consistent with our findings in the beam walk test and indicates better motor control and balance for the female animals. Taken together, the rotarod and beam walk test results provide evidence that female animals exhibited better functional recovery all the way to 120d post-injury.

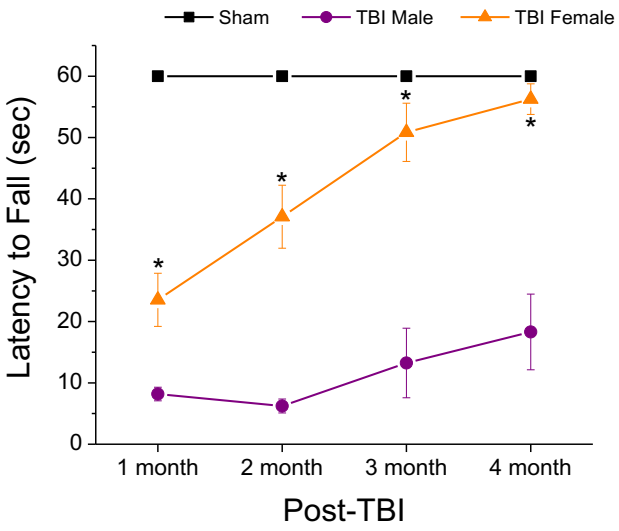

Figure 3. Rotarod Test Results

Elevated Plus Maze

There is some literature that links moderate-severe TBI with psychological issues (Hseih et al., 2012; Gould et al., 2011). One such issue is anxiety and has been identified as the primary indicator of negative psychosocial outcomes following TBI (Draper et al., 2007). The elevated plus maze (EPM) is a tool utilized to assess levels of anxiety-like behaviors as well as learning deficits. In this behavior test, a higher average number of entries into the open arms of the EPM apparatus as well as the percentage of time spent within those arms is indicative of lower levels of anxiety.

The data for this testing scenario did not show statistically significantly results for sham and female TBI cohorts. The TBI group showed both a decreased average number of entries into the open arms and a decreased percentage of time spent within those arms at nearly all timepoints. Although not statistically significant, these results were to be expected given the known link between TBI and anxiety.

In contrast, the male cohort had a significantly higher average number of open arm entries than their female counterpart throughout the 4-month experiment. Additionally, the male cohort spent a greater percentage of the 5-minute testing interval at all but one of the timepoints. These results may appear to be somewhat unexpected, as one may expect the male cohort to have displayed higher levels of anxiety given their poorer functional recovery. However, these results can be reconciled through the potentially decreased awareness and cognition experienced by male animals in comparison to the female cohort. Figures 4 and 5 depict the significant variation between male and female TBI cohorts and suggests that the female animals were better equipped to handle the negative cognitive consequences associated with TBI. This is consistent with the literature which links TBI to various cognitive deficits (Smith, 1998; Filley, 2018; Adamson et al., 2019; Stocchetti and Zanier, 2016). However, the relationship between cognitive impairment and TBI is not fully understood and remains under investigation.

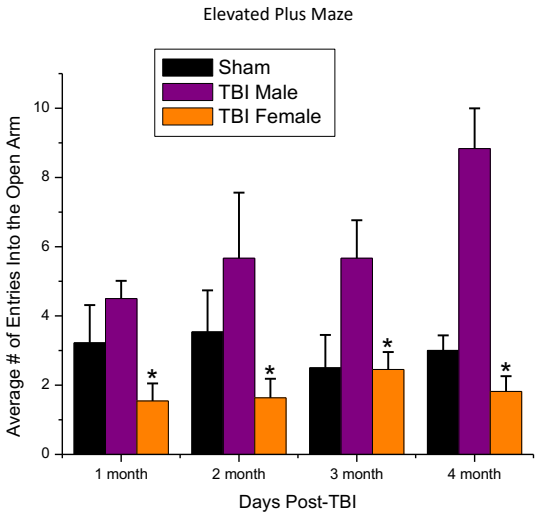

Figure 4. EPM Average Open Arm Entries

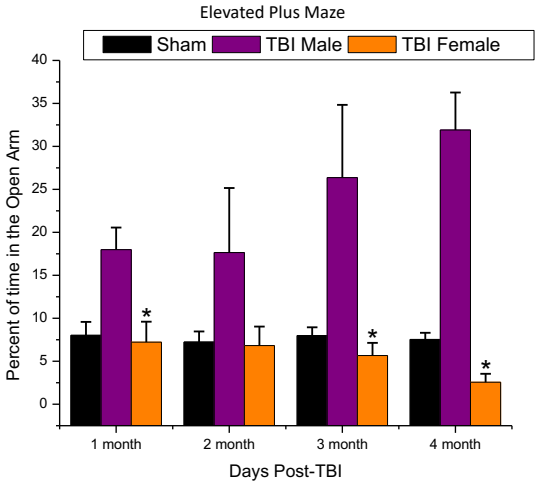

Figure 5. Percent of Time in Open Arm

EEG-Recordings and Seizure Analysis

Electroencephalography (EEG) is commonly implemented as a means of recording neuro-electrical activity and is commonly used as a diagnostic tool in clinical settings (Sharanreddy and Kulkarni, 2013). For seizure detection in this study, deep-hippocampal electrodes were inserted to monitor brain activity, and signals were recorded continuously throughout the duration of the study period. As one might expect, massive amounts of seizure

data was collected, and manual analysis of this data is both tedious and time consuming. So, we utilized a custom MatLab analysis software to detect electrical signals with an amplitude and frequency more than double their respective normal for a duration between 8 and 75 seconds. Additionally, seizure events were cross-referenced with video footage to confirm corresponding behavioral symptoms.

Figures 6-8 depict sample traces collected over the course of the study. As expected, the sham animals did not display significant electrical discharges with amplitudes above 2mV. However, the female and male TBI cohorts displayed seizure events throughout the experiment. Male TBI animals had substantially pronounced seizure activity with respect to both amplitude and duration compared to female TBI animals. This data is consistent with the behavioral and functional recovery tests performed and provide further evidence of a decreased seizure susceptibility in females individuals suffering from TBI.

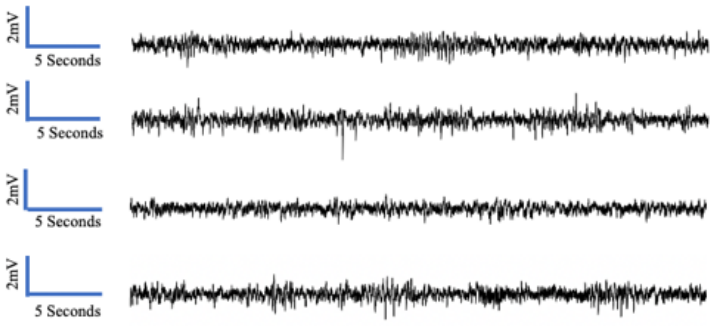

Figure 6. Sham EEG Recordings

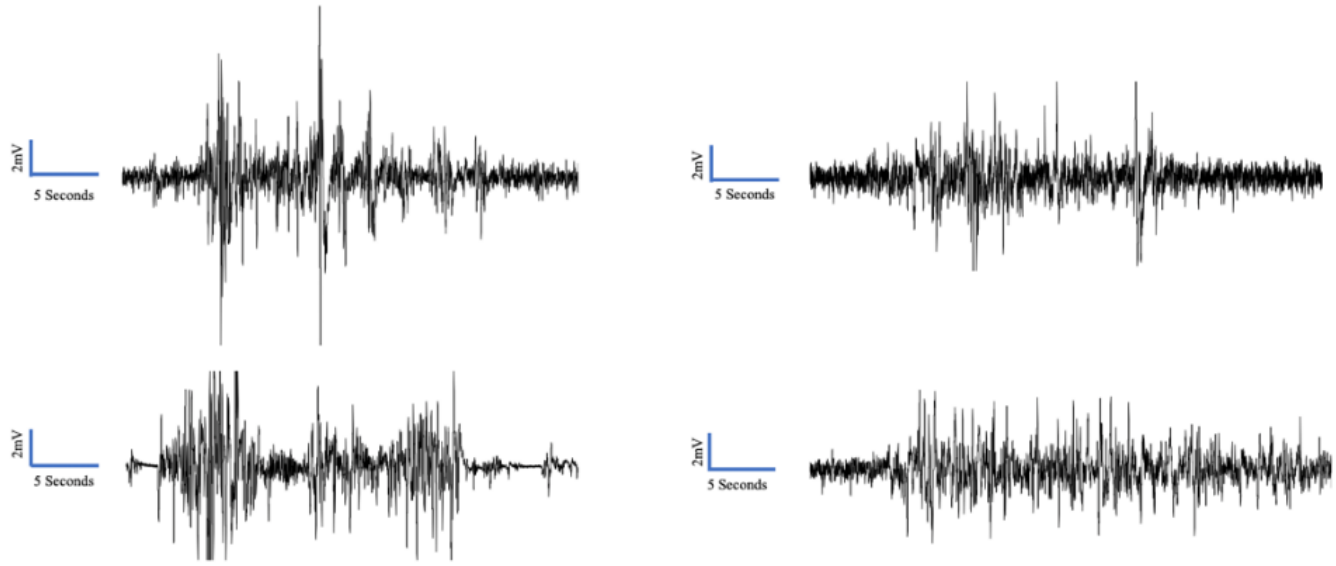

Figure 7. Female TBI EEG Recordings

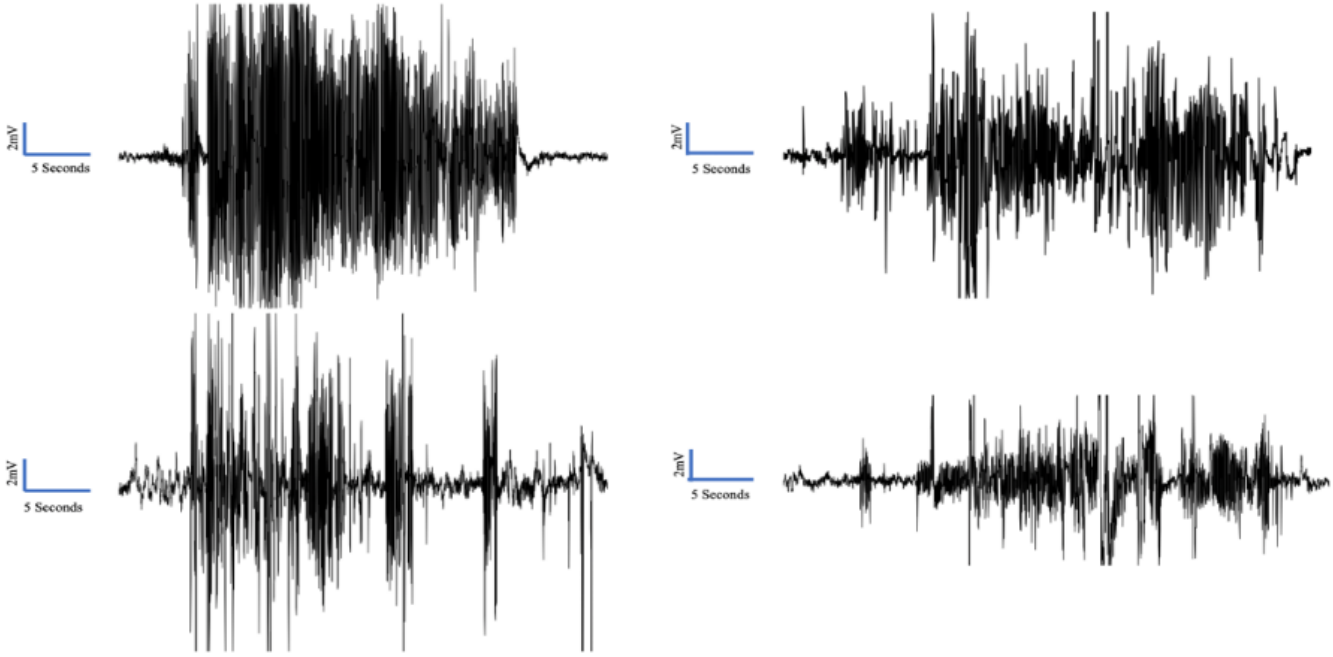

Figure 8. Male TBI EEG Recordings

CHAPTER IV

CONCLUSION

TBI and PTE are prevalent conditions in today's society. Additionally, women are now deploying in military operations and participating in high-contact sports more frequently than in previous generations. This means that a greater number of women are at-risk for TBI each year. Thus, there is a need for a better understanding of the differences between males and females in terms of recovery from TBI and development of PTE. In this study, we focused on whether females may be better equipped to handle the maladaptive processes associated with TBI than their male equivalents. Previous studies have largely centered on acute differences in seizure susceptibility in males and females. Instead, we performed quantitative analysis of behavioral, functional recovery, and EEG traces during the study period to explore long-term discrepancies and effects of TBI.

Female animals were found to have better outcomes in each of the tested criteria, and the results show a clear distinction between the two sexes pertaining to recovery from TBI. Further, female animals showed an increased resilience to TBI and post-traumatic seizures. The literature indicates that this increased seizure resilience occurs, in part, through neurosteroid metabolites of progesterone found in greater abundance within female animals. Allopregnanolone is one such metabolite that has been noted to have anticonvulsant properties for quite some time through its potentiation of extrasynaptic $GABA_A$ receptors and associated tonic inhibition. The results of this study are consistent with encouraging clinical trials for ganaxolone, a synthetic analog of allopregnanolone, and provide further insight to the clinical potential of neurosteroids for treating TBI and PTE. Such neurosteroids and their synthetic analogs are promising tools for

decreasing the maladaptive processes associated with TBI, and research of their efficacy will continue to develop in the coming decade.

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