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An Investigation of Anxiety- And Depression-Like Behavior after Head Trauma in Mice

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By

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Abstract

Every year around 2 million people in the United States (US) suffer a traumatic brain injury. Those people are often at a higher risk of post-TBI psychiatric illness, like anxiety and depression. Animal models of TBI are a useful way to measure such psychiatric illnesses in a pre-clinical setting. There were two purposes of this study: the first being to test the modified Marmarou TBI model, and second to investigate anxiety- and depression-like symptoms in C57Bl6/J mice following a TBI. The modified Marmarou model used different weights (95g, 30g, & sham) to see how severity effected the manifestation of anxiety-like and depression-like symptoms. Anxiety-like behavior was tested using the elevated zero maze (ZM), and the open field test (OF). Depression-related behavior was tested using the forced swim test (FST). Lastly, the effects that the TBI had on motor coordination was also tested using a rotarod. The mice were put through four trials in each test over seven days post TBI with a three day inter-trial interval between the first two trials and the last two. Results indicated that TBI did not cause anxiety or depression-like behaviors or any deficits involving motor coordination. In conclusion, future studies are needed in order to understand how effective this weight-drop model is, or if it was the behavioral tests that needed to be altered in order to measure anxiety and depression-like behavior more accurately.

Keywords: TBI, weight drop, anxiety, depression, zero maze, open field, forced swim test, Rotarod

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Traumatic brain injuries (TBI) are the product of the brain rapidly accelerating and decelerating against the skull after an impact to the head. While TBI's are most commonly associated with contact sports and military service, the general public is still at risk to suffer a TBI. The Center for Disease Control and Prevention (CDC) has reported that between 1.4 and 1.7 million emergency department visits, hospitalizations, and deaths occur each year because of TBI (Faul, Wald, Xu, & Coronado, 2010). In fact, from 2006 to 2014 rates of TBI related emergency department visits rose by 54% (Peterson, Xu, Daugherty, & Breiding, 2019). The symptomology of TBI can differ for each one experienced (Daneshvar et al., 2011). Symptoms can be dependent on personal factors such as age, gender, and previous history of traumatic brain injuries (Patterson & Holahan, 2012). Most symptoms of TBI fade away over time. However, some effects may persist for weeks, months, and in the most severe cases, even years (Evans, 2004; Kobeissy, 2015). Symptoms that continue to persist can end up becoming disabilities. n estimated 2% of the population lives with a TBI-associated disability (American Psychological Association, 2013).

Pathophysiology of TBI

The disabilities may be a result of a complex pathophysiological response that, while still relatively unclear, is thought to be a cascade of mechanical and chemical responses (Giza & Hovda, 2001; Giza, & Hovda, 2014; Vascak, Jin, Jacobs, &

Poylishock, 2017). Many mechanisms are occurring at the same time that can cause damage; physical damage that can cause contusion, intracranial hemorrhaging, or lacerations and diffuse brain damage that cause axonal stretching and the resulting diffuse axonal injury (DAI) (Giza & Hovda, 2001; Werner, & Engelhard, 2007; Giza, & Hovda, 2014).

Cerebral blood flow. To start, during a TBI the brain is put through a force that causes it to vigorously accelerate and then decelerate inside the skull. The permeability of the blood-brain barrier (BBB) as well as cerebral blood flow becomes impaired by both cerebral hypo-perfusion and hyper-perfusion (Werner, & Engelhard, 2007). Hypo-perfusion has been seen to develop immediately after TBI (Martin et al., 1997; Werner, & Engelhard, 2007), which would disrupt glucose metabolism by limiting the amount of glucose that can enter through the BBB (Chen et al., 2004). After the lowest levels of CBF, it rises and may exceed normal rates becoming hyper-perfusion (Kelly et al., 1997).

Spreading depolarization. The shearing force put on the neurons causes the membrane structure to become unstable, due to the instability, ions such as sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) can cross the membrane with much less resistance (Giza, & Hovda, 2001; Patterson, & Holahan, 2012; Giza, & Hovda, 2014). As K^+ flows out of the cell and extracellular K^+ increases, prolonged neuronal depolarizations are triggered. The neuronal depolarizations which increase intracellular Na^+ and Ca^{2+} also pulls water into the cell causing it to swell (Giza, & Hovda, 2001; Ayata, & Lauritzen, 2015). The swelling is theorized to trigger the release of all neurotransmitters in the affected area. And with glutamate being one of the neurotransmitters released, it spreads

to other cells and attaches to NMDA receptors, which allows for even more Ca^{2+} to enter the cell in already affected cells as well as to spread to cells that have not been affected (Neher, & Sakaba, 2008; Dong, Wang, & Qin, 2009; Vyleta, & Smith, 2011; Pietrobon, & Moskowitz, 2014; Ayata, & Lauritzen, 2015). This process is known as spreading depression (SD) (Ayata, & Lauritzen, 2015). SD could eventually lead to the disruption of the glucose metabolism and the tricarboxylic acid (TCA) cycle, as well as the possibility of induced excitotoxicity.

Glucose Metabolism. Glucose metabolism is designed to maintain homeostasis in the brain (Prins, Greco, Alexander, & Giza, 2013; Jalloh et al., 2015). The brain relies on glucose to fuel all of the processes that take place (Prins et al., 2013). Because many processes are dependent on glucose and adenosine triphosphate (ATP), it is difficult to accumulate ATP for storage, which is why a disruption of glycolysis is so damaging (Bonora et al., 2012). In a TBI, ATP supplies become low, and because glycolysis is disrupted the supply cannot be consistently replenished. The inconsistent supply of glucose and ATP leads to the highs and lows of glycolysis. Through glycolysis and the TCA cycle, ATP is formed to be used as energy. Following a TBI, cerebral glucose metabolism increases exponentially and enters a state of hyperglycolisis to try and cope with the SD, while reinstating equilibrium. However, the availability of glucose decreases exponentially, and while the cause remains unknown there are theories as to why there is a shortage.

Glucose initially crosses through the BBB and is transported to cells where glycolysis begins. However, one theory is that following a TBI, CBF decreases, which

also decreases the amount of glucose available to be metabolized into ATP (Giza, & Hovda, 2001; Chen et al., 2004; Prins et al., 2013). The glucose that does make into the cell is converted into pyruvate through several enzyme-catalyzed reactions (Bonora et al., 2012; Jalloh et al., 2015). Furthermore, once glucose is converted into pyruvate, it is transformed into acetyl CoA as it enters the TCA cycle inside the mitochondria. However, because there is less glucose there is also less pyruvate made. Nicotinamide adenine dinucleotide (NADH) assists the TCA cycle in moving electrons down the electron transport chain (ETC). The ETC sends protons across the inner mitochondrial membrane which maintains the concentration gradient. ATP is made as protons flow down through ATP synthetases (Bonora et al., 2012; Jalloh et al., 2015). A result of the process of converting glucose to ATP post-TBI is that oxygen free radicals increase. These free radicals are usually maintained by pairing agents that nullify their unpaired electrons (Prins et al., 2013; Giza & Hovda, 2014). After a TBI, those pairing agents are reduced and the free radicals increase, which can cause damage to DNA by activating lesion causing enzymes as well as damage to the plasma membrane of the cell (Prins et al., 2013). Following hyperglycolisis, glucose metabolism decreases and while there are several theories as to why, the mechanisms are not known (Prins et al., 2013).

Excitotoxicity. The final step towards cell death is excitotoxicity. Overstimulation from prolonged depolarization leads to the accumulation of intercellular Ca^{2+}; this begins the process of mitochondrial dysfunction. The mitochondria, through the TCA cycle produce ATP. During this process, the mitochondria use the electrochemical gradient made of protons sent down the ETC as a buffer in the removal

of Ca^{2+} (Duchen, 2004; Chávez-Castillo, Rojas, & Bautista, 2017). Excess inter-mitochondrial Ca^{2+} leads to a reduction in ATP synthesis and increases ATP usage as a way to remove Ca^{2+} (Szydlowska, & Tymianski, 2010; Chávez-Castillo, Rojas, & Bautista, 2017). However, during the early stages following a TBI there is not enough glucose available to make ATP to compensate for the increase of Ca^{2+} in the mitochondria (Szydlowska, & Tymianski, 2010). Dysfunction of the mitochondria leads to an increase in free radicals through the matching of unpaired electrons resulting in superoxide ions called reactive oxygen species (ROS) (Duchen, 2004; Szydlowska, & Tymianski, 2010). These ROS have the capability of damaging mitochondrial DNA as well as nuclear DNA, such as the genes that create the transports that regulate glutamate; although, this has not been documented in humans (Duchen, 2004; Chávez-Castillo, Rojas, & Bautista, 2017). Ca^{2+} excess, in congruence with the creation of ROS end up leading to mitochondrial dysfunction and cell death, though, it is theorized that once CBF increases and glucose is abundant again, the increase in the production of ATP could reduce the effects of the influx in Ca^{2+}.

Neuroinflammation. Neuro-inflammation is a secondary response to TBI. Neuro-inflammation is the activation of the brain's immune system in response to an inflammatory challenge like acute brain injury or a stressful event and leaves the brain vulnerable to a secondary injury (Hein & O'Banion, 2009). Yet, the role of the inflammatory response following central nervous system (CNS) injury remains unclear (Patterson & Holahan, 2012). In fact there have been instances where neuro-inflammation has contributed to neuroprotective efforts and the absence of neuro-

inflammation led to increased damage (Ziebell, & Morganti-Kossman, 2010). Microglia and astrocytes secrete pro and anti-inflammatory cytokines, chemokines (a type of cytokine that attracts white blood cells to sites of infection), growth factor (a substance that stimulates cell growth), as well as perform phagocytosis (the ingestion of dying cells) (Karve, Taylor, & Crack, 2016). Microglia have three states: resting state (state 1), an activated state (state 2), and a phagocytic state (state 3) (Streight, & Kincaid-Colton, 1995). The role of microglia in a resting state is to monitor the health of the cells around it (Streight, & Kincaid-Colton, 1995).The microglia change shape in an activated state when a disturbance in the environment is detected and in the Phagocytic state, microglia change shape again when dead cells are detected. In this state the microglia are attempting to degrade the dead matter (Streight, & Kincaid-Colton, 1995).

Astrocytes keep the amount of glutamate in the brain at a stable level. During excitotoxicity the transporters on astrocytes that store the glutamate are blocked leaving the levels of glutamate in the brain unmaintained (Karve et al., 2016; Chen & Swanson, 2003). Considering the functional properties of cytokines, it is thought that cytokines created from neurons are mostly involved in cellular communication, whereas cytokines from glial cells encourage neuronal growth, survival, and repair, but may also be involved in changes that are linked to different neurodegenerative diseases (Dantzer et al., 2008). However, the roles of anti-inflammatory cytokines in response to a concussion are poorly understood. In fact, anti-inflammatory cytokines are paradoxical in a sense as it relates to brain injury; some effects are beneficial while others are detrimental (Ziebell, & Morganti-Kossman, 2010; Patterson, & Holahan, 2012). The focus of past research has

been pro-inflammatory cytokines like interleukin-1α and β (IL-1α and IL-1β), IL-6, tumor necrosis factor-α (TNF-α), and IL-10. They mitigate the inflammatory response to trauma/infection. These peripherally produced pro-inflammatory cytokines act both in the peripheral immune system as well as in the brain where a chronic inflammatory response can cause abnormal behavioral symptoms and deficits in memory and motor coordination (Ziebell, & Morganti-kossman, 2010; Danzter, O'Connor, Freund, Johnson, Kelley, 2008).

*Interleukin-1***.** IL-1α and IL-1β are important initiators of the immune response, playing a key role in the onset and development of a complex hormonal and cellular inflammatory cascade (Ziebell, & Morganti-Kossman, 2010). IL-1α and IL-1β are pro-inflammatory cytokines that are thought to aid in defense against infection or disease (Carlson et al., 1999; Ziebell, & Morganti-Kossman, 2010; Patterson, & Holahan, 2012). However, IL-1α and IL-1β has been shown to play a role in neuronal degeneration, since IL-1β stimulates the release of other pro-inflammatory cytokines, the chronic release of IL-1β and thus the chronic release of other pro-inflammatory cytokines could be neurodegenerative. In fact, neuronal damage was reported to increase IL-1β, which was injected following ischemic or traumatic injury in rats (Loddick, & Rothwell, 1996).

Interleukin-6. IL-6 promotes both a pro-and anti-inflammatory responses to head trauma (Lenzlinger, Morganti-Kossman, Laurer, & McIntosh, 2001), and has been seen as a mediator of inflammatory responses to infections and tissue damage (Kopf et al., 1994). IL-6 has been seen to be neuroprotective; oxidative stress and apoptotic cell death in mice was reduced with the expression of IL-6 (Penkowa et al., 2003). Yet, this type of

response may be time and location-specific (Patterson, & Holahan, 2012). In several studies IL-6 was seen to not exhibit neuroprotective properties when neuro-inflammation was induced. In one such study neuro-inflammation was induced in two types of C57B mice (IL-6 $^{-/-}$ and IL-6 $^{+/+}$) through the injection of Lipopolysaccharide (LPS). During a water maze task, IL-6$^{+/+}$ mice injected with LPS performed worse than IL-6 $^{-/-}$ injected with LPS, as it was seen that IL-6 $^{+/+}$ mice took longer to find the platform. The results showed that IL-6 deficient mice were more resistant to deficits induced by neuro-inflammation than mice that were not deficient (Sparkman et al., 2006). Furthermore, neutralization of IL-6 led to a reduction in neuro-inflammation and mTBI, as well as a restoration of motor coordination on the rotarod (Yang et al., 2013). Even with the neurodegenerative responses seen using IL-6, its role in secondary neurodegeneration due to concussions is still unclear (Patterson, & Holahan, 2012).

*Interleukin-10***.** Interleukin-10 (IL-10) is produced by microglia and astrocytes in the Central nervous system (CNS) and the periphery by lymphopoietic cells (Ziebell, & Morganti-Kossman, 2010; Minagar et al., 2002). Interleukin-10 (IL-10) is a regulatory cytokine that maintains the anti-inflammatory environment within the CNS (Xin et al., 2011). The neuroprotective characteristics of IL-10 include the suppression of microglia and astroglia activation, as well as decreased production of pro-inflammatory cytokines (Ziebell, & Morganti-Kossman, 2010). IL-10 works through glial cells to regulate the anti-inflammatory environment to protect motor neurons (Xin et al., 2011). However, any neuroprotective effects of IL-10 appear to be dose- and site-specific, while the type of TBI model also seems to influence the effects of IL-10 (Patterson, & Holahan, 2012). The

role of IL-10 following concussion is not well understood. Due to the many different types of TBI seen in both clinical and nonclinical research, it is difficult to pinpoint the exact role IL-10 plays in response to a concussion (Patterson & Holahan, 2012).

Tumor necrosis factor- α. TNF is a pro-inflammatory cytokine that initiates and regulates the cytokine cascade during an inflammatory response (Perry, Collin, Weiner, Acton, & Go. 2001). The role of TNF in CNS inflammation is not well understood. Because of the low levels of TNF seen in the brain, it is difficult to determine the exact role it plays in physiological conditions. Though, it has been reported that TNF is released after a concussion occurs (Shojo et al., 2010; Patterson, & Holahan, 2012). And while it appears that the initial release of TNF-α following a concussion may be harmful, a delayed chronic release has been seen to be beneficial (Scherbel et al., 1999). Even still, the relationship between the severity of the concussion and the level of release of TNF and how that affects its overall properties is still unclear (Patterson, & Holahan, 2012).

TBI and Disabilities

Disabilities related to TBIs may not only be physical, but psychological as well. Psychological disorders related to anxiety and depression is commonly associated with the occurrence of TBIs, and as well as with each other. Studies have shown that anxiety disorders and depressive disorders co-occur over 50% of the time (Olfson et al., 1997; Hirschfeld, 2001). TBIs create an inordinate amount of both psychological and physiological stress on the body and mind. Behavioral changes as a result of TBIs are the manifestation of both psychological and physiological stress.

Depression. Depression is a disorder that manifests as feelings of sadness or worthlessness, lethargic behavior, and a lack of pleasure (American Psychiatric Association, 2013). There is a plethora of research that supports that TBI's increase the probability of experiencing depression (Kim et al., 2007; Whelan-Goodinson, Ponsford, Johnston, & Grant, 2009). In fact, Jorge et al., (1993) found that 42% of patients in their study experienced depression following a TBI. While another study found 53% of its patients suffered from depression (Bombadier et al., 2010). Furthermore, patients can still experience depressive symptoms even if they do not reach the clinical threshold. A majority of patients in two studies were experiencing depressive sympotoms (Seel, & Kreutzer, 2003; Seel et al., 2003).

While the prevalence of depression post-TBI is well documented, the predictors of post-traumatic depression are not as well documented. Many risk factors associated with the likelihood of post-traumatic depression have been tested, the most common being age, gender, education, TBI severity, marital status, history of psychiatric disorders, and history of alcohol and substance abuse (Guillamondegui et al., 2011; Cnossen, 2017). However, past research has yet to find a clear answer to what risk factors accurately predict depression. In fact, several human studies have found that TBI severity is not predictive of psychiatric illnesses like depression (Malec, Brown, Moessner, Stump, & Monahan, 2010; Bryant et al., 2010; Baecher et al., 2018). Furthermore, animal studies have found similar results (Schwarzbold et al., 2010; Washington et al., 2012), and while they found mild TBI resulted in depressive-like behavior, more severe TBI did not result in such behavior even though the more significant TBIs had noticeable

hippocampus damage with greater tissue and neuronal loss. On the other hand, Levin et al., (2005) found that TBI patients that had abnormal CT scan results were 8 times more likely to have depression than a patient with a normal CT scan result. However, some animal studies have found that extensive tissue damage from lesions, even those that are seen 90 days after the initial injury may not predict depression-like behavior (Schwarzbold et al., 2010; Bajwa et al., 2016).

The reason that some lesions do not produce depressive-like behavior could be due to the timeframe of the studies. For example, the study may have taken place after the acute trauma phase of the TBI. Jorge et al, (2003) found that lesion location no longer significantly correlated with the manifestation of depression after the first three months. The acute phase or hypoglycolisis phase takes place immediately after hyperglycolisis when glucose metabolism and oxidative metabolism are at their lowest. The location of the lesion may also be a reason (Vataja et al., 2004). Bajwa et al., (2016) noted that the mice that underwent closed cortical impact (CCI) were impacted directly above the motor cortex while the mice that underwent closed head injuries (CHI) exhibited depression like behavior after being impacted on the skull above the frontal-parietal region. In some human studies of depression, lesions were seen in the left hemisphere of the frontal lobe, particularly in the prefrontal cortex (Jorge et al., 2004; Levin et al., 2005). Reviews of depression neurobiology and TBIs have noted lesions and decreased grey matter volume and white matter volume in those areas as well as the basal ganglia (Dougherty, & Rauch, 1997; Jorge et al., 2004; Rao et al., 2010).

The pathophysiology of depression post-TBI is complex, and is not fully understood. However, the cascade of events that occur after a TBI leave the brain vulnerable to secondary injuries. Due to the increase in prevalence post-TBI the answers to how depression occurs may lie in the processes that lead the brain to become vulnerable. Post-TBI, the increased immune response following the TBI would increase stress leading to over activity in the sympathetic nervous system which would rapidly increase the release of pro-inflammatory cytokines (Michopoulos, et al., 2017; Bodnar, Morganti, & Bachstetter, 2018). The disinhibition of pro-inflammatory cytokines may further create an environment that produces symptoms associated with depression and anxiety by affecting the activity and connections of the regions associated with those disorders ((Dougherty, & Rauch, 1997; Miller, Haroon, Raison, & Felger, 2013; Rowe et al., 2016; Michopoulos, et al., 2017). These responses may become chronic over time due to the dysregulation of the neuroendocrine system and the HPA axis, causing a reduction of corticosterone, which may promote further inflammatory activity (Rowe et al., 2016; Michopoulos, et al., 2017).

Pro-inflammatory cytokines may alter the function of neurotransmitters metabolism involving monoamine such as serotonin, norepinephrine, and dopamine (Raison, Capuron, & Miller, 2006). Past research has shown that inflammatory cytokines reduces the expression of some monoamine transporters, while at the same time increasing the expression of dopamine and serotonin (raison et al., 2006). The reduced expression of some of the transporters limits the amount available for use leading to an eventual decline (Hasler, 2010).The decline of neurotransmitters such as serotonin and

dopamine would lead to what past research calls the monoamine deficiency hypothesis, which states that depressive pathology is caused by a shortage in available monoamines neurotransmitters (Hasler, 2010).The monoamine deficiency hypothesis could be viable post-TBI through the process of spreading depolarization. As was previously stated, during spreading depolarization the cells swell and release all neurotransmitters contained in the cell, and in this instance the pro-inflammatory cytokines could disrupt the transporters of the monoamine neurotransmitters which would eventually lead to a decline in expression once the monoamine neurotransmitters that had been dispelled by the cell were used.

Another neurotransmitter process that is affected by a TBI is the glutamate metabolism. Glutamate metabolism is a process by which glutamate (Glu) is put through a cycle of being metabolized to and from glutamine (Guerriero, Giza, & Rotenberg, 2015). During the process, Glu binds to NMDA and AMPA receptors, and then is removed by astrocytes. The binding of Glu to those receptors allows for Ca++to enter the neuron (Guerriero, Giza, & Rotenberg, 2015). Furthermore, past research has noted that the astrocytes that are responsible for the reuptake of Glu become blocked post-TBI (Gurrieroet al., 2015), which leads to unregulated levels of Glu. The unregulated levels of Glu leads to excitotoxicity, which past research has noted to exacerbate pro-inflammatory reactions through the creation of reactive oxygen species (Miller, & Raison, 2016).The inhibitory neurotransmitter GABA is created in the astrocytes that regulate Glu (Guerriero et al., 2015; Lener et al., 2017). Disruption of the Glu and GABAergic systems have been profiled in depression as an issue with increased levels of Glu, as well

as with anxiety (Swanson et al., 2005; Martin et al., 2009; Hasler, 2010; Shin, & Liberzon, 2010; Sanacora et al., 2012).

Anxiety. Anxiety is characterized by the DSM 5 as a person experiencing an excessive amount of fear, whether it is real or perceived (American Psychiatric Association, 2013). It is common knowledge that, like depression, the likelihood that a person experiences anxiety increases after a TBI (Hiott, & Labbate, 2002; Moore, Terryberry-Spohr, & Hope, 2006). In fact, a cohort of over 5000 participants experienced general anxiety disorder (GAD) at a rate of almost 6% (Kessler et al., 2005), while other studies have noted that the prevalence of GAD for people who had experienced a TBI ranged from 12% to 68% (Fann, Uomoto, Katon, & Esselman, 1995; Moore et al., 2006; Sharma, Sharma, Jain, Mittal, & Gupta, 2015).

Studies show that anxiety is highly comorbid with depression, and they share many of the same findings due to that high rate (Moore et al., 2006; Bryant el al., 2010). TBI severity has not been observed as a predictor of either anxiety or depression (Hiott, & Labbate, 2002; Bryant et al., 2010). In fact, Baecher et al., (2018) found that severity did not predict anxiety or depression; however, more severe TBIs were predictors for post-traumatic stress disorder (PTSD). Thus, it is plausible to believe the anxiety manifests itself in a variety of ways depending on the area of the brain that has been damaged. Evidence supports this theory, as anxiety has been observed in patients with lesions in both the left and the right hemispheres (Moore et al., 2006; Knutson et al., 2013; Sharma et al., 2015). In fact, a study found that lesions were present in 33% of patients that had been diagnosed with anxiety. However those lesions were not localized

to a singular area of the brain as multiple areas including the left frontal lobe, the subcortical of the left hemisphere, the right parietal lobe, and the right temporal lobe were observed to have those (Sharma et al., 2015). Animal models have also shown that anxiety is not predicated on lesions being localized to one area of the brain, as damage to both hemispheres has been observed in the presence of anxiety (Almeida-Suhett et al., 2014; Bajwa et al., 2016; Qu, Liu, Xie, Li, & Xu, 2016; Tucker, Fu, McCabe, et al., 2016).

Damage to the limbic system and the circuits that connect them could possibly lead to dysregulation of mood and behavior which could eventually lead to clinically diagnosable disorders such as those listed under anxiety disorders. Particularly, there is evidence that damage to the amygdala from TBI can result in anxiety (Bryant, 2008), as was seen in a study by Almeida-Suhett et al., (2014), where anxiety-like behavior in mice coincided with a decrease in the inhibitory neurotransmitter GABA in the amygdala after a TBI.

The physiology of fear and anxiety based disorders post-TBI is complex, as there are numerous possibilities in how they manifest themselves. The literature states that fear and anxiety symptomology appear in the months following a TBI (Jorge et al., 2003). Additionally, neuro-inflammation may play a key role in the manifestation of anxiety. Literature has linked aspects of the neuro-inflammatory response to the manifestation of fear and anxiety related symptomology and disorders (Michopoulos, Powers, Gillespie, Ressler, & Jovanovic, 2017). The increased immune response following the TBI would increase stress leading to over activity in the sympathetic nervous system which would

increase the release of pro-inflammatory cytokines (Michopoulos, et al., 2017). These responses may become chronic over time due to the dysregulation of the neuroendocrine system and the reduction of corticosterone, which may promote further inflammatory activity (Rowe et al., 2016; Michopoulos, et al., 2017).

Furthermore, the disinhibition of pro-inflammatory cytokines may further create an environment that produces symptoms of fear and anxiety disorders by affecting the activity and connections of the regions associated with those disorders (Rowe et al., 2016; Michopoulos, et al., 2017). Particularly, damage to the limbic system, which includes structures such as the hippocampus, the hypothalamus, and the amygdala could result in anxiety (Rajmohan, & Mohandas, 2007; Bryant, 2008). Damage to the hippocampus, the controlling mechanism for the HPA axis, coupled with the increase in norepinephrine leads to an increase in inflammatory cytokines (Rajmohan, & Mohandas, 2007; Martin, Ressler, Binder, & Nemeroff, 2009; Michopoulos, et al., 2017; Kokiko-Cochran, Godbout, &Tapp, 2019).

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A possible cause of Anxiety disorders resulting from the excessive release of pro-inflammatory cytokines could be their effect on Glutamatergic and GABAergic pathways in the Limbic system (Martin et al., 2009). As was previously stated, the cycles that create Glu and GABA are disrupted after a TBI causing an increase in Glu and a decrease in GABA. Furthermore, a decrease in GABA has been observed in patients that were diagnosed with GAD (Martin et al., 2009).

Animal Models of TBI

Animal models such as controlled cortical impact (CCI), fluid percussion, and impact acceleration models have been used considerably to study TBIs and advance the understanding of the neural pathology of brain injuries (Patterson & Holahan, 2012). The CCI procedure can be either an open head or closed head TBI. The open head model involves making an incision in the skin on the mouse's head to expose the skull and subjecting the mouse to a single impact on the exposed skull by an impactor (Laurer et al., 2001). Whereas in the closed head model of mTBI, the skin is not cut open and the skull is not directly impacted (Osier, & Dixon, 2017). The fluid percussion injury method involves the use of a small incision in the skull, above the parietal cortex. After the incision is made a small tube is placed into the brain, in which fluid pressure produces displacement and deformation of the neural tissue through degrees of shearing and contusion (Kabadi, Hilton, Stoica, Zapple, & Faden, 2010). The impact acceleration model is also known as the weight-drop model. Using this method, a weight is dropped through a tube, from any given height, onto the head of the subject (Xiong, Mahmood, & Chopp, 2013; Johnson, Meaney, Cullen, & Smith, 2015; Bodnar, Roberts, Higgins, & Bachstetter, 2019).

Weight-Drop Model. The Marmarou weight drop model is a common impact acceleration model used to induce TBI. Compared to the other models, the weight drop model is by far the least invasive of the models and when done correctly, the most efficient. The other models also require a form of surgery which makes them far more invasive and more likely that the rodent does not survive the procedure. While surgery

could be performed for a study that uses a weight drop, it is not a necessity. In the Marmarou weight-drop model, the rodent is anesthetized and an incision is made in the rodent's skin to expose the skull, once exposed a steel disc is attached. The rodent is placed on a foam pad underneath a Plexiglas tube from where a brass weight falls onto the steel disc attached to the head of the rodent (Cernak, 2005; Marmarou et al., 1994). Unfortunately the Marmarou weight drop model produces a mortality rate of 44%. And because of the high mortality rate, the Marmarou model was modified in an attempt to lower the mortality rate (Mychasiuk et al., 2014; Kane et al., 2012). The modified Marmarou model with free fall places the rodent on a sheet of aluminum foil under a plastic tube that a weight will drop through (Mychasiuk et al., 2014; Kane et al., 2012). Underneath the aluminum foil is a foam pad. When the animal is struck by the weight it drops through the sheet of aluminum foil onto the pad. The primary rationale for this method is to create a more efficient model that more accurately represents the pathophysiology and symptomology of a concussion or repetitive TBI while keeping the mortality rate at an acceptable level (Mychasiuk et al., 2014; Kane et al., 2012).

TBI Models of Animal Disability

To understand and work toward future treatments of these disabilities, it is important to be able to recreate them in a laboratory setting. While it is highly unethical to purposely give humans concussions by any means, animal testing, however, is an accepted way to mimic human concussion (Cernak, 2005). Nevertheless, animal models do have certain limitations since they cannot fully mimic human symptomology and behaviors. There is also no way for the researcher to know for certain if an animal is

feeling afraid, anxious, or depressed. Despite those limitations depression, anxiety, and irritability are serious outcomes of TBI in humans and require a continued and concerted effort with the methods at hand (Crawley, 2007).

Certain outcomes seen in human concussions are tested in animal models using different behavioral tests; these tests can gauge aspects of locomotor coordination, anxiety, and depression among others. Testing for anxiety and depression associated with mild or moderate TBI would ideally include no or very little impairment in locomotor activity, meaning that physically there would be no adverse effects due to the TBI. The hypothesis would be that if there isn't motor impairment, changes in movement can be interpreted to indicate anxiety- or depression-like behavior.

Anxiety. Emotional impairments following TBI have been studied with tests like the open field (OF) task and the zero maze (ZM). These tasks tap into the aversion that rodents (e.g., rats, & mice) have for bright and open spaces (Seibenhener, & Wooten, 2012). It is theorized to be a fight or flight response brought on by evolutionary processes to avoid predators. Hyperactive behavior has been noted in several studies that tested mice in the open field maze post-TBI. However, it is thought to be independent of anxiety-like behaviors since those same mice that have shown hyperactive behavior were seen to also exhibit anxiety-like behavior later on (Schwarzbold et al., 2010; Wakade, Sukumari-Ramesh, Laird, & Vender, 2010; Tucker et al.,, 2016). For example, Yu et al., (2012) witnessed that the mice travel a greater distance around the edges of the open field. However the mice also spent less time in the center of the open field, suggesting that even while hyperactive the mice were less willing to explore away from the edge.

The severity of TBI may not play a role in whether or not hyperactivity is displayed. Bajwa et al., (2016) found that mice subjected to more severe TBI did not display hyperactive behavior in the open field while less severe TBI did. Hyperactivity in rats is less common. O'Connor, Heath, Cernak, Nimmo, & Vink, (2003) found that rats subjected to TBI displayed anxiety-like behaviors, which lasted for the entirety of the study (4 weeks). Anxiety-like behavior similarly has been observed in several other studies using both rats and mice (Vink, O'Connor, Nimmo, & Heath, 2003; Fromm, Heath, Vink, & Nimmo, 2004; Ajao et al., 2012; Budde et al., 2013; Heldt et al., 2014; Semple, Zamani, Rayner, Shultz, & Jones, 2019).

The ZM has the same purpose as the OF task, as it also tests for anxiety-like behaviors, but instead of an arena it gives the rodents an option of exploring open areas or staying in the walled areas. Using the zero maze, the mice have the option of going into and staying in an enclosed space instead of one of the open sections. However, the ZM has not been utilized in many studies involving TBI. The elevated plus maze (EPM), which is the original version of the ZM has been utilized much more often across the literature. Out of the 12 single TBI studies listed in two literature reviews, the ZM is only used 4 times whereas the elevated plus maze is used 8 times (Malkesman, Tucker, Ozl, &McCabe, 2013; Bodnar et al., 2019). In a meta-analysis of over 400 animal studies related to TBI, the ZM is only mentioned briefly (Semple et al., 2019). However, the research seems to suggest that the ZM is a valid alternative to the EPM, as research has found the two tests to be comparable to each other (Shepherd, Grewal, Fletcher, Bill, &Dourish, 1994; Braun, Skelton, Vorhees, & Williams, 2011). Past research has shown

anxiety-like behavior to be present in rodents that kept to the enclosed sections of the ZM, which was similar to what was seen in rodents when tested in the OF task (Ajao et al., 2012; Tchantchou et al., 2012). The ZM was used instead of other models of anxiety, like the EPM, due to past research showing that the ZM promotes explorative behavior (Tucker, &McCabe, 2017); this may be due to the design of the ZM. The ZM allows for easier movement than the EPM because it allows the mice to keep moving instead of having to stop and turn around.

Depression. Depression, commonly observed after TBI, can be assessed using the Porsolt forced swim test (FST). The FST is a test of despair-like behavior that looks at the time it takes for the rodents to give up on escaping and become passive (Kimbler et al., 2012). Animal testing using the FST seems to provide support for the theory that TBI severity does not predict depression severity. Washington et al., (2012) found observed depression-like behavior in their mild, moderate, and severe TBI conditions, and a slight decrease in immobility in the sham injury group. Additionally, none of the TBI groups were significantly different from each other. A number of other studies have observed depressive like behavior in rodents after TBI as well (Semple et al., 2019; Zohar, Rubovitch, Milman, Schreiber, & Pick, 2011). While there are studies that have found null results there is evidence to suggest that the FST is a valid test of depression like behavior.

Motor Coordination. Some studies that tested for physical impairment used a rotarod device. Many such studies that have tested for motor coordination impairments after TBIs using the rotarod have observed deficits in the length of time the animals were

able to stay on the device (Mouzon et al., 2013; Yang, Gangidine, Pritts, Goodman, & Lentsch, 2013; Yang et al., 2013). As it was stated above, since it is impossible to ask the rodents whether they feel depressed or not, physical impairment brought on by TBI makes the interpretation of movement or lack thereof more difficult. Therefore, having multiple measures of motor coordination or locomotor activity is an important check to have when testing behavior post-TBI.

Current Study

The current study was designed to test the validity of the modified Marmarou weight drop model when only a single TBI is administered (Mychasiuk et al., 2014; Kane et al., 2012). Previous research sought to create a repeated TBI model that would be more replicable to lower the mortality rate of the rodents used. While the mortality rate of the original Marmarou model was 44% and 69% using two different weight levels (Marmarou et al., 1994), the new modified models succeeded in producing lower mortality rates: 3.4% with a single TBI (Mychasiuk et al. 2014), and 5% with a repeated TBI (Kane et al., 2012). Furthermore, the model used by Kane and colleagues (2012), when replicated in a single TBI model has produced a rate of 0% mortality (Yu, Wergedal, Rundle, & Mohan, 2014). The numbers support that this model has been very successful in reducing mortality. Yet, those previous studies have mainly looked at the replicability of administering multiple TBIs, but also have done little in the way of behavioral testing. The present study used a variety of behavioral tasks that are specific to anxiety and depression, as well as a motor coordination task, to create a starting point for future anxiety and depression research using this model of TBI. Specifically, the present

study used the zero maze (anxiety-like behaviors), the open field task (anxiety-like behaviors), the rotarod (motor coordination), and the forced swim task (depression/ despair-like behaviors) to assess the trauma recovery by the mice. The abnormal behaviors and deficits in motor coordination seen in past research support the theories that chronic neuroinflammation can have negative effects on brain function (Dantzer et al., 2008). Others have shown that rodents that have been put through either the open field or rotarod task experience motor deficits caused by the TBI (Mourtney et al., 2017; Yang, Gangidine et al., 2013; Yang et al., 2013). In the open field task, a lack of movement or freezing could mean an increase in the levels of anxiety; therefore, measuring changes in motor behavior is important. Additionally, mice that have been put through the zero maze have shown similar anxiety-like behaviors to those in the open field task (Elder et al., 2012). The open field and zero maze tasks can be used to test the validity of the other, since the behaviors seen in both tasks have been shown to correlate (Díaz-Morán et al., 2014). Studies that have used the forced swim task to test for depressive-like behavior have observed significant differences between control and TBI groups (Washingtn et al., 2012; Zohar et al., 2011). Using this version of Marmarou's impact acceleration model along with these behavioral tests will help identify the strengths and weaknesses of the model and whether it is appropriate to use for single TBIs.

Hypotheses

Hypothesis 1. It was predicted that the manifestation of anxiety-like behavior in the ZM would be dependent on the severity of the TBI. Anxiety-like behavior was

analyzed as the amount of time spent (duration) in the open area. Furthermore, it was predicted that the more severe TBI group would spend the least amount of time in the open area. Locomotor activity in the ZM was used as a control to measure whether the TBI negatively affected the ability of the mice to move, but locomotor activity among the groups was not expected to be significantly different.

Hypothesis 2. It was predicted that the manifestation of anxiety-like behavior in the OF task would be dependent on the severity of the TBI. Anxiety-like behavior was analyzed as the amount of time spent (duration) in the center of the arena. Furthermore, it was predicted that the more severe the TBI group would spend the least amount of time in the center of the OF arena. Locomotor activity in the OF was used as a control to measure whether the TBI negatively affected the ability of the mice to move, but locomotor activity among the groups was not expected to be significantly different.

Hypothesis 3. It was expected that the manifestation of depression/despair-like behavior would be dependent on the severity of TBI. Thus, mice exposed to more severe TBI would not only become immobile faster, but immobile longer, compared to the control and less severe TBI group.

Hypothesis 4. It was predicted that deficits in motor coordination would be severity dependent. The more severe the TBI groups who experience greater TBI will spend less time on the rotarod compared to those with no or less severe TBI.

Methods

Subjects

Thirty-two mice (C57bl6/j), at least 90 days old (24 to 35 grams) that were born in the Stephen F. Austin State University mouse colony were separated into three groups. The groups were split into the 95g TBI group (*n*=11), the 30g TBI group (*n*=11), and the sham injury group (*n*=10). The mice in the sham injury group, like the name suggests were not subjected to the weight drop, they were anesthetized and placed on the platform where they fell into the container, but they were not subjected to the weight drop. Mice were housed in groups of two in clear 10.25 inch x 6 inch x 4.5 inch (l x w x h) polycarbonate cages lined with Kaytee Kay-Bob pet litter. All mice were given free access to food and water. The colony room was temperature controlled at around 72 degrees Fahrenheit. The mice experienced a 12hr light-dark cycle. All sessions occurred during the light cycle. All experimental protocols were approved by the Stephen F. Austin State University Institutional Animal Care and Use Committee and followed the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996).

Materials

TBI model. The modified weight drop consisted of a 42.9 cm x 29.2 cm x 23.8 cm platform that had multiple layers; the top layer was a sheet of Reynolds Wrap aluminum foil that covered most of the top of the platform, the second layer consisted of 3 22.2 cm x 12.1cm x 6.4 cm foam sponges (Appendix E – 5). The present TBI model looked to replicate the modified Marmarou model used by Kane and colleagues (2012) in

a single TBI format. To do that the 95gm weight, along with the height of the drop were applied to the present study. Furthermore, past research has observed a mortality rate of zero when dropped at the current height (Yu, Wergedal, Rundle, & Mohan, 2014) as they found a mortality rate of zero in the mice subjected to just a single impact. Past research has also found the 30 gm weight to be among the most commonly used weights in the weight drop model (Bodnar et al., 2019). Therefore, we chose to use both the 95 and 30 gm weights for this study. The designated weight was dropped vertically through a tube (100 cm) made of PVC pipe held up by two clamps attached to a stand and were positioned 2.5 cm above the platform. A metal tip (2 x 10 mm) was attached to the bottom of the weight to minimize the initial impact area. The top of the weight was tethered to a metal beam that was stationed above the tube using Solutions Berkley Braided 30lb fishing line so that there was not a possibility of a second impact with the mouse's head.

Before the weight drop all cages were brought out of the colony and put in the designated testing space where one mouse from the first designated cage was weighed and then anesthetized by using a 20% isoflurane and 80% propylene glycol solution. The mice were anesthetized by placing them into a container for 2 to 5 minutes where a gauze was soaked in 5ml of the mixture. The mice were monitored until they were unable to right themselves for an extended period. Anesthesia was maintained during the weight drop by transferring the mouse to a 50 ml tube with a gauze inside it dampened with 2 ml of the isoflurane mixture and placing the head of the mouse into the tube. Mice in each condition (95 gm, 30 gm, and sham TBI) were anesthetized.

While anesthetized the head of the mouse was quickly placed in a jig stationed directly under the middle of the 100 cm tube. The jig made it easier to accurately place the mouse. Once the mouse had been accurately placed, the weight was dropped. After the mouse was struck by the weight, the mouse fell through the aluminum foil onto the pad. Once trauma was induced the mouse was placed into its cage. The mice were housed in pairs according to condition so that there would be no interactions between the conditions, which might confound the study. After exposure to TBI the mice were continuously monitored until the mice were out of danger. Furthermore, the mice were tested on the 1st, 2nd, 6th, and 7th day after the weight drop. On each testing day, the mice were run through the zero maze, open field task, rotarod, and the forced swim task in that order. All tests were conducted after weight drop; there were no training phases done beforehand.

Procedure

Several assessments were performed by the mice on multiple days post-TBI to test depression and anxiety-like behaviors. Previous research has stated that the sequence of tests should place the least invasive or aversive tests first followed by the more invasive or aversive tests (Voikar, Koks, Vasar, & Rauvala, 2001; Voikar et al., 2004), this is was done to reduce stress levels in the mice so that the least aversive tests were not affected by the stress placed upon the mice in the more aversive tasks. So testing order went as followed: First was the Zero Maze (ZM), then the Open Field Test (OF), the Rotarod, and finally the Forced Swim Test (FST). Testing was performed first on days 2 and 3, then a three day inter-trial interval with testing resuming on days 7 and 8 post-TBI.

The apparatus' will be cleaned between each trial for every test. The Noldus EthoVision XT tracking program was used to track the mice in the zero maze, open field test and forced swim test as well as do partial analysis for those tasks.

Zero maze. The zero maze apparatus was an elevated (94 cm) circular platform measuring 52 cm in diameter which included a 7 cm path (Appendix E – 1). The apparatus included closed and open sections, with the open sections of the maze being comprised of sets of walls measuring 20.3 cm high on both the outer and inner edges of the platform. The maze was illuminated in such a way that it provided similar light to both open and closed quadrants. At the start of each trial a mouse was placed at the entrance to one of the closed sections, facing an open section of the Zero maze, and allowed to roam for 5 minutes. The Noldus separated the maze into four sections and designated where the center of the mice was. The Noldus then tracked where, as well as how long the mice stayed in a particular section. The mouse was considered in one section or another if the majority of the mouse was in that section. After 5 minutes had passed the mouse was placed back into its cage. Anxiety-like behavior was measured and interpreted as the amount of time spent in the closed area.

Open field test. The open field apparatus was a square metal pan (30.5 cm x 30.5 cm x 7.62 cm) (Appendix E – 2). The pan was covered with a transparent panel made of Plexiglas. Mice were placed on the edge of the arena and allowed to roam for 5 minutes. Once 5 minutes had passed the mouse was placed back in its cage. Anxiety like behavior is the main dependent variable for this task. The mice were scored in two areas of interest; the outer area that was closest to the wall of the pan, and the center area. The

size of the center area was around 15cm x 15cm. The Noldus tracked the center of the mice for time and location on the edge of the arena, and in the center of the arena. Anxiety-like behavior in the mice was determined by the amount of time spent in the center of the arena

Rotarod. A rotarod device (ENV-571M) was utilized to test motor coordination deficits (Appendix – 3). The device was comprised of a singular (7.62 cm) lane, given the current space on the rotarod, only one mouse was run at a time. Motor coordination in the rotarod was measured as the amount of time the mice were able to hold on to the rod. The mice were placed on the rotarod while it was set to the lowest setting, and then it was switched over to the run setting to begin the trial. Once the trial had begun, the rod gradually increased its rotational speed from 4 rotations per minute (rpm) to 40 rpm over 300 seconds or until the mouse fell off.

Forced swim test. The Porsolt forced swim test (Porsolt et al., 1977) was used to assess depression-like behavior. Mice were individually placed into a cylindrical container (diameter 10.95 cm, height 25.24 cm) and forced to swim (Appendix E – 4). The container contained enough water so that the mouse could neither escape the container nor touch their tail to the bottom of the container to hold themselves up. The water in the container was at around 25 degrees Celsius. The Noldus was used to track immobility in the FST. The Noldus used the programs activity detection software to track the center of the mouse as well as differentiated between highly mobile (swimming and climbing/escaping) and immobile (floating).

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software. Multiple mixed design factorial analysis of variance (ANOVA) were performed to test dependent variables across the four testing days. The assumption of sphericity for running a mixed-design ANOVA was tested using Maulchy's test of sphericity. If the assumption of sphericity was found to be violated, the conservative estimate of Greenhouse-Geisser was used in the tests of within-subjects results (Field, 2013).

Results

Zero Maze

Hypothesis 1. It was predicted that the manifestation of anxiety-like behavior in the ZM would be dependent on the severity of the TBI. A measure of locomotor activity was used to determine how well the groups could move by observing the distance travelled in the ZM. The purpose of these measures was to ensure that the behavioral anxiety measures were not confounded by motor deficits. Anxiety-like behavior was analyzed as the amount of time spent (duration) in the open area.

Firstly, A 3 (Condition: 95 gm, 30gm, sham) x 4 (Testing day: 1, 2, 3, 4) x 10 (Time: ten 30 second bins) mixed design ANOVA was run to analyze distance travelled by each group to see if the locomotor activity was impacted by TBI severity. There was a significant main effect of day, $F(2.28, 63.94) = 8.69$, $p < .001$, $\eta_p^2 = .237$ (Figure 1.), showing a downward trend after day 1, with a slight increase in the distance travelled after the 3 day inter-trial interval on day 3. There was a significant main effect of time, $F(4.14, 115.80) = 4.04$, $p = .004$, $\eta_p^2 = .126$ (Figure 2.), There was a decrease in the distance travelled after the first bin in which the mice travelled around the same distance each of the remaining bins. The main effect of condition was not significant, $F(2, 28) = 1.31$, $p = .287$, $\eta_p^2 = .085$, meaning that TBI severity did not play a role in the distance travelled in the zero maze (APPENDIX A-4).

The two-way interaction between day and condition was not significant, $F(4.57, 63.94) = .92$, $p = .471$, $\eta_p^2 = .061$ (APPENDIX A-1). The two- way interaction between

time and condition was also not significant, $F(8.27, 115.80) = .83$, $p = .584$, $\eta_p^2 = .056$ (APPENDIX A-2), and the two-way interaction between day and time was not significant either, $F(12.71, 355.90) = .97$, $p = .476$, $\eta_p^2 = .034$ (APPENDIX A-3). Lastly, the three- way interaction between day, time, and condition was not significant, $F(25.42, 355.90) = .85$, $p = .678$, $\eta_p^2 = .057$.

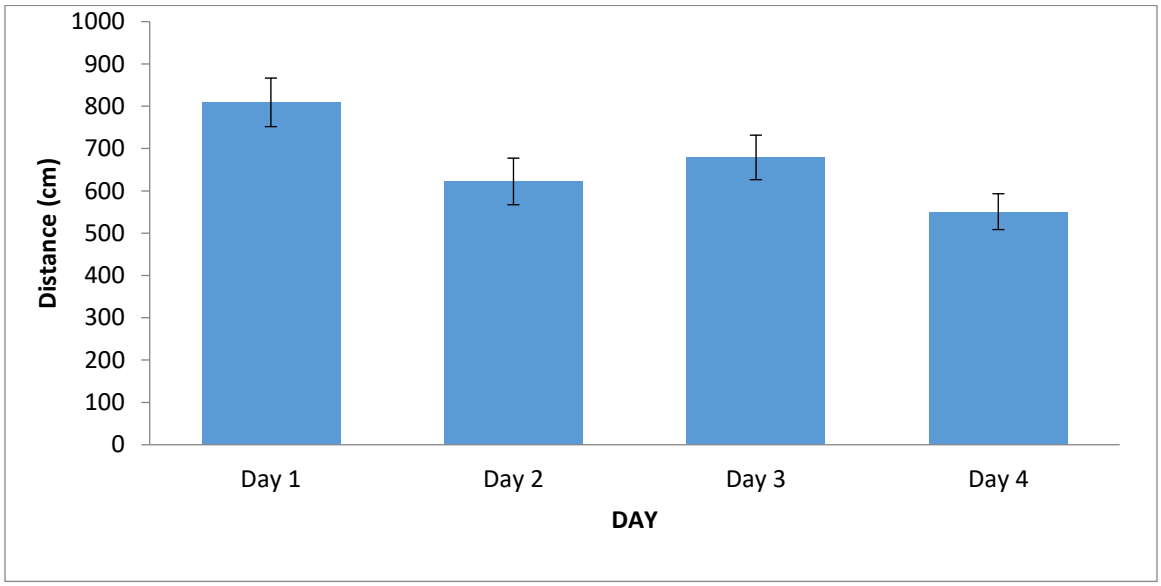

Figure 1. Mean of total distance travelled and standard error across testing days in the ZM. Error bars indicated +/- range of one standard error.

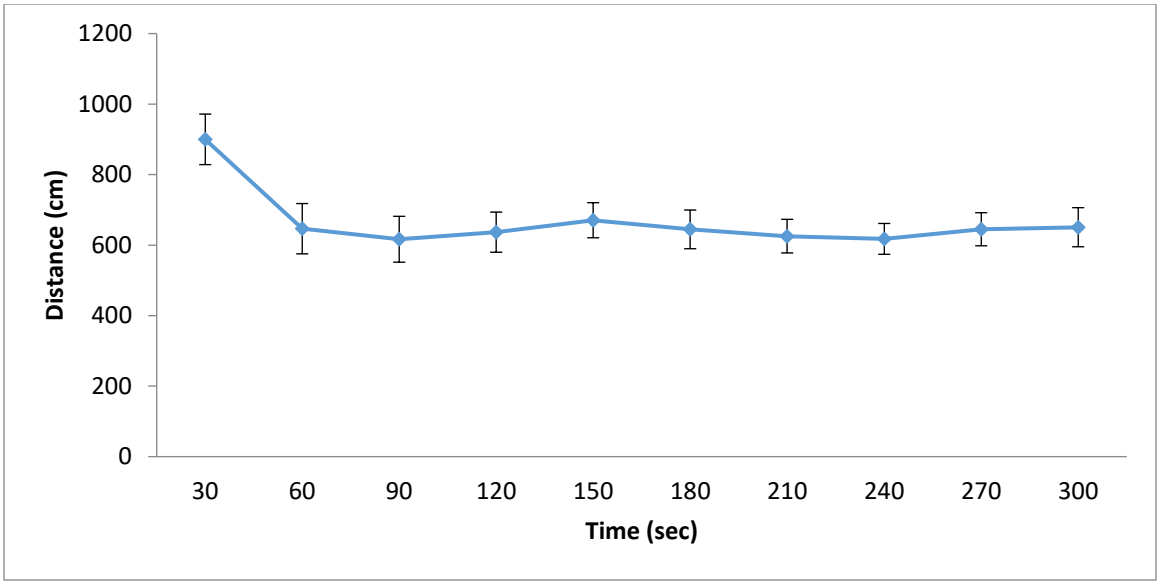

Figure 2. Mean of total distance travelled and standard error across each 30 second bin in the ZM. Error bars indicated +/- range of one standard error.

A 3 (condition) x 4 (day) x 10 (time) mixed design ANOVA was used to analyze the time spent in the open area of the ZM over the four day testing period. There was a main effect of day, $F(2.\ 06, 57.67) = 42.06$, $p < .001$, $\eta_p^2 = .600$ (Figure 3.). The time that the mice spent in the open area dropped significantly after the first day of testing, and then continued to drop even after the inter-trial interval. There was also a main effect of time**,** $F(9, 252) = 5.85$, $p < .001$, $\eta_p^2 = .173$ (Figure 4.). The mice spent the most time in the open area at the start of the trial during the first 30 seconds, and then the time spent in the open area became similar in every bin for the rest of the trial. However, the main effect for condition was not significant, $F(2, 28) = 1.57$, $p = .227$, $\eta_p^2 = .101$ (APPENDIX A-7), meaning that there were no differences between the conditions.

The two-way interaction between day and condition was not significant, $F(4.12, 57.67) = .49$, $p = .750$, $\eta_p^2 = .034$, showing that the conditions did not impact the

behavior over the 4 trials (APPENDIX A-5). The two-way interaction between time and condition was also not significant, $F(18, 252) = .93$, $p = .544$, $\eta_p^2 = .062$, showing that condition did not impact behavior over the course of each trial (APPENDIX A-6). Furthermore, the two-way interaction between day and time was significant, $F(10.79, 302) = 2.04$, $p = .025$, $\eta_p^2 = .068$. The interaction shows that the average time spent in the open area during each bin significantly dropped after the first day of testing, and continued to drop with each subsequent trial (Figure 5.). The three-way interaction between day, time, and condition was not significant, $F(21.57, 302) = .89$, $p = .609$, $\eta_p^2 = .060$.

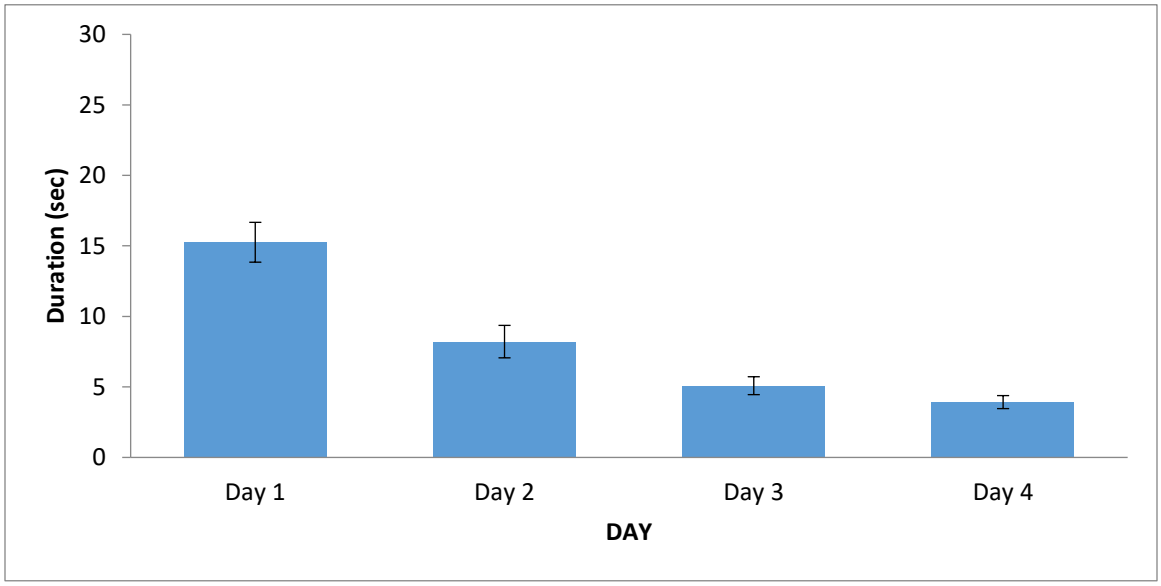

Figure 3. Mean of total time spent in the open area and standard error based across each testing day in the ZM. Error bars indicated +/- range of one standard error.

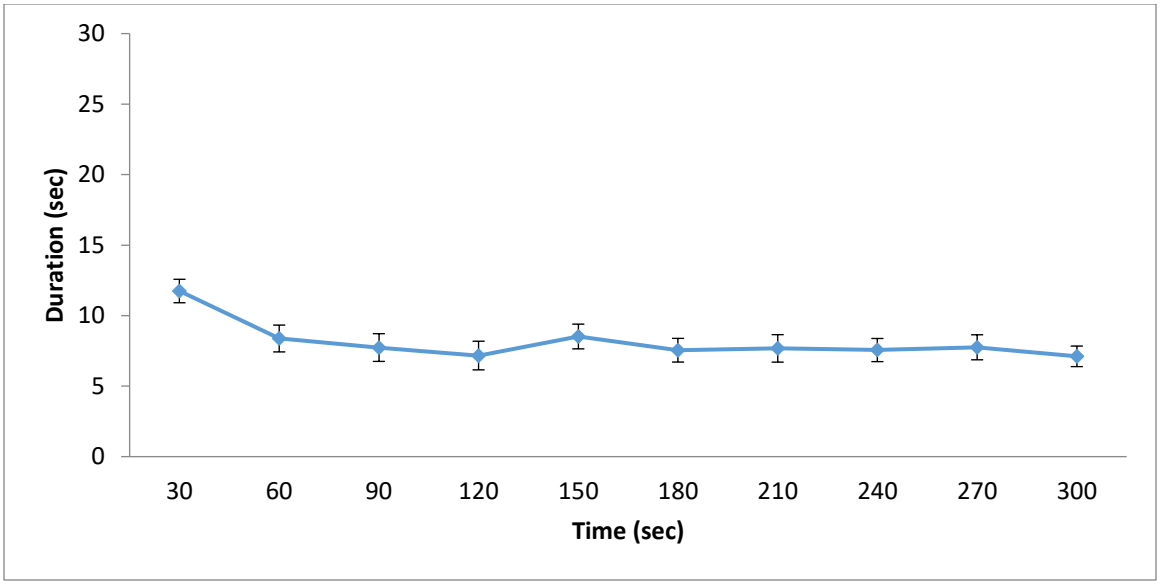

Figure 4. Mean of total time spent in the open area and standard error across each 30 second bin in the ZM. Error bars indicated +/- range of one standard error.

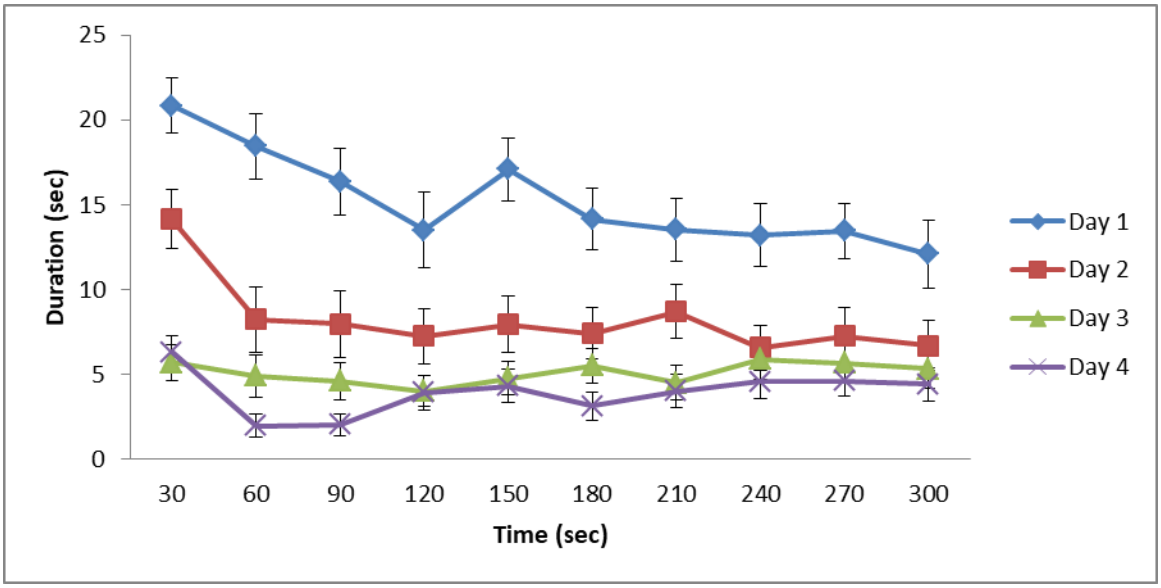

Figure 5. Mean of total time spent in the open area and standard error across Time and Day in the ZM. Error bars indicated +/- range of one standard error.

Open Field Task

Hypothesis 2. It was predicted that the manifestation of anxiety-like behavior in the OF task would be dependent on the severity of the TBI. Anxiety-like behavior was analyzed as the amount of time spent (duration) in the center of the arena. Deficits in locomotor activity to determine if the TBI negatively affected how much the subjects were able to move was determined by total distance travelled (movement) in the arena. Distance travelled was added to further measure the effects of the TBI on the mice and their ability to move. If the TBI negatively effected the mice' ability to move then the chance of incorrectly interpreting the behavior as anxiety-like behavior would increase. Furthermore, it was predicted that the more severe the TBI (95gm vs 30gm) the longer the subjects would stay in or near the edge of the arena as opposed to the center of the arena, thus exhibiting higher levels of anxiety-like behavior.

A 3 (Condition) x 4 (Day) x 10 (Time) mixed design ANOVA was run to analyze distance travelled by each group over the four days and across the entirety of the trial to see if the locomotor activity was impacted by TBI severity. There was a significant main effect of day, $F(1.8, 52.25) = 76.84$, $p < .001$, $\eta_p^2 = .726$ (Figure 6.). The distance travelled after the first day of testing dropped significantly, and then was steady on day 2 and day 3 before decreasing again on the final day of testing. There was also a significant main effect of time, $F(4.54, 131.63) = 3.60$, $p = .006$, $\eta_p^2 = .11$ (Figure 7.). The average distance travelled by the mice was significantly greater during the first 30 seconds of the trials, and then became reduced during the rest of the trials. However, the main effect for

condition was not significant, F(2, 29) = 2.58, p = .093, η_p^2 = .101 (APPENDIX B-3.), showing that there were no significant differences between conditions.

The two-way interaction between day and condition was not significant, *F*(3.60, 52.25) = .159 , p = .947, η_p^2 = .011, showing that the distance travelled was similar in each condition across each day (APPENDIX B-1). The two-way interaction between time and condition was also not significant, *F*(9.08, 131.63) = .93, p = .499, η_p^2 = .061, showing that condition did not impact distance traveled over the length of trials (APPENDIX B-2). There was a significant two-way interaction between day and time, *F*(13.57, 393.48) = 4.36 , p < .001, η_p^2= .131. The significant interaction shows that the average distance travelled across time was significantly less after the first testing day (see Figure 8.). Lastly, the three – way interaction between day, time, and condition was found to be not significant, *F*(27.14, 393.48) = .70, p = .972, η_p^2 = .043.

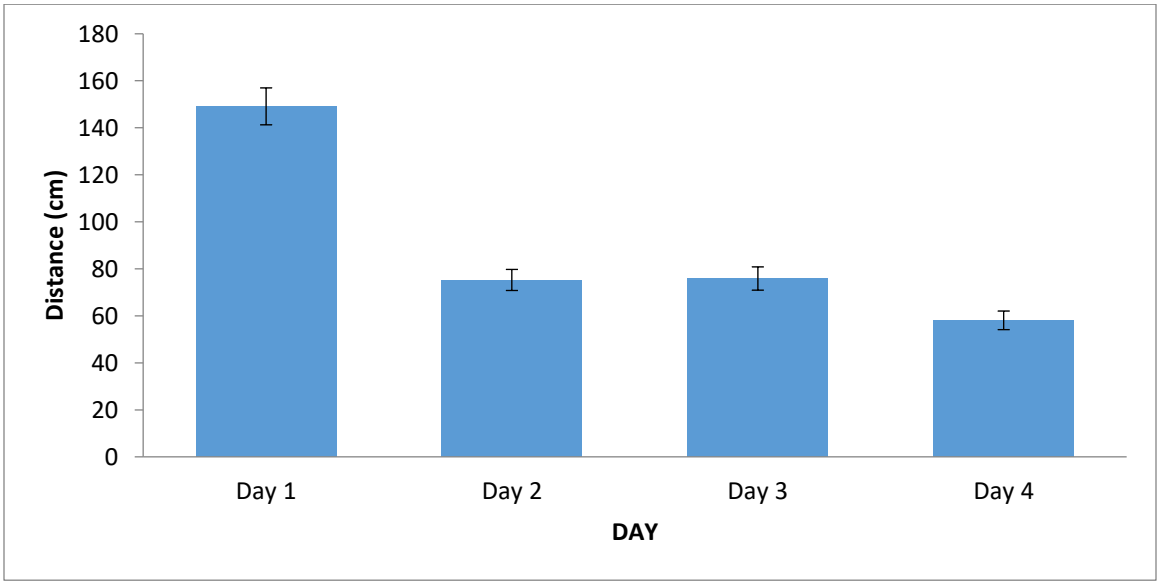

Figure 6. Mean of total distance travelled and standard error based across testing days in the OF task. Error bars indicated +/- range of one standard error.

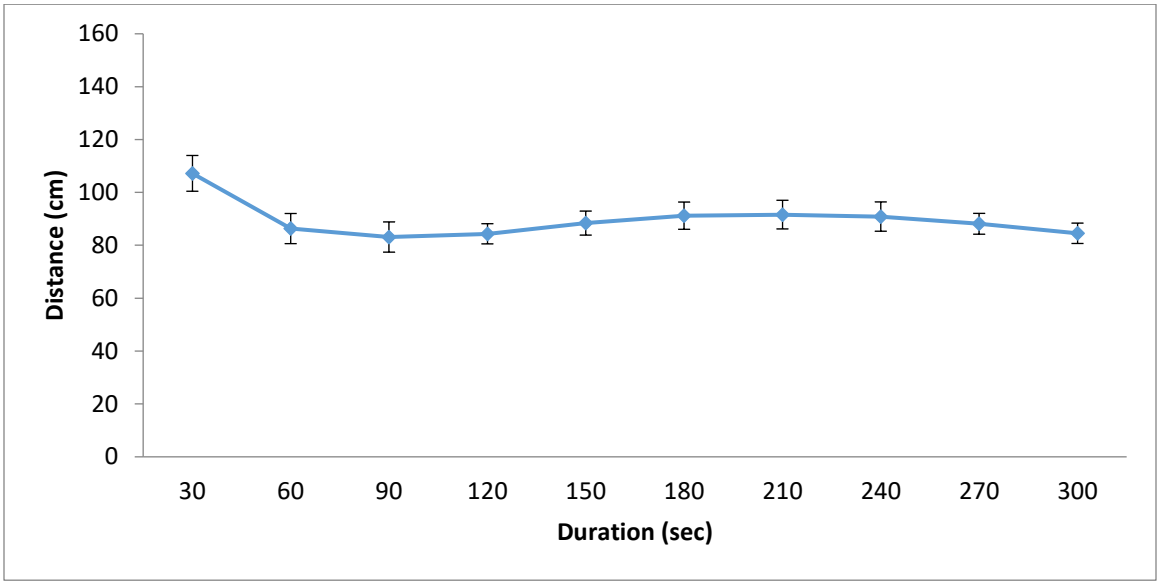

Figure 7. Mean of total distance travelled and standard error across Time in the OF task. Error bars indicated +/- range of one standard error.

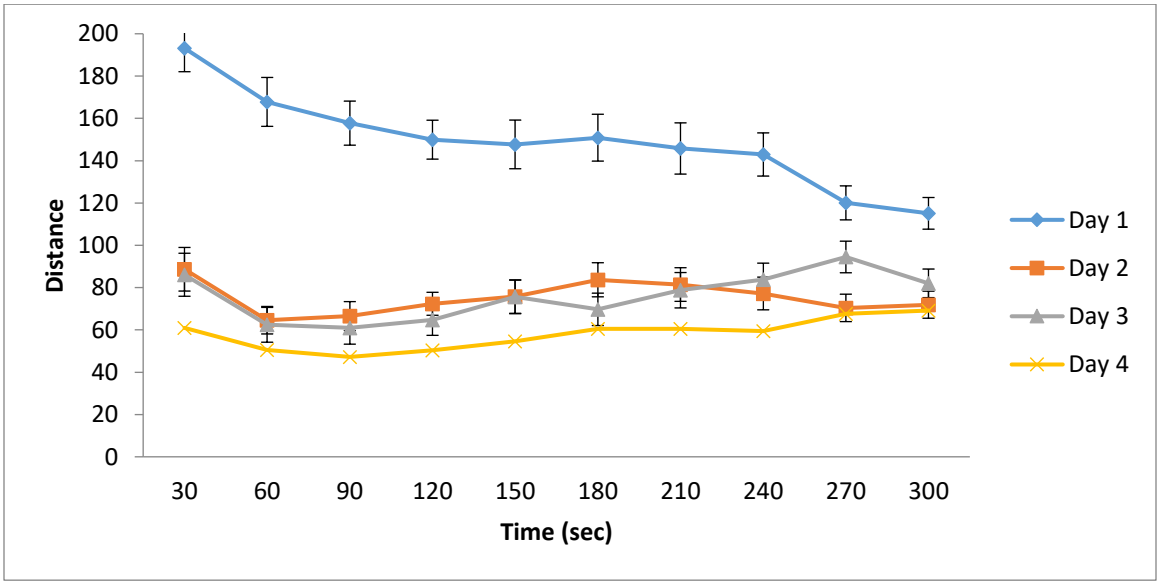

Figure 8. Mean of total distance travelled and standard error across Day and Time in the OF task. Error bars indicated +/- range of one standard error.

A 3 (Condition) x 4 (Day) x 10 (Time) mixed design ANOVA was used to analyze the amount of time that the mice spent in the center of the OF arena over the entire testing period. A main effect of day was observed for the amount of time spent inside the center of the OF arena, $F(3, 87) = 15.57$, $p < .001$, $\eta_p^2 = .349$ (Figure 9.). The time spent in the center of the arena decreased after the inter-trial interval on day 3 and then again on day 4. There was also a significant main effect of time, $F(9, 261) = 11.50$, $p < .001$, $\eta_p^2 = .284$ (Figure 10.). The average amount of time spent in the center increased from the start of the trials, showing a slight increase in explorative behavior. Furthermore, the main effect for condition was not significant, $F(2, 28) = 1.565$, $p = .287$, $\eta_p^2 = .101$ (APPENDIX B-7), meaning that there were no group differences in the amount of time spent in the center of the arena.

The two-way interaction involving day and condition was not significant, $F(6, 87) = .79$, $p = .578$, $\eta_p^2 = .052$ (APPENDIX B-4). The two-way interaction between time and condition was not significant, $F(18, 261) = .75$, $p = .762$, $\eta_p^2 = .049$ (APPENDIX B-5). The two-way interaction between day and time was not significant $F(11.71, 339.53) = 1.37$, $p = .181$, $\eta_p^2 = .045$ (APPENDIX B-6). Lastly, the three- way interaction between day, time, and condition was not significant $F(23.42, 339.53) = 1.01$, $p = .446$, $\eta_p^2 = .049$.

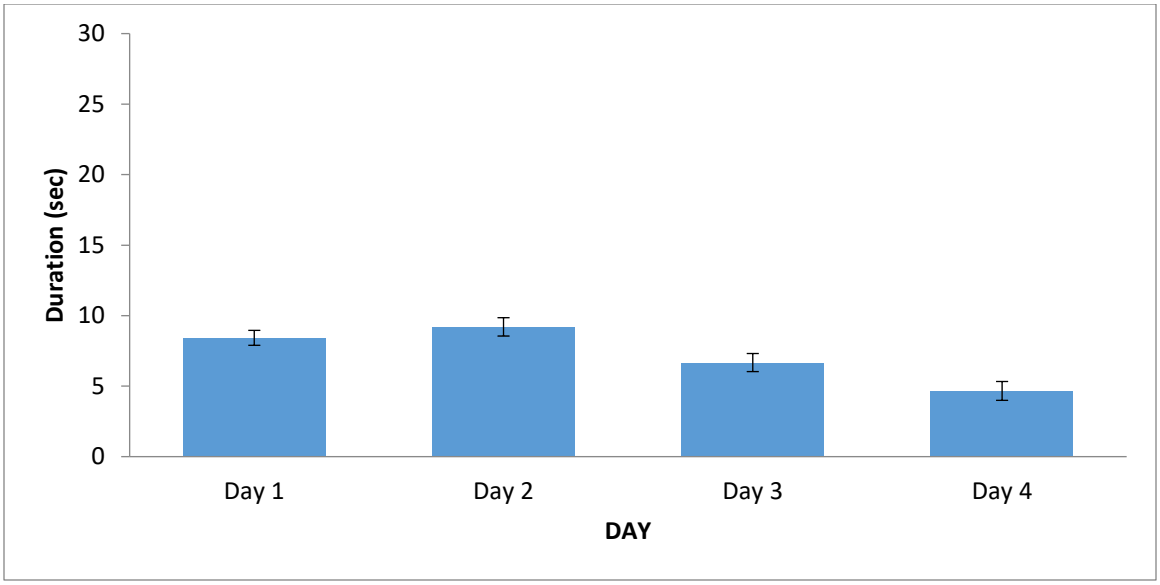

Figure 9. Mean of total time spent in the center and standard error across testing days in the OF task. Error bars indicated +/- range of one standard error.

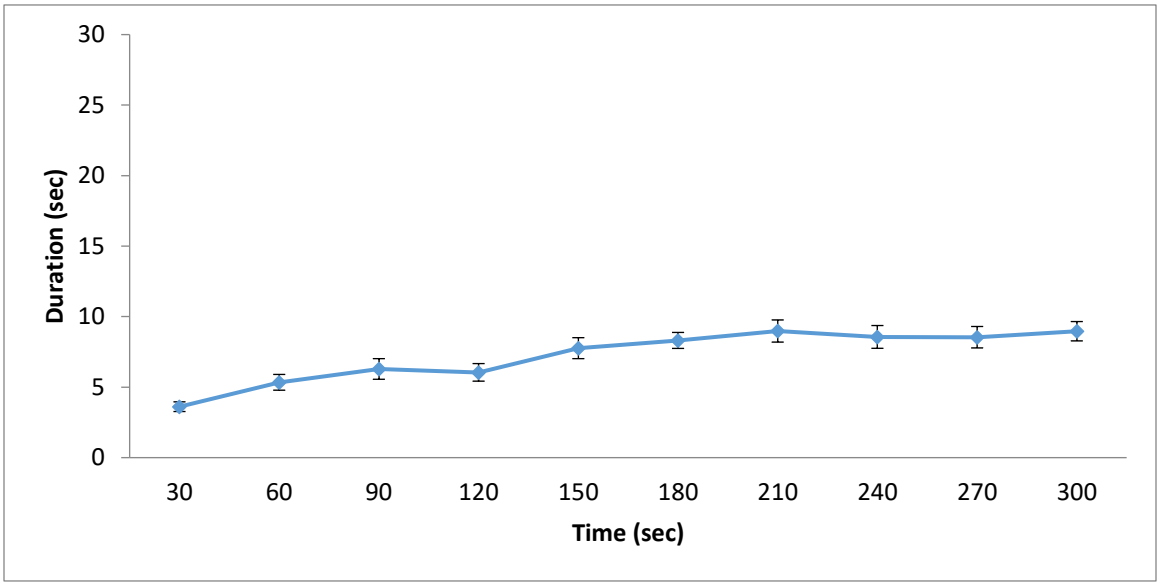

Figure 10. Mean of total time spent in the center and standard error across Time in the OF task. Error bars indicated +/- range of one standard error.

Rotarod

Hypothesis 3. It was predicted that deficits in motor coordination would be severity dependent. The more severe the TBI, the more severe the impairment, the less time would be spent on the rotarod. Furthermore, recovery of motor coordination was hypothesized to be severity dependent as well. The subjects in the 30 gm condition were expected to recover faster than the 95 gm condition.

A 3 (Condition) x 4 (Day) mixed design ANOVA was run to test the motor coordination of the mice. There was a significant main effect of Day, $F(3, 87) = 25.40$, $p < .001$, $\eta_p^2 = .467$) (Figure 11.), showing that the average amount of time the mice stayed on the rotarod increased each day. The main effect for condition was not significant, $F(2, 29) = .639$, $p = .535$, $p = .639$, $\eta_p^2 = .535$ (APPENDIX C-2). Furthermore, the interaction between day and condition was also not significant, $F(6, 87) = 1.30$, $p = .264$, $\eta^2 = .082$ (APPENDIX C-1).

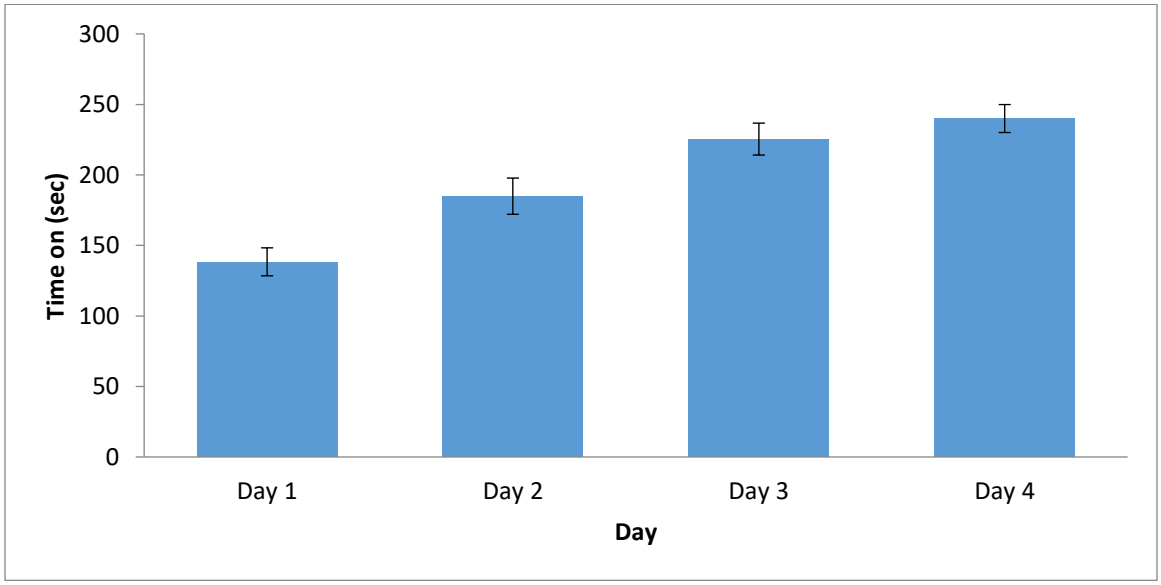

Figure 11. Mean of total time spent on rotarod and standard error across testing days. Error bars indicated +/- range of one standard error.

Forced Swim Test

Hypothesis 4. It was expected that the manifestation of depression/despair-like behavior in the FST would be dependent on the severity of TBI. Thus, mice exposed to more severe TBI would not only become immobile faster, but immobile longer.

A 3 (Condition) x 4 (Day) x 10 (Time) mixed design ANOVA was used to analyze the amount of time that the mice spent immobile in the FST over the entire testing period .There was a significant main effect of Day, $F(2.38, 68.95) = 26.35$, $p < .001$, $\eta_p^2 = .476$ (Figure 12.). Immobility significantly increased after the first day of testing and then started to level out after the inter-trial interval on testing days 3 and 4. There was also a main effect of time that was significant, $F(5.26, 152.59) = 24.08$, $p < .001$, $\eta_p^2 = .454$ (Figure 13.). The average immobility increased during the first few 30 second bins until leveling out towards the middle of the trials. Furthermore, the main effect of condition was not significant, $F(2, 29) = .64$, $p = .536$, $\eta_p^2 = .042$, indicating that immobility did not differ between TBI groups (APPENDIX D-3).

The interaction between day and condition was not significant, $F(4.75, 68.95) = .83$, $p = .527$, $\eta_p^2 = .054$, showing that immobility among groups did not differ across testing days (APPENDIX D-1). The interaction between time and condition was not significant, $F(10.52, 152.59) = 1.04$, $p = .416$, $\eta_p^2 = .067$, which means that immobility did not differ among groups across time (APPENDIX D-2). Yet, The interaction between day and time was significant, $F(12.03, 349) = 9.90$, $p < .001$, $\eta_p^2 = .254$,

meaning that mice became immobile faster on days 2, 3, and 4, while on day 1 the mice spent less time immobile (Figure 14.). Lastly, the three-way interaction between day, time, and condition was not significant $F(24.07, 349) = .792$, $p = .748$, $\eta^2 = .52$.

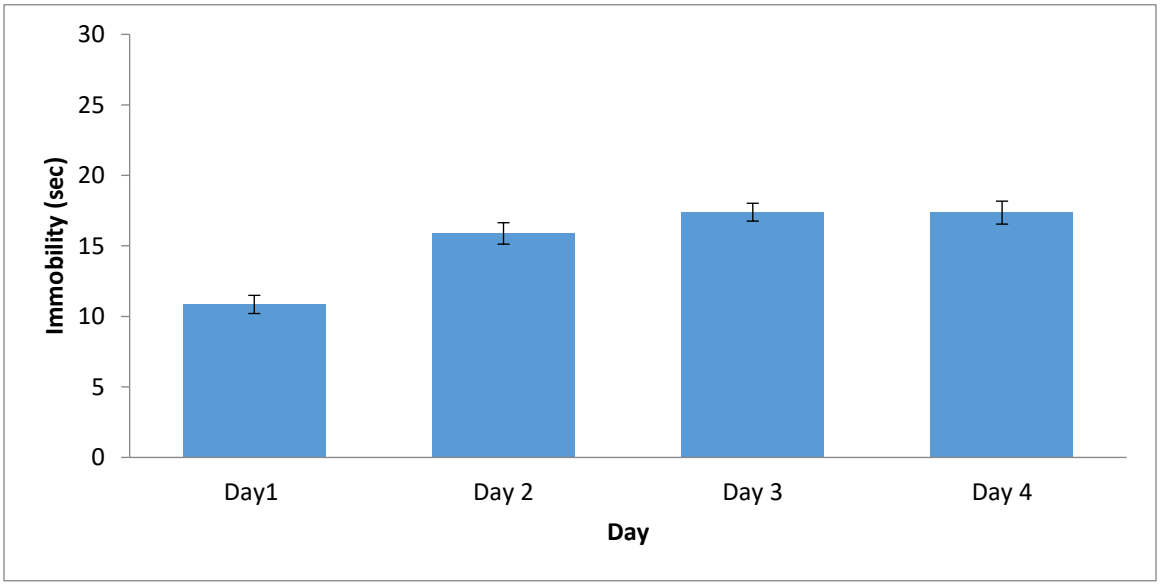

Figure 12. Mean of total time spent immobile and standard error across testing days in the FST. Error bars indicated +/- range of one standard error.

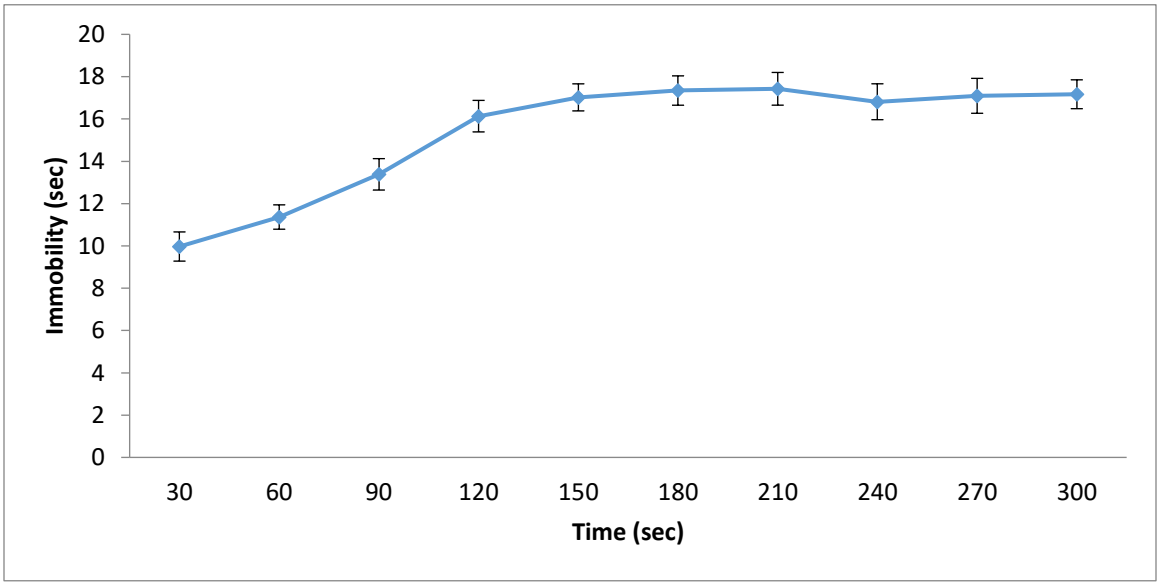

Figure 13. Mean of total time spent immobile and standard error across Time in the FST. Error bars indicated +/- range of one standard error.

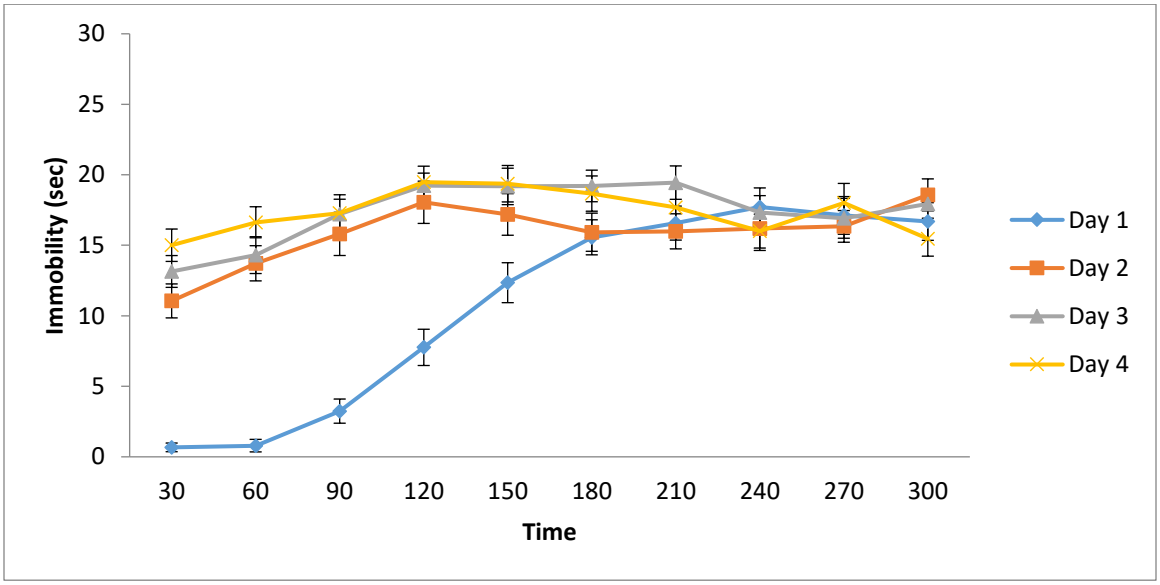

Figure 14. Mean of total time spent immobile and standard error across Day and Time in the FST. Error bars indicated +/- range of one standard error.

Discussion

The current research project examined the practicality of the fall method weight drop model using a height and weight combination that is seen sparingly in the literature. The current study looked at the effects of the TBI through the lens of anxiety and depression using several common models of anxiety and depression; the OFT, the ZM, and the FST. The Rotarod was used as a measure of motor coordination, and was the main test that looked at how the ability to move was affected by TBI. The main hypotheses were that the more severe the TBI the higher the probability of anxiety- and depression-like behavior manifesting in the mice. Additionally, it was hypothesized that the mice would recover according to the level of severity that was sustained: the more severe the TBI, the longer the recovery time. Several hypotheses were made with specific dependent variables in mind to flesh out the main hypothesis. And while the findings do not support the main hypotheses, the implications and limitations of this study could be used to improve further research.

Anxiety. The expectation based on the hypothesis was that varying levels of anxiety-like behavior would be exhibited by mice that had experienced either of the TBI groups. The anxiety-like behavior as the hypothesis states was that the mice in the TBI groups would spend less time in the open area of the ZM. Furthermore, Mice in the 95 gm condition would show more anxiety-like behavior compared to the 30 gm condition. However, the groups did not show significant differences in anxiety-like behavior as the amount of time spent in the open area did not differ among the groups.

The mice spent increasingly more time in the closed area as the trials were run, keeping in line with the theory of habituation. This was also seen in a study run by Cook, Crounse, and Flaherty (2002), they found that the time spent in the open area by C57BL/6J mice decreased significantly after the first day of testing, and decreased again after the second day. Additionally, they also found that latency to leave the closed area increased significantly after the first testing day. However, a significant drop off in time spent in the open area is not always present. Tucker and McCabe (2017) saw a gradual decline instead of a significant drop off after the first day of testing. Placement at the start of each trial may be the reason for the absence of a significant drop-off. The mice in Tucker and McCabe's study were randomly placed at one of the openings to the closed section, while the mice in Cook et al., (2002) and the current study were placed at the same opening to the closed section. The reason that random placement may create different results could be that the mice habituate to that particular area. The mice may have been be spatially aware that they were placed in the same area over and over again, and decreased their explorative behavior. Random placement may have reduced habituation, and while the mice may still become accustomed to the maze over time, a different starting point each trial could slow the habituation down. In conclusion, there is no evidence that TBI caused anxiety-like behavior in the ZM.

There was an expectation based on previous research that the motor skills of the mice subjected to TBIs would not be hindered. And while distance traveled by the mice did decrease over time, the three groups were not significantly different from each other supporting that the TBIs did not have an impact on motor skills in the ZM. The mice did

appear to habituate after day 1of testing as indicated by the significant drop in distance traveled. Rankin et al., (2009) stated habituation is one of the simpler types of learning that the mice can achieve, and defined it as a response to repeated stimulation that results in a decline in some aspects. The behaviors seen in the ZM do seem to be explained by some of the characteristics of habituation. One characteristic of habituation is spontaneous recovery, which is the recovery of a behavior to a stimulus if the stimulus is taken away after repeated exposure (Rankin et al., 2009). The spontaneous recovery in this study is the explorative behavior, which was the distance traveled, that decreased on testing day 2 but then increased on testing day 3 after a three-day inter-trial interval.

The expectation in the OF regarding the distance traveled by the mice was that the TBIs would not hinder their locomotor activity. That expectation was met due to the three groups' not traveling significantly different distances. Similar to the ZM, the mice appear to have habituated just as quickly to the OF test as distance traveled was significantly less after the first day, and throughout the rest of the trials. The findings of the present study align with past research as habituation in the OF due to repeated exposure has been observed; mice exposed to the OF repeatedly are less active (Bolivar, Calderone, Reilly, & Flaherty, 2000; Cook et al., 2002; Voikar, Vasar, & Rauvala, 2004; Gould, Dao, & Kovacsics, 2009). The hypothesis for the OF stated that the mice in the TBI conditions would spend less time in the center of the OF arena, which would signify higher levels of anxiety. While the time spent in the center was low throughout the study, there were no significant differences between the TBI groups and the control group. Therefore, there is no evidence that TBI caused anxiety-like behavior in the present study. And, even though

anxiety-like behavior was not observed in the period that the mice were tested, the presence of anxiety cannot be ruled out. The decrease in activity seen in the mice may be one explanation for why anxiety-like behavior was not observed. The time spent in the center was low throughout the trials, and decreased further after the inter-trial interval which mirrored the decrease in activity on those days. Furthermore, Cook et al., (2002) also observed the decreases in time spent in the center along with the reduction in mouse activity. However, the time spent in the center increased on day 2, which does not align with any of the other behavioral tests in the study. An interpretation of day 2 could be that the size of the OF may have had an impact. The small arena used in the present study may have restricted how movement was interpreted. The same movement could be interpreted differently in a larger arena than in a small arena. And due to that difference, if a larger arena had been used then the distance traveled on the second day may have been in line with what was seen in the other behavioral tests. Furthermore, the differences in the amount of time spent in each zone may have been clearer due to the effort it would take to travel into and out of each zone. Contrary to what was seen on day 2, the behavior of the mice following the inter-trial interval aligned with what was seen in the other behavioral tasks during the same span. The time spent in the center of the arena, as well as the distance traveled decreased during the testing days after the inter-trial interval providing evidence that the mice had habituated to the OF.

Depression. The FST was used to create an environment that would make depression-like behavior manifest in the mice. It was expected that the mice in the TBI conditions would be immobile for longer periods than the sham group. However, there

were no differences between groups during the FST, leading to the assumption that the weight drop did not affect depression-like behavior. Similar to other studies (De Pablo, Parra, Segovia, & Guillamón, 1989; Boucher et al., 2011), levels of immobility increased when subjected to repeated trials or exposures to the FST. During the first several minutes of the first trial, immobility rapidly increased, and then leveled out towards the end of the trial. This rapid increase in immobility during the first trial is also in line with what has been seen in past research (De Pablo et al., 1989; Mul, Zheng, & Goodyear, 2016; Wang, Wee, Chio, Hu, & Kuo, 2016). Furthermore, the behavior that was witnessed on the subsequent days seems to be a learned reaction that was brought on by exposure to the FST on the first day. Some critics believe the behavior the rodent's exhibit is not related to depression-like behavior, but is instead a coping mechanism that is activated when they cannot escape the container of water (De Pablo et al., 1989; Steimer, 2011; Commons, Cholanians, Babb, & Ehlinger, 2017). The present findings suggest that the TBI did not increase depression-like behavior because the amount of immobility did not differ among the groups.

Motor-coordination. The rotarod was the main test for whether the TBIs affected the mice's ability to move. It was predicted that mice that were in the TBI conditions would spend less time on the rotarod compared to the sham, which would suggest that the TBI was severe enough to cause a deficiency in motor coordination. However, the results failed to align with the initial hypothesis. There were no differences between any of the groups in regards to time spent on the rotarod, suggesting that the mice had recovered by the time the first trial took place, or that the weight drop did not cause any detectable

motor deficits. Furthermore, the amount of time spent on the rotarod by the mice increased significantly each day. The increase may be due to the mice clinging to the rotating apparatus. The mice in the present study began to cling more as the rotational speeds increased. Previous research has noted that clinging is a reaction that the mice have to prolonged time, as well as multiple trials on the rotarod (Wahlsten et al., 2003). With that said, the ability to move on day 1 of testing was demonstrated by all groups making it unlikely that the TBI had a significant negative effect on the locomotor activity of the mice.

Limitations

The expectations for the present study were that anxiety and depression-like behaviors would manifest in several behavioral tasks. However, the results of this study demonstrated that the hypotheses in the current study were not supported. And while the current weight drop model was able to reduce some of the limitations of the Marmarou weight drop model, specifically the mortality rate, which was zero, the modification to the model in which limited mortality also may have played a role in its ineffectiveness. With no significant differences between the groups, the appropriate conclusion to make is that the weight drop had no impact on the mice during testing. A concern of the study is that the TBI may not have affected the mice in ways that could be measured by the behavioral or physical tasks that the mice were put through. There may also have been a limitation in the sequence of testing, as the sequence was not counterbalanced. Counterbalancing would have removed confounds related to order effects, such as fatigue during later compared to earlier tests.

There are several limitations of the ZM and the OFT in this study; those limitations involve how the study was run and how the inter-trial interval affected the behavior of the mice, as well as how the behavior should be interpreted. The behavior of the mice was affected by the repeated exposures to the ZM and OF, as well as the short inter-trial interval. The intention of the inter-trial interval was geared to look at the recovery of the mice in the TBI conditions, as no expectation repeated exposures to the tests would affect the mice. Introducing longer inter-trial intervals such as a week or a month, similar to those seen in past research may help slow down habituation or eliminate it. And while there is still uncertainty surrounding whether or not anxiety-like behavior would manifest, the lack of habituation in the behavioral task would allow for a clearer interpretation of the behavior seen even if the behavior is not anxiety-like. Past research such as Tucker and McCabe (2017) successfully slowed down habituation in the ZM using repeated exposure in conjunction with longer inter-trial intervals. Furthermore, Schneider, Ho, Spanagel, and Pawlak (2011) observed less habituation almost a month after the first trial. Additionally, other animal studies that did not use repeated exposure had inter-trial intervals between each trial, whether the same behavioral test was being run multiple times or not (Watanabe et al., 2013; Mishra et al., 2017). Animal studies that have longer inter-trial intervals more closely resemble human studies of psychopathological disorders which look at the long term disorders over months and years. Studies that look at anxiety and depression after TBI often find that anxiety and depression are still present during follow-up examinations months after the studies had begun (Whelan-Goodinson et al., 2009; Bryant et al., 2010; Sharma et al., 2015).

Therefore, if the present study had used a longer inter-trial interval, it may have been able to capture some of these long-term effects that other studies have observed.

Anxiety-like behaviors in the ZM and the OF are thought to be present if the mice spent more time in the closed section, and keeping to the outside of the arena respectively. In each test the distance traveled significantly declined after the first trial, and trended downward during the remaining trials. Furthermore, the mice spent more and more time in the areas of the apparatus that theoretically signified anxiety. And while the behavior would seem to be in line with what would be considered anxiety-like behavior, this may not be the case. Voikar et al. (2004) specifically mentioned that the mice in their study habituated after repeated exposure to the OF, and was not the result of elevated levels of anxiety-like behavior. The research has shown that there have been mixed results, partly due to different interpretations of what behaviors anxiety manifests as well as the context at which the behaviors occur (e.g. repeated trials). The different interpretations have researchers wondering if the models are enough to diagnose anxiety in animals

Critics of the animal models of psychiatric illness argue that because diagnoses are done by a set of criteria, one behavioral test cannot exemplify a disorder (Lampis, Maziade, Battaglia, 2011). Furthermore, they argue that the best way to come close to interpreting behavior in rodents as similar to humans is by using a battery of tests to look at anxiety under many different conditions (Nestler, & Hyman, 2010; Steimer, 2011). And though the present study included two measures of anxiety it would have been more

appropriate to include a multitude of other tests that might test for other aspects of anxiety (e.g. dark and light box, and the urine test).

A limitation strictly for the OFT is that the arena size may have affected the results gathered. The mice spent the most time in the center of the arena on the second day of testing despite the distance traveled on day 2 dropping significantly, which may have been a result of a smaller arena. The smaller arena may have made it easier for the mice to move into the center without having to travel very far. A larger arena could eliminate this potential anomaly.

There are several limitations that may have affected the behavior seen in the FST, as well as the understanding and interpretation of the behavior as it relates to depression. Critics of the FST have questioned whether it accurately measures depression-like behavior at all, instead believing that it more accurately measures coping strategies (Kloet, & Molendijk, 2016; Commons et al., 2017). They argue that the behavior seen in the FST may not be depression, or at least may not be similar to depression in humans. Furthermore, immobility seen in the FST is thought of as a reaction to an acute stressor such as being placed in a container filled with water without escape while human depression is a complex chronic emotional state. Researchers agree that, while animals may experience emotions that are similar to humans, without communication it is difficult to know what forms those emotions take, and currently the forms those emotions take are unknown (Kloet, & Molendijk, 2016; Commons et al., 2017). Therefore, if the FST measures coping under stressful circumstances than depression-like behaviors, then we cannot conclude that depression did not occur in the groups that experienced a TBI.

While these are fair criticisms, more research should be done to test whether the FST is a good model of depression.

Another aspect that may limit the validity of the FST as it relates to depression-like behavior or just a coping mechanism is how many trials are run. In some studies that use rats, a 15-minute preconditioning phase is used the day before, while studies using mice have done single trials that don't require preconditioning (Parra, Caerols, Monleón, & Simón, 1999; Slattery, & Cryan, 2012; Bogdanova, Kanekar, D'Anci, & Renshaw, 2013). Research shows that just a single exposure to the FST may increase immobility levels (De Pablo et al., 1989; Cryan, & Mombereau, 2004), which is what was seen in the present study as immobility rapidly increased during the first several minutes until it became steady for the rest of the trial. The amount of time that the mice were immobile also increased during the testing days.

Finally, limitations of the rotarod were seen in how the test was run. The mice were allowed to stay on the rotating rod through the length of the 5 minutes or until they fell off, per the methods. However, the mice began to cling to the device while it rotated which may have increased the amount of time that they were able to stay on the rotarod device. This is supported by Wahlsten et al. (2003), which stated that it became much more difficult to score the data once the mice started to cling to the rotarod device. The data also suggests that the mice began to cling more as the testing days passed, which furthers the assumption that the validity of the test was compromised.

Any future TBI studies should look to be longitudinal to capture the aspect of anxiety and depressive-like behavior that has appeared after weeks and months in other

studies (Watanabe et al., 2013; Mishra et al., 2017). Additionally, future studies should look at limiting habituation as much as possible. A lengthy inter-trial interval should be deployed to eliminate any habituation in the behavioral tests. This follows one of the characteristics of habituation that states that if the stimulus is removed for a given period then the reaction to the stimulus will return (Rankin et al., 2000). While different inter-trial intervals have been used in past research that used both rats and mice (Dawson, Crawford, Stanhope, Iversen, & Tricklebank, 1994; Schneider, Ho, Spanagel, & Pawlak, 2011; Tucker, & McCabe, 2017), a standardized inter-trial interval does not exist. Therefore, setting a standard could be the subject of future research. Additionally, lack of standardization may be responsible for contradictory results across studies. For example, the mice in a study by Tucker and McCabe (2017) tested groups daily and weekly in the ZM, and observed that the behavior was fairly consistent for several trials. However, in both the present study and Cook et al., (2002), which tested the mice on consecutive days, the behavior was contradictory to that of Tucker and McCabe and instead, fit the characteristics that define habituation. These mixed results may be due to the difference in inter-trial intervals.

Another option would be dishabituation. One of the characteristics of habituation is that if another stimulus is introduced after habituation is observed then the reaction to the original stimulus will increase (Rankin et al., 200). For example, a room change may be effective for reversing the effects of habitation, as seen in Schneider et al., (2011). Another common form of dishabituation is the habituation/dishabituation task which exposes the mouse to different odors. Olfaction is how the mice explore environments,

due to their poor vision. Exposing the mice to different odors has been seen to suppress habituation (Arbuckle, Smith, Gomez, & Lugo, 2015), which can be done in conjunction with behavioral tasks to limit habituation.

Interpreting the results of the OF during the first part of the study is difficult due to the results from day 2. During the other behavioral tasks forms of habituation or coping were observed after day 1. One explanation is that the size of the arena may have had an impact on the ability of the mice to move into the center of the arena. Future studies should look to increase the size and depth of the arena so that behaviors and differences between groups will be easier to perceive. Due to the limitations of the FST, a future study may look to include a more appropriate test of depression. Critics of the FST suggest that better models of depression should focus more on symptoms that might relate better to human depression, like anhedonia (lack of pleasure), alterations in the amount of food consumed, and alterations in sleep cycles (Nestler, & Hyman, 2010). Future studies could test for anhedonia using the sucrose preference test. Previous research has found that mice subjected to repetitive TBIs had reduced preference towards sucrose (Klemenhagen, O'Brien, & Brody 2013). Though there is conflicting evidence to the contrary (Tucker, Burke, Fu, & McCabe, 2017), that only provides more of a reason to study the sucrose preference test in conjunction with TBI since there is not a consensus on its applicability as a model of depression.

Conclusion

In summary, the current study did not provide support for the modified Marmarou weight drop model when performing a single TBI. Furthermore, the current study showed

no support for any of the hypotheses. However, it is not clear that the null findings are because of the weight drop model or ways in which behavioral testing was implemented. The TBIs in this study did not produce anxiety or depressive symptoms in the mice, though the results may have been impacted by certain limitations of the study which prevented such symptoms from manifesting. However, the current study does support the idea that habituation increases in repeated trials when the inter-trial interval is not long enough. Furthermore, decreased reaction to the stimuli was seen across behavioral tests regardless of the stimulus the mice were exposed to. Hopefully, future research will provide better answers than the current study was able to provide.

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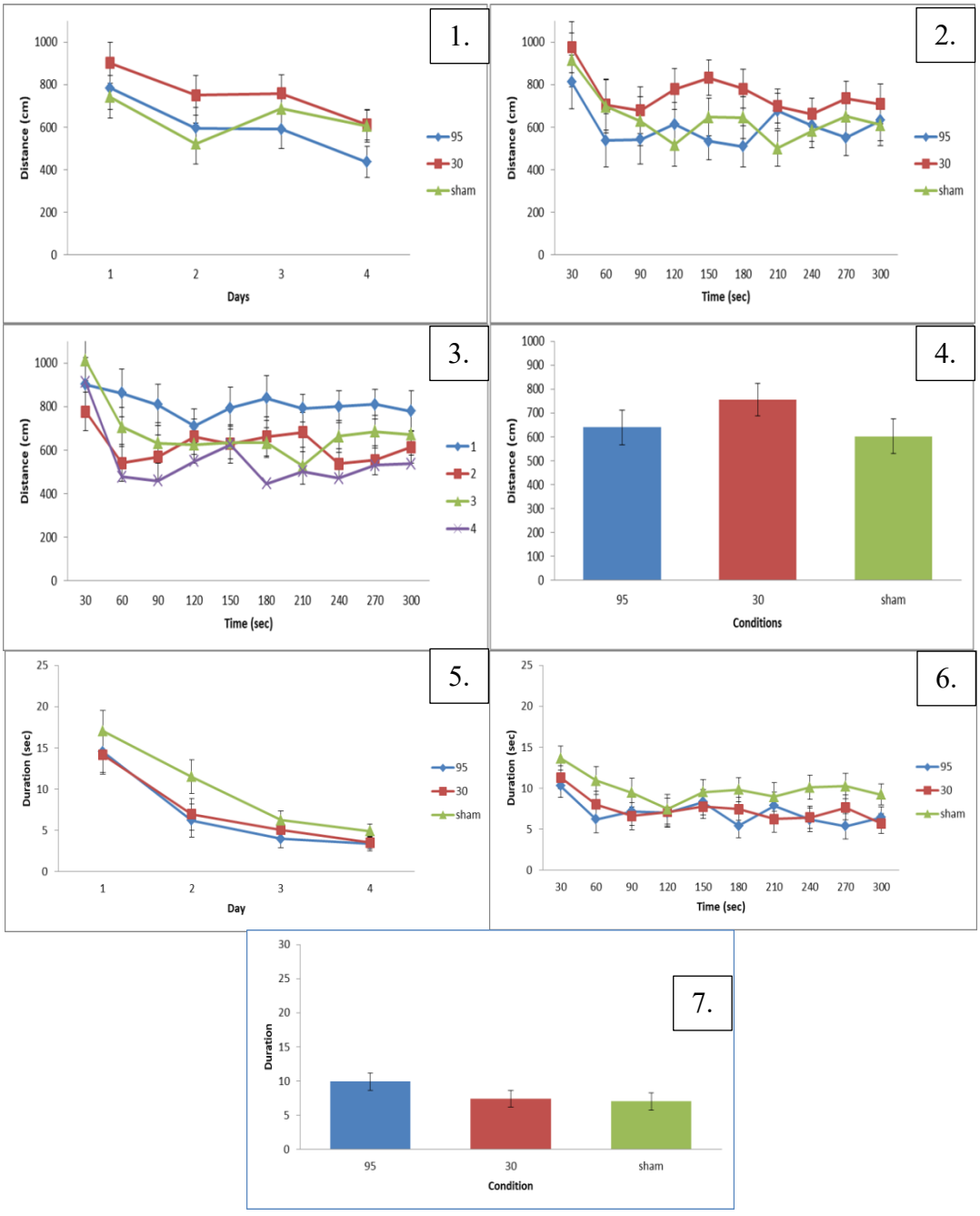
Footnotes

[1]All figures included in the results section depict any main effects or interactions that came out to be significant. Additionally, all the charts in the appendix were main effects or interactions that came out to be not significant.

APPENDIX A

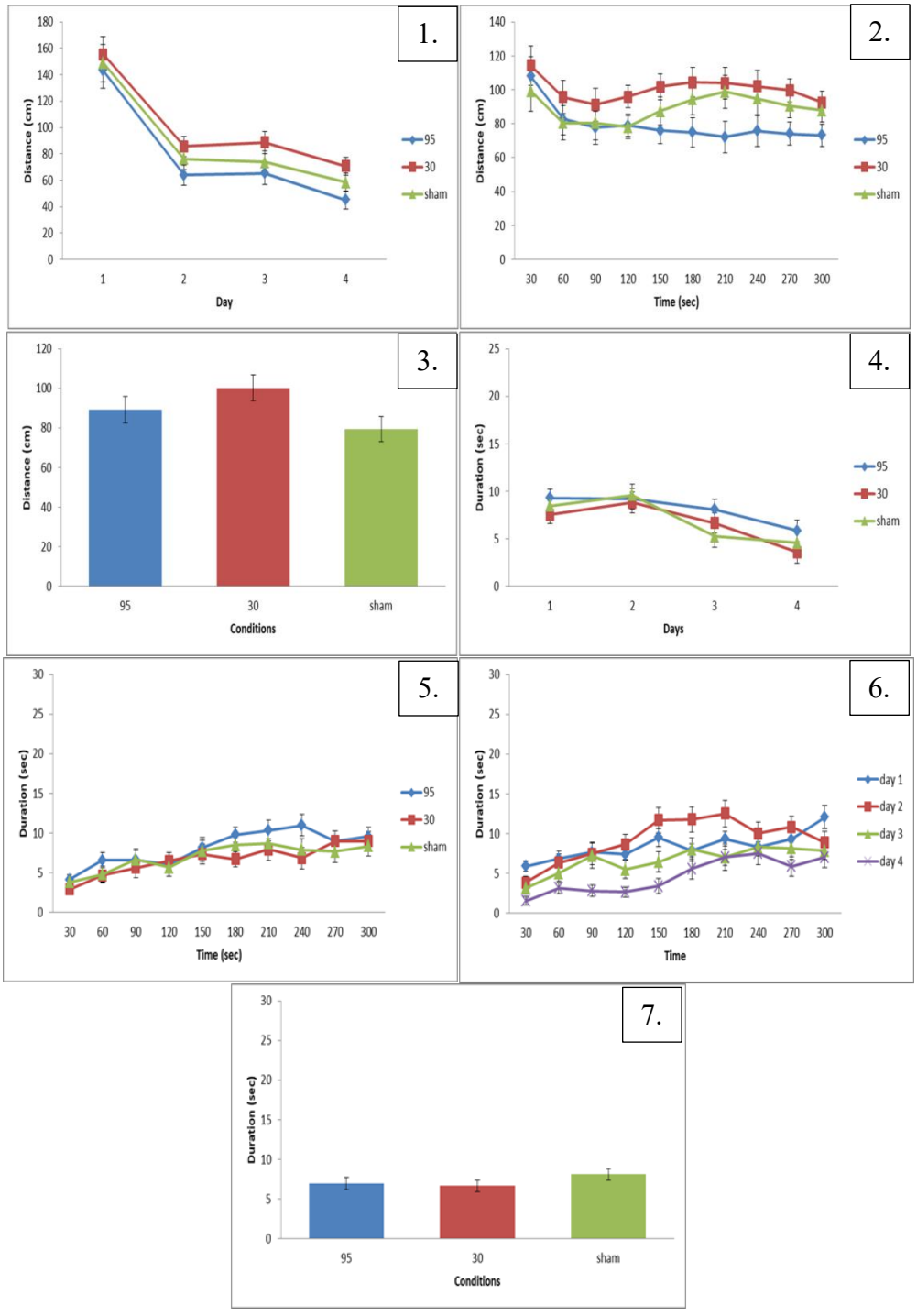
Zero Maze (Distance): 1. Day*Condition, 2. Time*Condition, 3. Time*Day, 4. Condition. (Duration): 5. Day*Condition, 6. Time*Condition, 7. Conditions
Error bars indicated +/- range of one standard error.



APPENDIX B

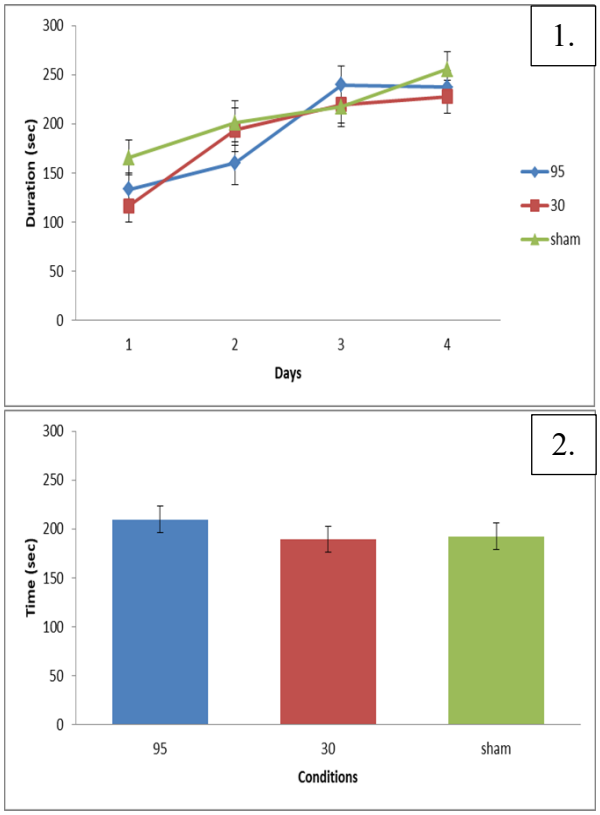
Open Field (Distance): 1. Day*Condition, 2. Time*Condition, 3. Condition. (Duration): 4. Day*Condition, 5. Time*Condition, 6. Time*Day, 7. Conditions

Error bars indicated +/- range of one standard error.



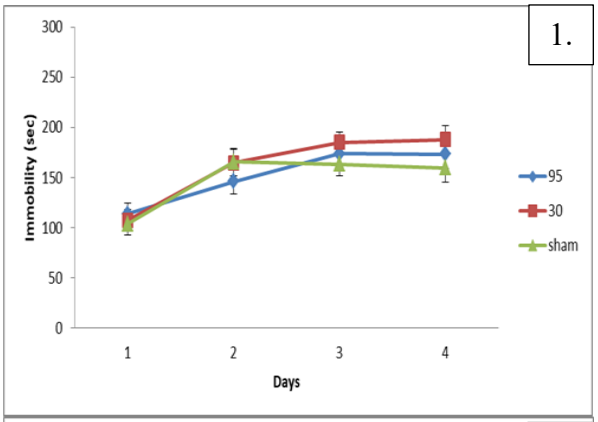
APPENDIX C

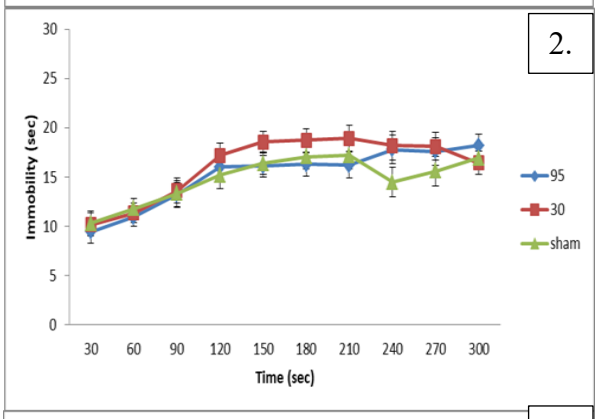
Rotarod: 1. Day *Condition, 2. Conditions. Error bars indicated +/- range of one standard error.

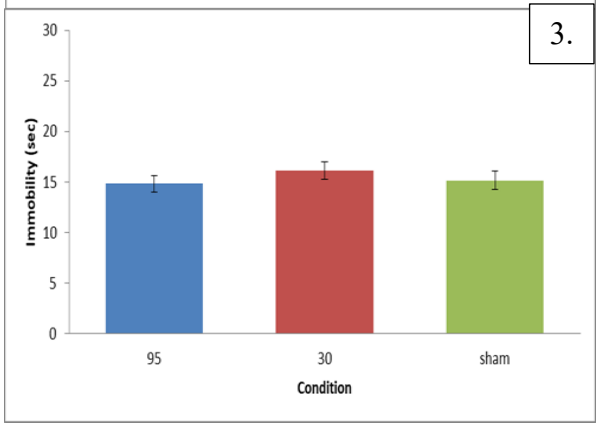


APPENDIX D

Forced Swim Test: 1. Day*Condition, 2. Time*Condition, 3. Conditions. Error bars indicated +/- range of one standard error.

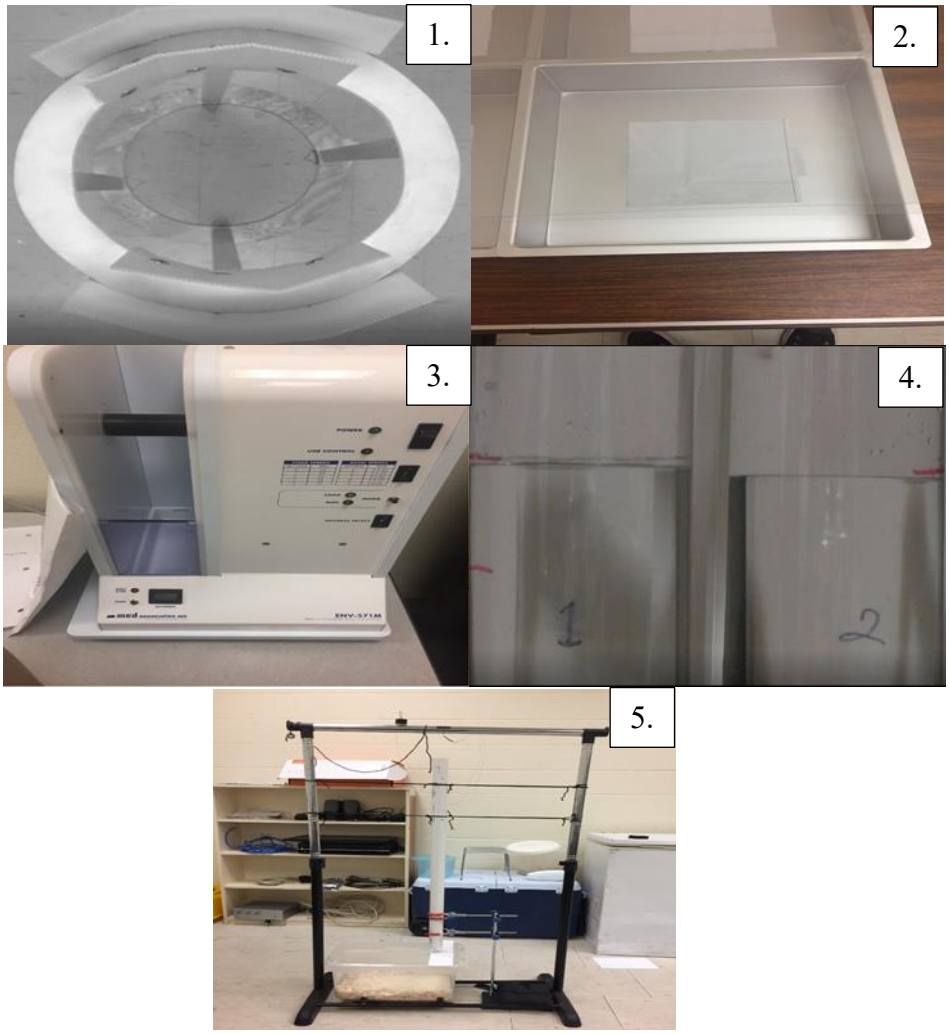






Appendix E

Apparati: 1. Zero Maze, 2. Open Field, 3. Rotarod, 4. Forced Swim Test, 5. Weight Drop. Error bars indicated +/- range of one standard error.



VITA

After completing high school at Plano East Senior High School in Plano, Texas, Sean went on to attend Texas Tech University. He would receive a Bachelors of Arts in Psychology from Texas Tech in December of 2016. The following fall, he would enter into Stephen F. Austin State University's graduate psychology program where he would earn his Master of Arts in Psychology in May 2020.

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