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Longitudinal Behavioral Assessment of Neonatal Traumatic Brain Injury

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Longitudinal Behavioral Assessment of Neonatal Traumatic Brain Injury

by

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Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree Master of Arts.

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ABBREVIATIONS

TBI	Traumatic Brain Injury
jTBI	Juvenile Traumatic Brain Injury
CCI	Controlled Cortical Impact
BBB	Blood Brain Barrier
EM	Electromagnetic
Px	Post-natal day x
ADHD	Attention Deficit Hyperactivity Disorder
SADHD	Secondary Attention Deficit Hyperactivity Disorder
LLU	Loma Linda University
MRI	Magnetic Resonance Imaging
RPM	Revolutions per Minute
ANOVA	Analysis of Variance

ABSTRACT OF THE THESIS

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By

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Traumatic brain injury (TBI) in children and infants is a primary cause of cognitive and behavioral problems that can persist through adulthood. In this study, the long-term behavioral effects of neonatal and juvenile TBI (jTBI) were characterized using mice. At an age of post-natal 7 or 10 days, mice underwent moderate or severe closed skull impact or sham surgery. Behavioral testing was conducted at 6 and 8 months post-injury. Tests administered included the open field activity (general activity levels), zero maze (anxiety), forced swim (depression), rotarod (coordination and balance), and water maze (general/spatial learning). jTBI mice showed elevated activity levels, impaired sensorimotor abilities, impaired spatial learning, and less efficient spatial search strategy use compared with sham animals. These differences were consistent and stable up to 8 months post-injury, suggesting that deficits acquired as the result of a TBI can have long-lasting behavioral impacts.

Introduction/Literature Review

Introduction

Traumatic brain injury (TBI) is a debilitating condition, often requiring supportive care as the only treatment (IOM, 2009) due to the lack of other options. It is estimated that 4,000 persons in the United States experience a TBI each day, ranging in severity from mild to fatal (Morales et al., 2005). To put it another way, 5.3 million Americans – or nearly 2% of the population – live with this type of injury (Thurman, Alverson, Dunn, Guerrero, & Sniezek, 1999).

Because of ethical concerns regarding the study of brain injury with human subjects, animals – often mice or rats – are used to study this condition. While much research has been done with TBI using animals, few studies have looked at the neurobehavioral effects of neonatal TBI (jTBI) over a long-term span of time. This study seeks to fill this gap in the literature.

A TBI can be described as any physical trauma to the brain, whether the result of a closed head-type or penetrating head injury, and can cause damage through several pathways. A common explanation (Wallesch, Curio, Galazky, Jost, & Synowitz, 2001) regarding the physical mechanisms of TBI describes two primary types and one secondary type of brain damage: (1) Focal damage from the local impact, (2) diffuse axonal injury – a shearing of axons or severing of white matter pathways in the brain, resulting from rapid head acceleration and deceleration, and (3) secondary types of damage related to factors such as mass compressive effects, fluid retention or edema, and hemorrhage (Gennarelli, 1994). These three types of physical injury are often seen in

motorbike or bicycle accidents, in which the head struck the pavement. Because the meninges and fluid surrounding the brain allow some movement within the skull, suddenly striking one's head in this manner can lead to all three of the above mentioned types of damage.

Human Studies

In humans, TBI is associated with a wide array of symptoms. Cognitive dysfunction is often observed (H. S. Levin, Eisenberg, Wigg, & Kobayashi, 1982), with the severity and nature of symptoms hinging on the severity and location of injury. Problems with memory, concentration, and attention are common. Common neurological deficits include loss of coordination, limb weakness, seizures, slurred speech, epilepsy, restlessness, or agitation. A large meta-analysis of long-term TBI consequences also noted that victims have problems with social functioning and conduct problems (IOM, 2009).

Location and Mechanism

Both primary types of TBI (diffuse axonal injury and focal lesions) can have similarly devastating effects (Wallesch, Curio, Galazky, Jost, & Synowitz, 2001). Because executive, memory, and behavioral/personality regulation functions are mediated by the frontal lobes, any type of damage in this area due to a TBI, whether focal or the result of more diffuse mechanisms, is often the primary reason for less-than-optimal outcomes after an injury (Mazaux et al., 1997). This type of damage relates to

the patient's long-term social and occupation functioning, which can predict their long-term outcomes (Mazaux, et al., 1997).

In a study examining differences between focal and diffuse types of injury, however, it was determined that, whereas frontal lobe-type symptoms were common to both primary mechanisms of TBI, the quality and character of these symptoms differed (Wallesch, et al., 2001). For example, the presence of more diffuse types of axonal injury was associated with problems in interference-type cognitive tasks, like the Stroop task, and with semantic fluency. Additionally, those with frontal lobe contusions often had problems with visuomotor planning and performance. Focal injuries generally affected specific functions in a more particular way, such as problems with concept formation and behavioral symptoms – depending on the location of the injury – but not with interference problems (Wallesch, et al., 2001).

Animal Models

Animal models are necessary to test hypotheses concerning the mechanisms and symptoms of TBI and to develop clinical therapeutic interventions in the laboratory (Laurer & McIntosh, 1999; Shohami, Novikov, & Bass, 1995). Through the characterization of TBI models and subsequent pre-clinical treatment trials using animals, help for those who experience this injury can be made available.

The physiological and behavioral effects of TBI in animal models often closely mirror what is seen in clinical patients. Graded injuries tend to produce graded effects, with less severe injuries producing similar symptoms to more severe injuries, but to a lesser degree as a result of more subtle morphological changes (Zohar et al., 2003). The

initial damage can then trigger a cascade of neurochemical and physiological changes in the brain that result in reproducible patterns of behavioral manifestations (Fujimoto, et al., 2004; Milman, Zohar, Maayan, Weizman, & Pick, 2008; Zohar, et al., 2003). The following sections delineate results often seen in several animal models, as well as the mechanism and types of injury often used in laboratory settings.

Mechanism of Injury

In animals, the controlled cortical impact (CCI) method is often used experimentally to cause the injury because of its consistent and reproducible histological and behavioral deficits (Brody, et al., 2007). This model has been shown to best replicate clinical effects of impact events (Fujimoto, et al., 2004). For this technique, animals are fully anesthetized, the skull is exposed, and a partial removal of the skull or craniotomy is performed (Brody, et al., 2007). An impactor is then used to damage the exposed brain with a fixed weight at a specified height and fixed velocity. This technique has been shown to cause consistent performance differences in multiple domains, including spatial learning and memory, motor functioning, and spontaneous activity (Fujimoto, et al., 2004; Saatman, Feeko, Pape, & Raghupathi, 2006). Furthermore, adjusting the depth and location of impact can affect behavioral deficits and cell death (Saatman, Feeko, Pape, & Raghupathi, 2006).

Another TBI model used is the fluid percussion model. This technique uses a pulse of fluid to create inertial forces that act on the brain. A fluid-filled tube is fed into the part of the brain chosen for TBI, and a pressure wave inside this fluid is created to act on the exposed brain. Fluid percussion models in rodents have been shown to cause

massive brain changes such as blood flow, cerebral metabolism, blood brain barrier (BBB) breakdown, and cell death (Fujimoto, et al., 2004), and cause more diffuse-type injures than the CCI model..

A third model used to experimentally study TBI is the impact-acceleration model. This technique works by resting the anesthetized animal's head on a foam pad, allowing for movement after it is impacted by a weight (Marmarou et al., 1994). This creates focal and acceleration-type injuries, including cell death and axonal injury in areas devoid of focal lesions (Foda & Marmarou, 1994). Although this model of damage most closely mirrors that which is seen in clinical TBI patients, especially motor-vehicle accidents (Fujimoto, et al., 2004), few long-term behavioral experiments using animals have implemented this model (Adelson, Dixon, & Kochanek, 2000). This is because the forces needed to create replicable analogues of human TBI behavior in animal models using this method cause extremely high rates of mortality in the subject animals (Fujimoto, et al., 2004). Using a less-severe injury model to retain an appropriate numbers of experimental subjects results in a disappearance of the behavioral effects of the TBI.

Studying TBI

Brody et al., (2007) described a new, electromagnetically (EM) CCI device that allows for fine control of the impact location and depth, without need for the frequent calibration necessary in other devices. This device uses an EM coil to deliver cortical impact at a very high, precise velocity and was shown to cause reproducible

behavioral and histological deficits in adult mice; similar to what is seen using other, pneumatic CCI devices (Brody, et al., 2007).

Several types of neurobehavioral test are often administered in TBI experiments (Fujimoto et al., 2004). The Morris water maze test of spatial learning and memory (Morris, 1984) and the rotating cylinder (rotarod) test of motor coordination and balance (Crawley, 2000) are often used in the literature. Other tests, such as the open-field activity test (Hall & Ballechey, 1932) measure spontaneous activity, and can provide a measure of hyperactivity-like behaviors.

These and other neurobehavioral tests are often used to assess brain injury constructs that clearly map to specific neurobehavioral deficits seen in human TBI patients. Even symptoms like depression can be experimentally modeled among TBI animals using learned helplessness-type paradigms (Milman, Rosenberg, Weizman, & Pick, 2005). Assessing animals across time points allows observation of changes over time, and whether consistent performance gaps exist between TBI and control groups.

According to some researchers, there are certain time periods during which these tests are sensitive to discern brain injured from control animals (Fujimoto, et al., 2004). Regardless of the exact protocol used, control mice will exhibit improvements in rotarod and water maze performance over time, presumably due to improvements in motor and spatial learning. The water maze task (Morris, 1984) in particular makes a good case that improved performance on the spatial paradigm is the result of improved spatial learning attribute, due to the fact that the platform location and release point of the animal into the water are counterbalanced and change across trials.

Improved performance over time on the rotarod and water maze is also noted among TBI animals. Brody et al. (2007) found effects for test day on both the water maze and rotarod tasks across groups (TBI and control), suggesting that the injured animals improved their performance over time. Other research has confirmed this hypothesis and found that mice suffering from neuron decortication (which presents similar symptoms to those of a brain injury) improved their performance on both the water maze and rotarod over time, although these gains in performance were not enough to match the performance of the control animals (Cendelin, Korelusova, & Vozeh, 2008). In most cases, then, the increase in performance across time for the water maze and rotarod by TBI mice is not as great or sufficient enough to match that of the control animals, presumably due to the present brain injury (Colombel, Lalonde, & Caston, 2002; Milman, et al., 2005; Saatman, et al., 2006).

Dogs and Pigs

Larger mammals, such as dogs and pigs, are less-often used when doing behavioral TBI research (Fujimoto, et al., 2004). Although plenty of studies examining the physiology of TBI using dogs or pigs have been published, there is little in the literature that involves the behavioral aspects of such models. This is because behavioral tests are not well established in large animals for TBI (Fujimoto, et al., 2004). Similarly, animals housing and financial considerations – which are considerably higher with larger animals like dogs or pigs – also serve as deterrants to using these animals. Finally, although physiological data are important to consider, behavior remains the final criterion by which a study or model should be judged; if a model does not produce clinically

relevant behavioral results, its connection to clinically-relevant therapeutic techniques is limited.

Rats

Of the many behavioral tests used to study TBI, most began by using rats as an analogue (Fujimoto, et al., 2004; Morris, 1984). Rats that undergo TBI have also been found to display more prolonged injury effects compared to other animals (Fujimoto, et al., 2004). It is unclear if this effect is due to a difference in injury models, or if other animals like mice recover faster (Fujimoto, et al., 2004). Either way, rats make excellent subjects for doing TBI research.

Behavioral deficits in rats that have undergone TBI show consistent deficits between control and injury groups (Fujimoto, et al., 2004; Morris, 1984; Piot-Grosjean, Wahl, Gobbo, & Stutzmann, 2001; Prins, Lee, Cheng, Becker, & Hovda, 1996). In particular, large group differences on learning-based tasks like the Morris water maze suggest that brain regions such as the hippocampus are especially vulnerable to TBI (Hamm et al., 1992). These effects in rats are very similar to learning-based deficits seen in clinical TBI populations (Hamm, et al., 1992).

Similarly, rat research has demonstrated experimentally-produced mood disturbances in rats after TBI, which are very similar to the long-term effects seen clinically (N. C. Jones et al., 2008). These findings suggest that long-term anxiety in TBI patients may be due, at least in part, to neurobiological factors relating to their injury.

Rats have been associated with studying the behavioral effects of brain damage since the formation of the classic neurobehavioral tests in this subject area (Hamm, Pike,

O'Dell, Lyeth, & Jenkins, 1994; Morris, 1984). Rats provide a great deal of consistency between animals, allowing the researcher to use fewer rats to achieve the same statistical power, compared to a greater number of mice (Fujimoto, et al., 2004).

Mice

Mice are often used for TBI research (Brody et al., 2007; Fujimoto, et al., 2004; Hartman et al., 2001; Milman, et al., 2005; Saatman, et al., 2006; Zohar, et al., 2003). They often produce similar behavioral profiles to those seen in rats, and are testable using most of the same neurobehavioral tests (Fujimoto, et al., 2004). The primary benefit to using mice over rats is the availability of testing transgenic mice in a TBI model, which can allow for the examining of the effects of a particular gene mutation or genetic predisposition to brain injury (Fujimoto, et al., 2004; Saatman, et al., 2006). Additionally, the lowered cost of housing needed for doing research with mice make them an attractive option.

The Effects of Age

Humans and animals that experience a TBI at a young age such as infancy may face worse long-term outcomes (Donders & Warschausky, 2007; Prins, Povlishock, & Phillips, 2003). Non-accidental head trauma is the leading cause of traumatic death during infancy (Gerber & Coffman, 2007). The short-term mortality rate is 15%-38%. Half of the survivors suffer from cognitive/neurological deficits; only 30% recovered with no measurable deficits (Gerber & Coffman, 2007). In a characterization of adults that had experienced early (age 6-12) or late (minimum age of 16) TBI, researchers found

that the participants with early TBI had worse cognitive, psychosocial, and neurobehavioral outcomes the later group. Another study that looked at TBI in children and adolescents found similar results; after controlling for severity, the younger children exhibited persistent cognitive impairment, while the adolescent group showed greater recovery over time (H. S. Levin, et al., 1982).

TBI early in life can greatly affect the developing brain. Children who had experienced a head injury had greater activation of many brain areas while sustaining attention during MRI relative to controls, signifying that the brain of a child or infant who experiences a head injury can have permanent structural changes in the brain (Kramer et al., 2008). Thus, imaging studies and neuropsychological tests suggest that TBI patients face worse outcomes if the injury is experienced at a younger age.

Animal models have also described the hazards of TBI at a young age. Neonatal rats that experienced focal damage and diffuse axonal injury as the result of a TBI were found to have reduced resilience for healing and normal reorganization following injury compared to older mice (Prins, Povlishock, & Phillips, 2003). Additionally, an increase in dopamine production in the brains of infant pigs that experienced a TBI compared has been noted compared to juveniles that had a TBI and controls; this increase can have devastating long-term effects and lead to increased neuronal injury (Walter et al., 2004). In humans, young human TBI patients had worse long-term outcomes than patients that experienced a head injury as older children or adolescents (Donders & Warschausky, 2007).

These findings are in opposition to the so-called "Kennard effect" (Kennard, 1938), which suggests that recovery is often more extensive after youthful brain damage

than after similar damage later in life. Some studies have noted that young age confers an advantage in recovering from focal vascular lesions (H. S. Levin, 2003). Similarly, (Kolb & Gibb, 1991) demonstrated increased dendritic branching for post-natal day 10 (P10) rats with frontal lobe lesions, but less branching for post-natal day 1 (P1) rats with the same lesions. These differences in age were corroborated through additional research done by (Kolb, 1987) in (Prins & Hovda, 2003), who posited a "narrower definition" of the Kennard effect to account for the improved outcomes of P10 rats compared to their P1 neonatal counterparts.

However, although this principle may be useful as a general guideline, there are many exceptions to the "rule." For instance, some research has shown that young age confers no advantage for severe diffuse brain injury (H. S. Levin, 2003). This is possibly due to alterations in white matter connectivity, which is a hallmark effect of TBI (Gennarelli, 1994; Wallesch, et al., 2001). Similar studies have shown that, in age-matched groups, children and adolescents that experienced a severe diffuse head injury exhibited memory deficits, and that these effects were more pronounced and long-lasting in children (H. S. Levin, et al., 1982).

Further evidence posits that the younger brain can demonstrate abnormal growth, depending on the regions affected. For instance, children with left-hemisphere brain damage were found to vary on how much language they could learn, depending on the cause of injury (Curtiss, de Bode, & Mathern, 2001). Similarly, removal of the anterior portion of an infant rat cortex was found to cause abnormal development of the posterior portion (Kolb & Holmes, 1983). It seems, then, that although the young brain retains

more plasticity than the adult brain, it is also more vulnerable to disruptions in development and organization (Kalat, 2004).

Activity

Increased rates of hyperactivity are often seen in children and adolescent TBI patients (Geraldina et al., 2003; Kramer, et al., 2008; H. Levin et al., 2007; Max et al., 2004). Symptoms of these secondary or acquired ADHD symptoms (SADHD) are mutually exclusive to a prior diagnosis of ADHD (Max, et al., 2004), and are positively related with worse TBI outcomes (Max et al., 1998). SADHD symptoms after TBI in children are also related to transient personality changes, but not to the age of injury or gender.

In a study of children ages 0-18 years that experienced a TBI, the age of the patient when injured tended to vary the presence or absence of psychological problems (Geraldina, et al., 2003). Younger patients tended to exhibit more internalizing problems such as anxiety or depression, where as the older groups (ages 6-13 and 14-18) demonstrated high levels of hyperactivity after injury, with the 6-13 age group showing hyperactive symptoms in 30% of patients regardless of TBI severity, compared to 3-7% in the general population.

There are few studies which describe the effects of TBI on activity levels in animals. One of the few studies which have examined activity levels created an animal model to study this effect, using adult gerbils as a model. 30 animals were divided into three groups, one sham surgery and two TBI (mild and moderate injury) (Li et al., 2006). Activity levels were assessed using the open field and T-maze tasks. Whereby the mild

injury group only demonstrated transient hyperactivity after injury, the moderate group showed prolonged hyperactive symptoms throughout the 7 days of follow-up testing (Li, et al., 2006). This evidence points to a positive link between hyperactivity and severity, similar to the results of pediatric clinical studies (Max, et al., 2004).

Affective Symptoms

Affective symptoms like anxiety and depression are common after a TBI (Geraldina, et al., 2003; Jorge, 2005; Jorge et al., 2004; Jorge, Robinson, Starkstein, & Arndt, 1994; Jorge & Starkstein, 2005; H. S. Levin, et al., 1982). Younger patients tend to express more internalizing symptoms after a TBI than adolescent patients, and can experience social issues as a result of affective symptoms (IOM, 2009). The incidences of depression and anxiety after TBI have been reported as high as 77% and 70%, respectively (Granacher, 2003; Jorge & Starkstein, 2005).

Affective symptoms are also present in experimental models of TBI. Anxiety after an injury, as measured by several neurobehavioral tests, was present up to 6 months following a TBI (N. C. Jones, et al., 2008). Although less-often reported than other domains like sensorimotor or spatial learning (Fujimoto, et al., 2004), pervasive affective symptoms are an important aspect to any clinically-relevant model.

Motor Deficits

Sensorimotor deficits are common in most TBI patients, and often include ataxia, weakness, seizures, and a general lack of coordination (IOM, 2009). Motor problems are similarly common in infants and young children who experience a TBI (Ewing-Cobbs et

al., 1998). These motor problems were noticeably more severe in this younger age group than in older adolescents, and included relatively severe hemiparesis that interfered with daily activities. Motor deficits in TBI patients are thought to arise from the disruption of any of a host of relevant vestibulomotor processes in the brain, and may involve fine motor coordination as well as more severe and directly observable deficits like hemiparesis (Ewing-Cobbs, et al., 1998; Fujimoto, et al., 2004).

Similarly, motor deficits have been shown to be a relevant domain for studying and treating TBI in the animal literature (Hamm, et al., 1994). Vestibular tests like the rotarod (B. J. Jones & Roberts, 1968) are sensitive to fine motor deficits by requiring animals to use complex and coordinated muscle movements, rather than simple strength. Motor deficits have long been studied in TBI research, and show the potential for accurate detection using neurobehavioral tests (Fujimoto, et al., 2004; Hamm, et al., 1994). Similarly, increased exposure to a motor task can result in learning and "rehabilitation" in animal models, mimicking the results obtained in physical therapy by TBI patients, and can demonstrate improvement of motor performance in TBI animals over time (Hamm, et al., 1994). However, the rate of recovery is not as swift with more severely injured animals (Hamm, et al., 1994).

Cognitive Symptoms

Cognitive deficits, such as problems with memory, are common after TBI (Donders & Warschausky, 2007; IOM, 2009; H. S. Levin, et al., 1982). Younger children tend to exhibit more severe cognitive deficits than adolescents exposed to the same level of injury, and cognitive symptoms tend to persist longer in younger patients

(H. S. Levin, et al., 1982). Cognitive problems in infants may receive less therapeutic attention early on due to the relative difficulty in assessing the cognition and memory of an infant or young child relative to an adolescent or adult.

Cognitive and memory deficits after TBI are very well documented in the experimental literature (Brody, et al., 2007; Cendelin, et al., 2008; Fujimoto, et al., 2004; Hamm, et al., 1992; Hicks, Smith, Lowenstein, Saint Marie, & McIntosh, 1993; Morris, 1984). Behavioral tests are often employed to test an animal's rate or retention of learned information. The best grounded of these measures use an animal's ability to spatially navigate towards a goal. Spatial memory tasks are often used to assess declarative hippocampally-mediated memory in animal models, and are a good measure of general memory abilities (Morris, 1984). This is because declarative memory is dependent on the hippocampus, which also mediates spatial memory. Experimentally, memory deficits in animals have been observed with different ages and injury severities (Adelson, et al., 2000; Brody & Holtzman, 2006; Dixon et al., 1999; Fujimoto, et al., 2004; Morris, 1984). Some research has suggested that memory deficits can occur up to a year after injury in animals, which is analogous to several decades of development in humans (Dixon, et al., 1999).

Search Strategy

The Morris water maze, described below, is considered to be a good test of spatial learning and memory (Brody & Holtzman, 2006; Morris, 1984). However, although the data extracted from the test is useful for showing the degree of spatial learning and memory possessed by an animal or group of animals, other factors that may impact test

performance are not assessed. These factors include the search strategy used by an animal to find the hidden platform. Search strategy refers to an animal's apparent utilization of different methods (spatially-mediated, random, exterior only, etc.) to find the platform in the "spatial" portion of the water maze. Thus, two groups of animals may appear to have similar spatial memory abilities, while one group uses a more efficient search strategy to find the platform (Brody & Holtzman, 2006; Janus, 2004). Search strategy analysis tests "compensation effects," or the tendency of many clinical TBI patients to make up for cognitive deficits using other means, such as mediating poor memory through the use of reminders or alarms.

Objectives/Hypotheses

There are few studies that characterize jTBI in a long-term capacity. Whereas most behaviorally-oriented studies assess periods of between a few days and 6 weeks or so, none look at the long-term behavioral effects of jTBI (Fujimoto, et al., 2004). Indeed, the few studies that describe long-term effects of TBI experimentally focus on adult models (Fujimoto, et al., 2004). This study characterized the behavioral effects of jTBI over an 8-month period, at which time a mouse is in the same developmental age as a middle-aged person. Short-term deficits have been demonstrated experimentally in animal models (Fujimoto, et al., 2004). However, the paucity of long-term studies in the literature gives credence to the current study. Similarly, few studies have exclusively looked at jTBI as a paradigm.

The main aim of this study was to characterize the long-term effects of jTBI. This was done through analysis of several neurobehavioral tests, described in the methods section. Thus, hypotheses surrounding each of the relevant domains were assessed.

Regarding activity levels, it was hypothesized that jTBI animals will demonstrate higher activity levels for the open field test compared to sham animals, because of the increases in activity seen in both humans and animals (Li, et al., 2006; Max, et al., 2004). Similarly, because of the worse prognosis associated with earlier injury (Donders & Warschausky, 2007; Prins, et al., 2003; Walter, et al., 2004), it was hypothesized that younger (P7), more severely injured TBI mice will exhibit hyperactivity compared to other groups.

Regarding affective symptoms, it was hypothesized that jTBI animals will demonstrate elevated anxiety-like symptoms on the zero maze, and higher depressive symptoms on the forced swim test, because of the increases in anxiety and depression noted both clinically and experimentally (N. C. Jones, et al., 2008; Jorge & Starkstein, 2005). Because of the tendency for earlier injuries to produce greater affective symptoms (IOM, 2009), it was also hypothesized that younger (P7) TBI animals will show greater anxiety-like and depressive behaviors compared to older (P10) TBI animals.

Regarding sensorimotor abilities, it was hypothesized that jTBI animals will exhibit impaired sensorimotor performance compared to shams. Similarly, more severe injuries have the strongest link to sensorimotor performance (Hamm, et al., 1994). Thus, it was hypothesized that more severely injury animals would exhibit impaired performance compared with moderately injured animals.

Finally, regarding cognitive abilities, it was hypothesized that jTBI animals will exhibit impaired cognitive abilities (spatial memory) compared to sham animals, because of the incidences of cognitive problems associated with TBI in both humans and animals of this age group (Donders & Warschausky, 2007; Hicks, et al., 1993; H. S. Levin, et al., 1982; Morris, 1984).

A second aim of this study is to assess the efficacy of search strategy employed by mice during the Morris water maze. Previous research has shown that brain-injured mice use fewer systematic and non-spatial search strategies, and use more repetitive looping (Brody & Holtzman, 2006). No age/severity differences were noted. Thus, it was hypothesized that jTBI mice as an overall group will use less effective search strategies than their sham surgery counterparts. Similarly, more efficient search strategies should mean better spatial performance on the water maze. Thus, it is hypothesized that search strategies will correlate with cognitive performance on the water maze.

Methods

The subjects for this study were 50 neonatal wildtype mice from Washington University in St. Louis, MO that experienced a TBI via EM CCI device with either a mild, severe, or sham injury before arriving at LLU. The experimental mice experienced the TBI at either P7 or P10 and were tested several months later, approximating the motor and cognitive abilities of an adult who experienced a TBI as a child (Craig et al., 2003; Yager & Thornhill, 1997). Approximately half of the total number of animals was controls that underwent a sham surgery, with the other half having experienced a TBI. Half of the TBI mice received the injury at P7 and half at P10, creating a total of five groups: Sham, P7 moderate, P7 severe, P10 moderate, P10 severe.

Materials

Injury model. TBI is operationally defined for this study as the amount of damage caused by an electromagnetically controlled cortical impact device set to a certain impact force, 5.2 m/s. A "moderate" injury was specified as a 2.0mm impact depth, and a "severe" injury was defined as 2.5mm impact depth, per previous findings suggesting the clinical relevance of these two depths (Brody, et al., 2007). Research has suggested that the degree of injury (an independent variable) scales well with the degree of brain damage and observed neurobehavioral deficits (Brody, et al., 2007; Fujimoto, et al., 2004; Saatman, et al., 2006).

This injury model is both valid and reliable. It is valid in replicating TBI in humans because the method of injury, through impact, is very similar to what is often

seen in human TBI patients, both in behavior and in conducting MRI studies (Saatman, et al., 2006). The EM method produces similar behavioral results to conventional weight-drop methods of TBI (Brody et al., 2007). Furthermore, the CCI method in general has been shown to differentiate between TBI and control animals, with greater impact forces scaling well and associating with more severe neurobehavioral differences (Brody, et al., 2007; Fujimoto, et al., 2004; Saatman, et al., 2006).

Concerning the reliability of this model, there exist several potential problems in regards to accurately detecting behavioral differences. Potential issues include the performing of a craniotomy prior to injury to expose the cortex (Brody, et al., 2007), which is not a feature present in accidental TBI and thus must be questioned. Similarly, completing this procedure involves anesthetizing the animals prior to the surgery; isoflurane will be used for this purpose. However, isoflurane was found to have neuroprotective qualities in response to TBI (Statler et al., 2006), which could reduce the model's ability to show accurate behavioral differences. The CCI method using isoflurane, however, is standard and has been shown to have good sensitivity and specificity in studying TBI (Brody, et al., 2007; Fujimoto, et al., 2004; Saatman, et al., 2006). Consistent and repeated use of this model across different experiments using mice has shown consistent, reproducible patterns of brain damage and neurobehavioral deficits (Brody, et al., 2007; Fujimoto, et al., 2004; Saatman, et al., 2006). The current experiment uses similar methods to what has been done before, thus showing good reliability for this model.

The independent variables for this experiment are whether the animal being tested is a control or TBI mouse as well as whether the TBI was inflicted at P7 or P10 and a

mild or severe injury. Thus, this project features two independent variables: severity and injury timepoint.

Activity. One behavioral construct that was examined is activity. Using the open-field test (Hall & Ballechey, 1932), animals were placed in plastic bins 49cm long and were uninterrupted for 30 minutes. The total distance the animal traveled during a trial, as well as its percentage of trial time spent moving was analyzed using the same computer system as the water maze. These two variables are highly correlated, thus analysis focused on the animal's distance traveled. The latency of the animal to move has been noted as one of the most informative sets of data obtainable using this test (Royce, 1977; Stanford, 2007).

Anxiety. Another construct studied was affective symptoms. Anxiety symptoms after TBI have been shown to develop in both humans and animals (Granacher, 2003; N. C. Jones, et al., 2008). This experiment tested for anxiety using data collected from the zero maze (Shepherd, Grewal, Fletcher, Bill, & Dourish, 1994). The zero maze consists of a horizontal ring, approximately 7cm wide. Half of the ring has walls surrounding the platform, making half of the surface "out in the open" and half "enclosed." The dependent variable is the percentage of time spent in the enclosed area. Animals are placed on the ring for 5 minutes.

Depression. Along with anxiety, depression after TBI was examined as a facet of the affective component. This construct was tested using the forced-swim model of learned helplessness (Lahmame, Grigoriadis, De Souza, & Armario, 1997; Milman, et al., 2005; Porsolt, Le Pichon, & Jalfre, 1977). This test was developed to examine the antidepressive effects of pharmaceutical compounds, and has been found sensitive to

variations in depression-like behaviors between TBI and sham animals (Milman, et al., 2005). Animals are placed in a 30cm wide, 50cm tall glass cylinder for 10 minutes. The trials were recorded and analyzed visually for movements suggesting a desire to escape the cylinder (active swimming or climbing up the side), or depression-mediated learned helplessness (purposeless swimming, no escape attempts). An animal's initial time to become helpless was recorded as the primary dependent variable.

Motor performance. Another construct for this experiment is motor performance, specifically defined as the length of time it takes for an animal to fall off a rotating cylinder called the rotarod (Dunham & Miya, 1957; B. J. Jones & Roberts, 1968). Thus, the dependent variable is simply the latency of time before an animal falls from the beam. The speed and acceleration of the cylinder can be manipulated to bring out motor deficits in TBI mice. If an animal falls, an electric eye will stop the timer allowing for exact measurement of how long the trial lasted. Through extensive use and testing, and by obtaining reproducible results, this test has been shown to be reliable (Fujimoto, et al., 2004; Saatman, et al., 2006). Many experiments have used this test with TBI mice, and have obtained reliable, consistent results (Brody, et al., 2007; Hamm, et al., 1994).

This test has also been shown to be a valid measure of motor ability. Motor skill, coordination, and balance are tested through the animal's attempts to remain on the beam, even as it moves and accelerates. This sort of procedure is a test of motor skill, and has been shown to be able to differentiate between animals with both TBI and other motor-type injuries and their respective controls (Brody, et al., 2007; Colombel, et al., 2002; Fujimoto, et al., 2004; Saatman, et al., 2006).

To run the rotarod test, animals were placed on the cylinder at one of 3 conditions: stationary (0 RPM), constant velocity (5 RPM), or accelerating velocity (5 RPM + 5 RPM/5 sec). Each iteration is administered to each animal for every rotarod test. The three speeds allow the test greater sensitivity to detect differences, as well as allowing animals to acclimate to the test. The accelerating paradigm is the most sensitive and useful for studying TBI (Brody, et al., 2007; Fujimoto, et al., 2004; Saatman, et al., 2006).

Cognitive/spatial performance. Another construct for this experiment is cognitive performance, specifically defined as spatial learning and memory performance tested using the Morris water maze (Morris, 1984). The main dependent variable for this task is how long it takes for an animal to find a platform, hidden under the surface of an opaque pool of water. The water maze itself is a circular tank 110cm in diameter filled with opaque water and containing a platform approximately 10cm wide. The animal is released from counterbalanced points along the wall of the tank and recorded using Ethovision behavioral tracking software, which visually tracks the animal's trial. The first task administered to the subjects, the cued task, features a clearly visible platform that can test for motor deficits and other physical limitations such as vision problems, swimming ability, and motivation to find the platform. This task was administered for 10 trials during a single day. Following the cued task, the platform is submerged slightly under the water's surface; the animal must rely on its spatial learning and memory to find the platform. Release points vary for this spatial component, which lasted for 10 trials a day for 3 days. The primary dependent variable for the cued portion was distance moved, or the total distance moved by an animal as recorded by Ethovision software. For the

spatial paradigm, cumulative distance is the dependent variable, defined as the summation of the animal's distance from the platform, measured 5 times per second. In this way, cumulative distance is sensitive to both distance and time.

Animals subjected to TBI by CCI – both by pneumatic impactor and electromagnet device – show clear spatial learning and memory deficits on this task compared to controls (Brody, et al., 2007). The water maze is a standard test that has been used repeatedly (Fujimoto, et al., 2004; Saatman, et al., 2006) and has provided consistent results across studies to show spatial deficits in mentally impaired rats or mice. Thus, it is reliable for use with a TBI model.

This test is also a valid measure of spatial learning. It has shown to be able to differentiate between injured and control animals in many protocols, including those looking at TBI (Brody, et al., 2007; Fujimoto, et al., 2004; Saatman, et al., 2006). Furthermore, it forces subject animals to learn and then recall the location of a submerged platform, which involves using the hippocampus and other cortical areas involved in spatial learning and memory (Morris, 1984). By controlling for swimming deficits through the cued portion of the task (which involves a visible platform), spatial learning and memory differences can be accurately ascertained, and confounds/mediators like ability to swim are taken out the equation.

Search strategy. To assess to efficacy of search strategy used for the Morris water maze, a pathway analysis was conducted. A bank of 9 search strategies, similar to those used in past studies (Brody & Holtzman, 2006; Janus, 2004) were used to test an animal or group's use of effective spatial strategies, using visual cues located in the testing environment, to locate the platform. The first and last blocks from each day of

water maze testing were reviewed, and each track was assigned a search strategy based on previously published criteria (Brody & Holtzman, 2006). The strategies were ranked on a 1-9 scale from most effective to least; data analysis involved a repeated-measures ANOVA to assess each group's strategy usage. The schema's search strategies (Figure 1) include: Spatial direct, swimming directly towards the platform; spatial indirect, taking a meandering but relatively expedient path towards the platform; focal correct, searching in the general vicinity of the platform; focal incorrect, searching in the incorrect area of the tank, but staying in a relatively small search area; scanning, searching the whole interior portion of the tank for the platform; random, having a random and disorganized strategy; focal incorrect, searching the wrong portion of the tank; chaining, or swimming in circles in the tank's interior; peripheral looping, or swimming in circles around the outside perimeter of the tank; and circling, or swimming in tight, concentric circles. To better organize the strategies, the first three strategies (spatial direct, indirect, and focal correct) can be conceptualized as "spatial" strategies, the second three (interior scan, random, focal incorrect) were grouped as "systematic: non-spatial" strategies, and the last three (chaining, peripheral looping, and circling) were grouped as "looping" strategies (Brody & Holtzman, 2006). The efficiency and efficacy of the strategies is roughly linear, with spatial direct being the best strategy, and circling the worst (Brody & Holtzman, 2006).

Procedure

The mice were tested using the activity, zero maze, forced swim, rotarod, and water maze tests in the Behavioral Neuroscience Laboratory at LLU. Shortly after

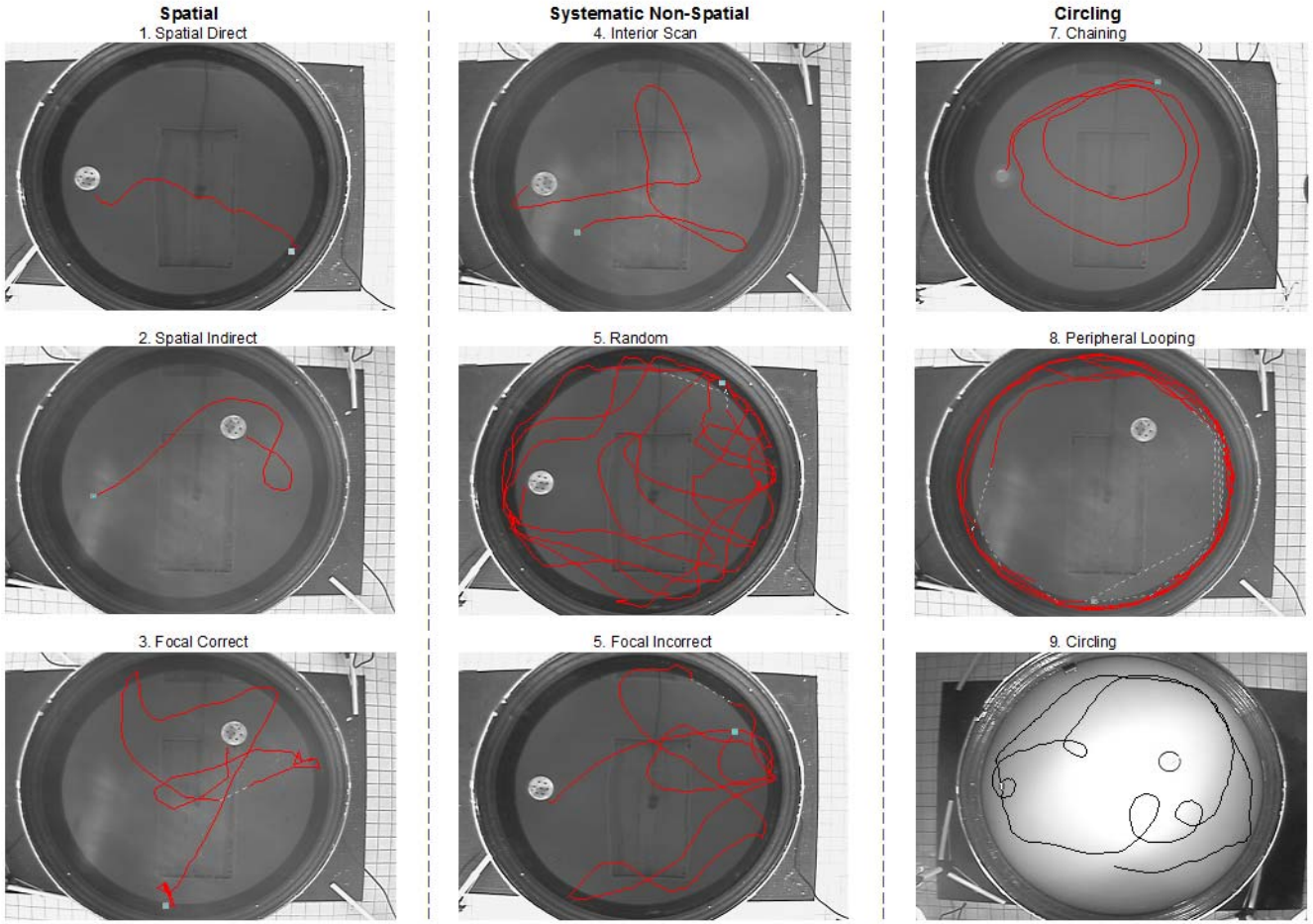

Figure 1. Strategy examples

arrival, at 6-months post-injury, the animals were run through a behavioral testing battery. This battery contained the tests described above over a two-week time period, with the open-field, rotarod, forced swim, and zero maze tests being administered the first week, and the water maze taking up the entire second seek. All tests except the water maze were run twice at two day intervals to provide a greater quantity of data and to assess the animals' relative performance over time. Approximately 7-8 weeks later, at 8-months post-injury, the same battery was repeated in the same manner as before.

Analyses

To test the hypotheses that TBI animals would display worse long-term behavioral outcomes, a series of repeated-measures mixed-model ANOVA were run. These were completed using SPSS 17 as well as Statistica 6.0. The nesting feature of the Statistica package was used for the water maze spatial analyses, allowing for analysis of overall group differences across testing timepoint (6 and 8 months), testing day (3 per timepoint), block (5 per day), and trial (2 per block). The nesting parameter allowed different within-groups levels to be compared, such as timepoint x day, as well as more complicated analyses (timepoint x day x group). This feature allowed better qualitative interpretation of the data based on findings outside the stated hypotheses.

Power Analysis

An a priori power analysis was completed using G*Power 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007). The analysis was completed using previously collected pilot data and substituting current N's to compute required N's needed for the current study.

Data was available for the rotarod and open field tests that fit the parameters of the present study (at least 8 months post-injury). Achieved results from the pilot data were tested to identify a necessary N for the current study. A required N of 48 was suggested for the present study, based on a power equal to at least .8.

Results

The hypothesis that TBI animals would exhibit higher activity levels compared to shams for the open field test was confirmed (Figure 2). The interaction between trial and group was not significant, but there was a main effect overall for group. TBI animals (M=711.78, SD=136.08) displayed overall higher activity levels compared to shams (M=591.68, SD=172.12), $F(1, 47)=5.56$, $p<.03$, power=.66, $\eta^2=.11$.

The hypothesis that severely injured P7 animals would exhibit more activity (hyperactivity) compared to other groups was confirmed (Figure 3). The interaction between group and day/trial of testing was not significant. There was a main effect for group $F(4, 44)=4.707$, $p<.01$, power=.93, $\eta^2=.30$. Follow-up testing was done by analyzing individual group differences using the Bonferroni to avoid artificially inflating type-I error. Post-hoc testing revealed that P7 severe animals (M=836.61, SD=113.08) exhibited significantly higher overall activity levels than sham animals (M=591.68, SD=172.12), $F(2, 17)=14.14$, $p<.005$, power=.95, $\eta^2=.44$. Similarly, P7 severe animals exhibited significantly higher overall activity levels than P10 severe animals (M=662.81, SD=130.91), $F(2, 17)=10.09$, $p<.005$, power=.85, $\eta^2=.36$. The more liberal Least Significant Difference (LSD) test suggested that P7 severe animals exhibited higher activity than all groups except for P10 mild animals.

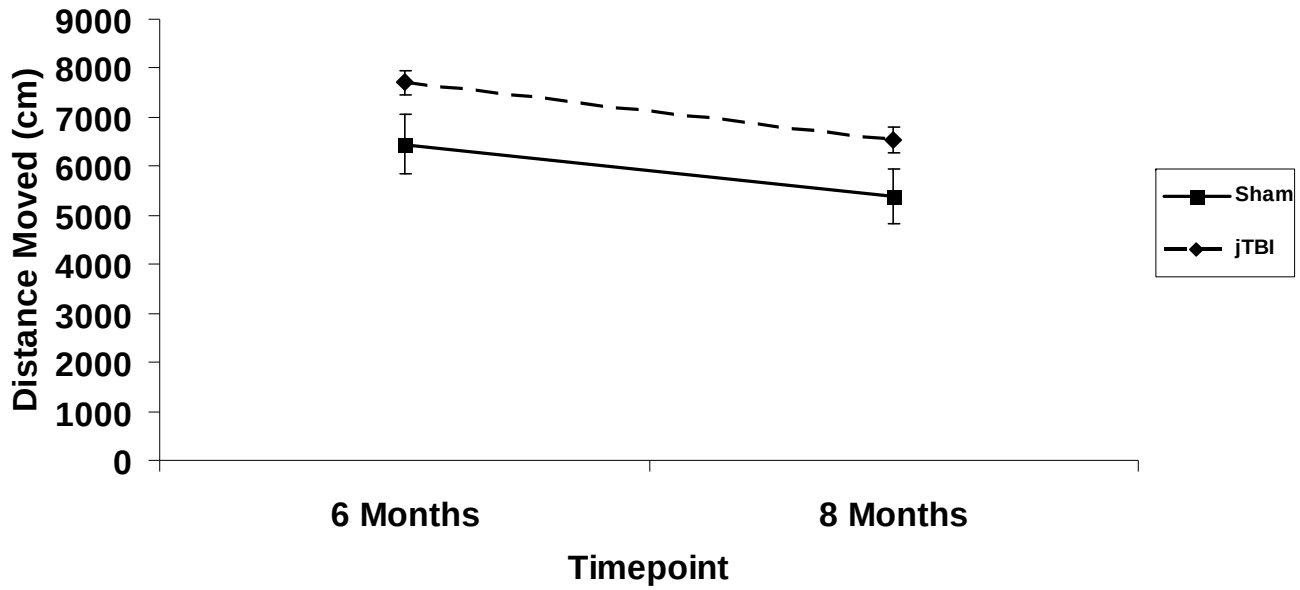

Figure 2. Activity levels over time – open field

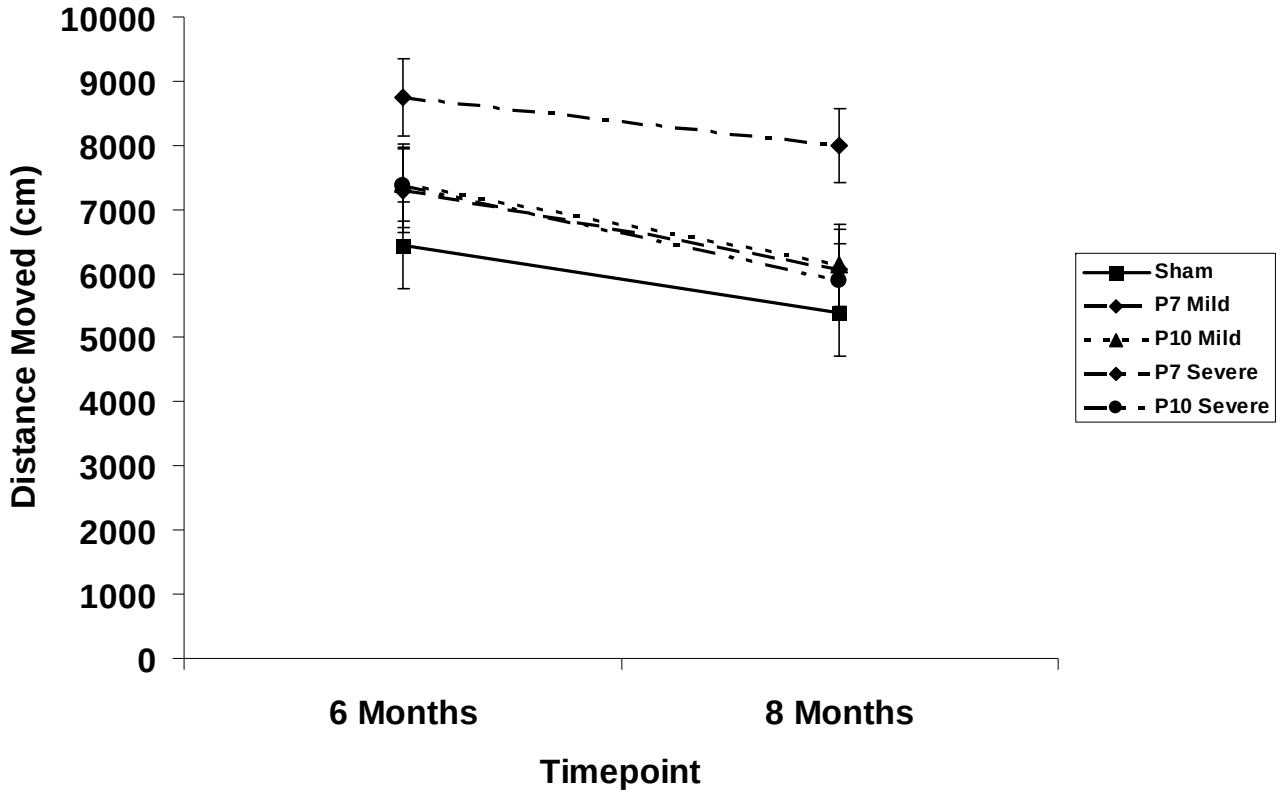

Figure 3. Activity levels for individual groups – open field

The hypothesis that TBI animals would show more anxiety-like differences than shams on the zero maze was not confirmed (Figure 4). The interaction between testing timepoint/day and the animal's status as an injured or sham animal was not significant. TBI animals (M=0.57, SD=0.12) displayed fewer overall anxiety-like behaviors than shams (M=0.67, SD=0.12), $F(1, 47)=5.29$, $p<.03$, power=.62, $\eta^2=.10$ rather than the hypothesized higher anxiety-like behavior in TBI animals.

The hypothesis that younger P7 TBI animals would show more anxiety-like differences than older P10 TBI animals on the zero maze was not confirmed (Figure 5). The interaction between testing timepoint/day and the animal's status as an injured or sham animal was not significant. The main effect between P7 and P10 animals was also not significant, $F(1, 37)=.00$, $p=.99$, power=.05, $\eta^2=.00$.

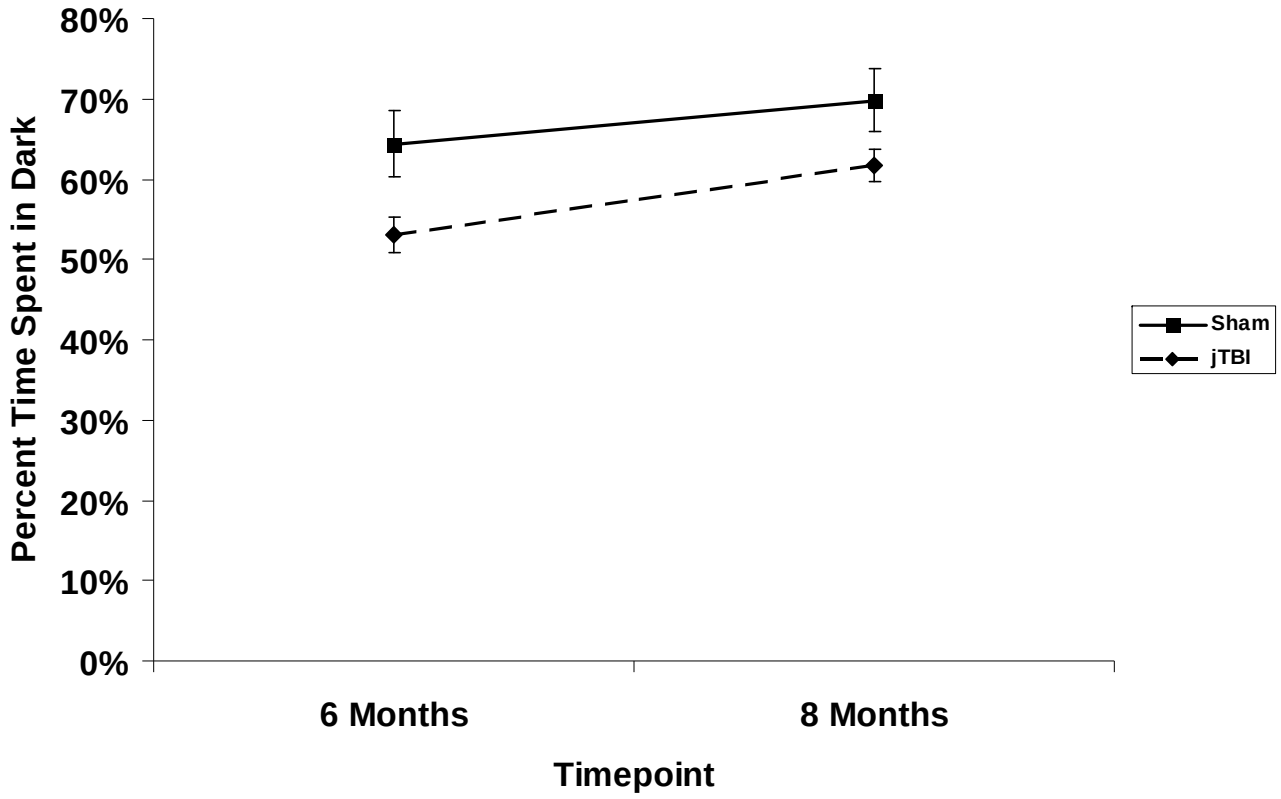

Figure 4. Anxiety-like behaviors over time – zero maze

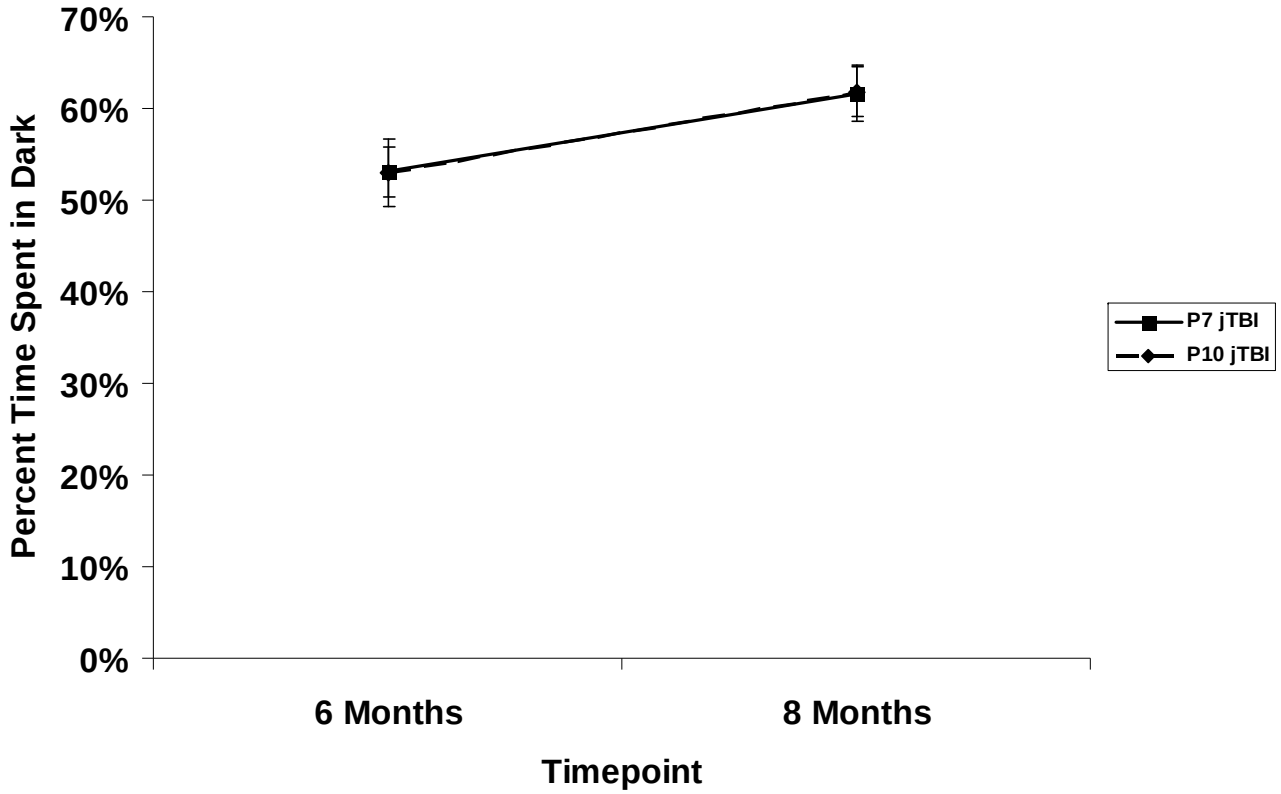

Figure 5. Anxiety-like behaviors for P7P10 animals – zero maze

The hypothesis that TBI animals would show more depressive behaviors than sham animals was not confirmed (Figure 6). Because the sphericity assumption was violated, $\chi^2(2)=9.23$, $p<.01$, the Huynh-Feldt correction statistic was used. The interaction between TBI/sham and day of testing was not significant. There was a main effect for test day $F(1.35, 63.36)=43.75$, $p<.001$, power=1.0, $\eta^2=.48$. The main effect for group was not significant, $F(1, 47)=.22$, $p=.65$, power=.07, $\eta^2=.01$.

The hypothesis that younger P7 TBI animals would show more depressive symptoms on the forced swim test than older P10 TBI animals was not confirmed (Figure 7). The interaction between testing day and age was not significant. The main effect for age was not significant, $F(1, 37)=.32$, $p=.58$, power=.09, $\eta^2=.01$.

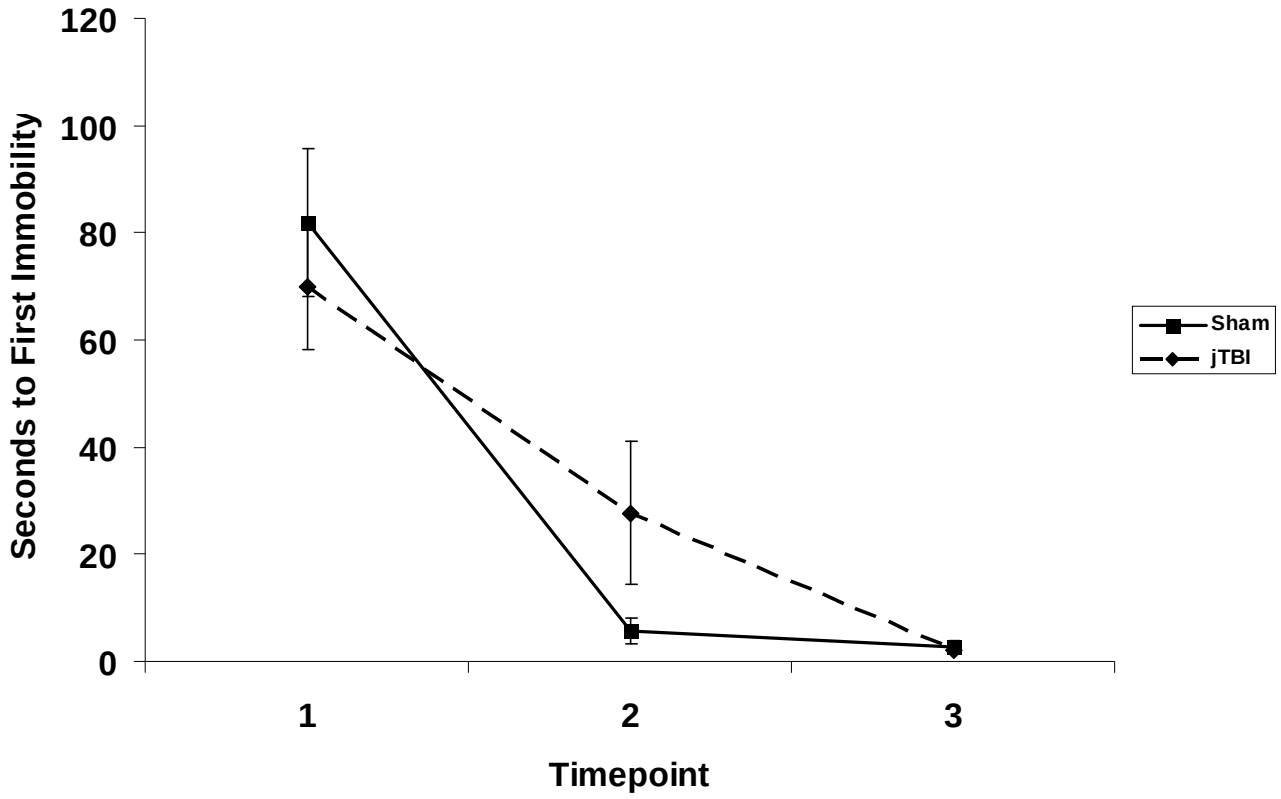

Figure 6. Helplessness behaviors over time – forced swim

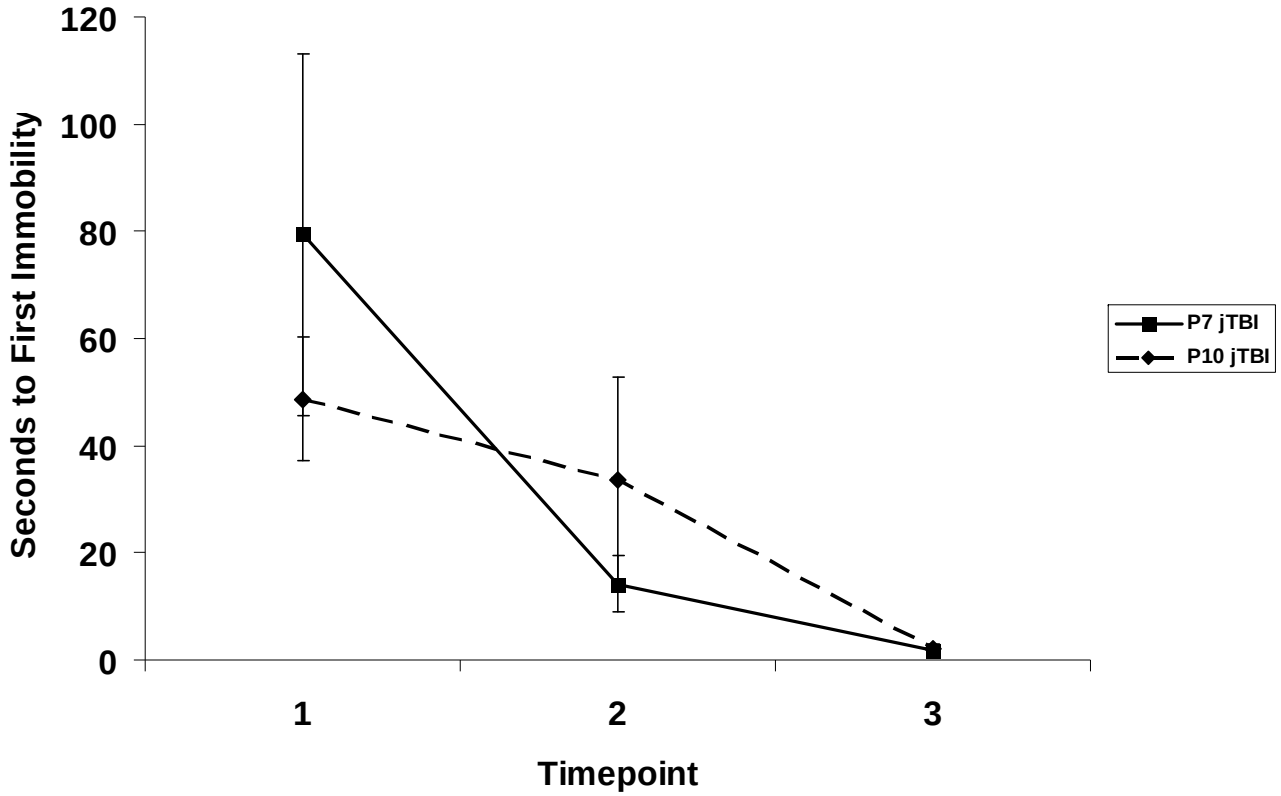

Figure 7. Helplessness behaviors between P7/P10 animals – forced swim

The hypothesis that TBI animals would exhibit impaired sensorimotor abilities on the rotarod compared to shams was confirmed (Figure 8). Sphericity assumptions were not violated, so the sphericity assumed metrics were used. The interaction between group and timepoint was significant, $F(1, 47)=10.621$, $p<.01$, power=.89, $\eta^2=.18$. Additionally, there was a main effect for timepoint $F(1, 47)=162.08$, $p<.001$, power=1.0, $\eta^2=.78$. Sham animals ($M=26.14$, $SD=5.21$) demonstrated overall greater sensorimotor abilities than TBI animals ($M=16.93$, $SD=6.11$), $F(1, 47)=25.64$, $p<.001$, power=1.0, $\eta^2=.35$.

The hypothesis that severely injured TBI animals would show greater sensorimotor deficits on the rotarod than less severely injured TBI animals was not confirmed (Figure 9). Sphericity assumptions were not violated, so the sphericity assumed metrics were used. The interaction between day/timepoint and severity level was not significant. The main effect for timepoint was significant, with both groups demonstrating better sensorimotor performance at 8 months than at 6 months, $F(1, 37)=102.07$, $p<.001$, power=1.0, $\eta^2=.73$. The main effect between mild/moderate and severe animals was not significant, $F(1, 37)=.09$, $p=.77$, power=.06, $\eta^2=.002$.

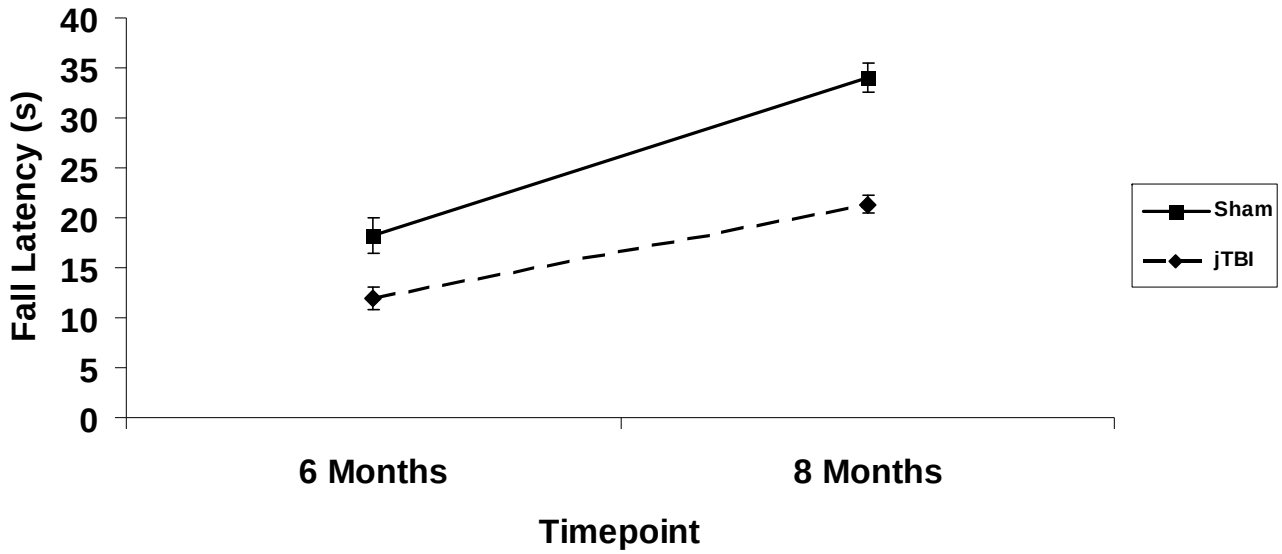

Figure 8. Sensorimotor performance over time – Rotarod

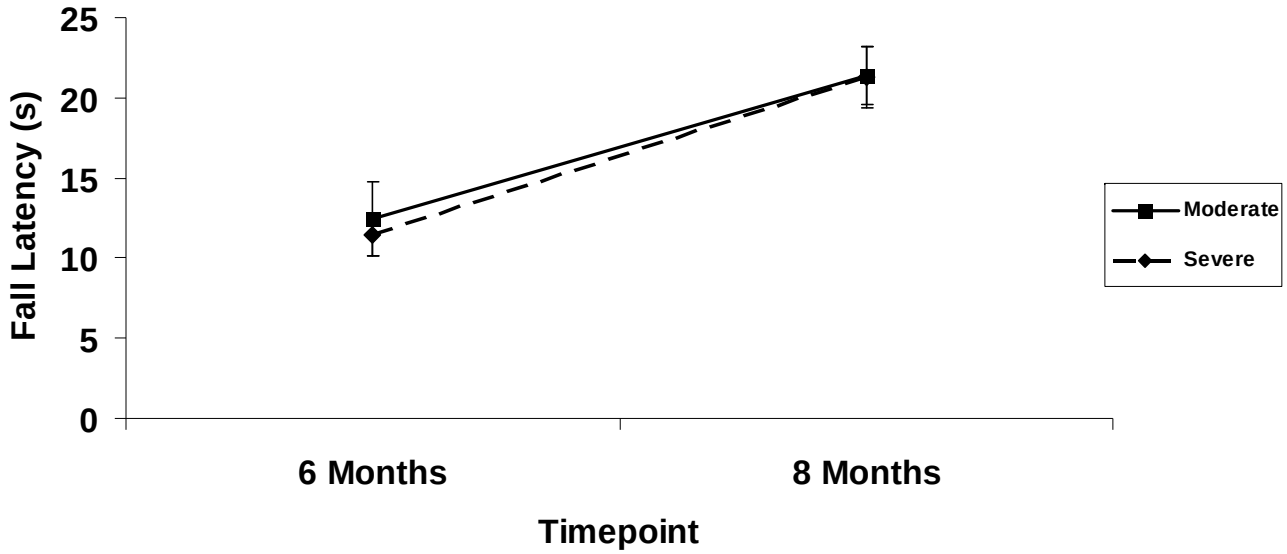

Figure 9. Sensorimotor performance for moderate/severe jTBI animals – Rotarod

The hypothesis that TBI animals would exhibit impaired spatial learning on the water maze compared to shams was confirmed (Table 1 & Figure 10). The interaction between group and day/ of testing was not significant. There was a main effect for timepoint, with both groups performing better at 8 months compared to 6 months, $F(1, 47)=37.92$, $p<.001$, power=1.0, $\eta^2=.08$. There was a main effect for group, with TBI animals ($M=7177.49$, $SD=1855.37$) exhibiting higher overall cumulative distances from the platform than sham animals ($M=5109.32$, $SD=1855.37$), $F(1, 47)=9.89$, $p<.01$, power=.99, $\eta^2=.03$.

Table 1

Mixed-model repeated measures nested ANOVA for spatial water maze

	Degrees of Freedom	F	Significant?
Experimental Group	1	9.89	Yes
Timepoint	1	37.92	Yes
Test Day	2	2.71	No
Block	4	19.81	Yes
Trial	1	10.12	Yes
Group x Timepoint	1	1.38	No
Group x Day	2	2.17	No
Group x Block	4	0.64	No
Group x Trial	1	0.65	No
Timepoint x Test Day	2	10.12	Yes
Timepoint x Block	4	1.98	No
Timepoint x Trial	1	0.00	No
Day x Block	8	2.58	Yes
Day x Trial	2	2.17	No
Block x Trial	4	2.54	Yes
Group x Timepoint x Day	2	1.78	No
Group x Timepoint x Block	8	0.34	No
Group x Timepoint x Trial	1	1.07	No

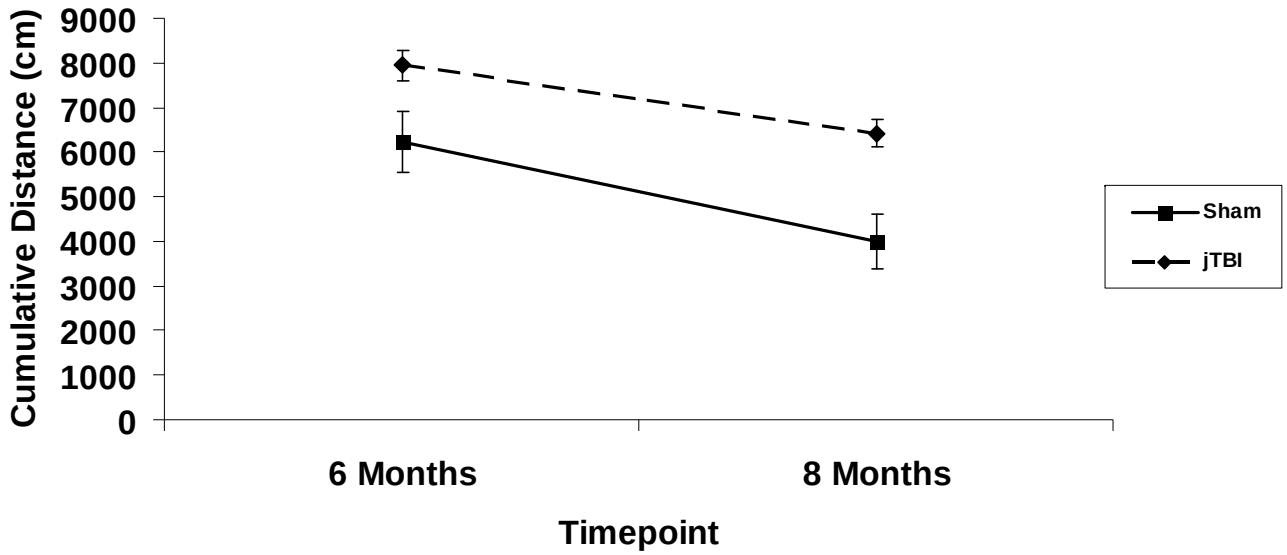

Figure 10: Spatial memory performance over time – water maze

The hypothesis that TBI animals would show less effective search strategies on the Morris water maze compared to sham animals was confirmed (Figures 11 & 12). The sphericity assumption was violated $\chi^2(275)=367.39$, $p<.01$ and thus the Huynh-Feldt corrected statistic was used. The interaction between timepoint and TBI/sham was not significant. There was a main effect for timepoint, $F(19.56, 919.52)=2.23$, $p<.02$, power=.99, $\eta^2=.05$ with both groups using more spatial strategies as time went on. There was also a main effect for group, with sham animals ($M=1.57$, $SD=.15$) utilizing more overall spatial strategies than TBI animals ($M=1.76$, $SD=.26$), $F(1, 47)=5.35$, $p<.03$, power=.62, $\eta^2=.10$.

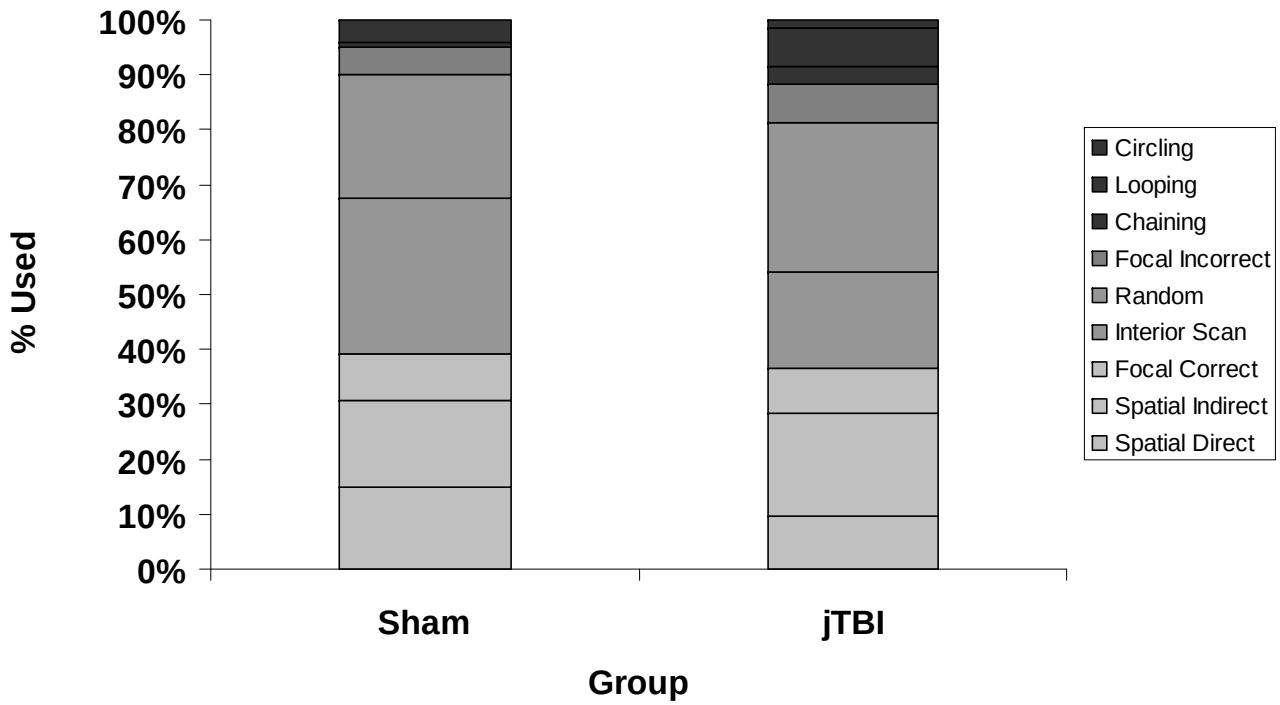

Figure 11. Water maze search strategy – 6 months post-injury

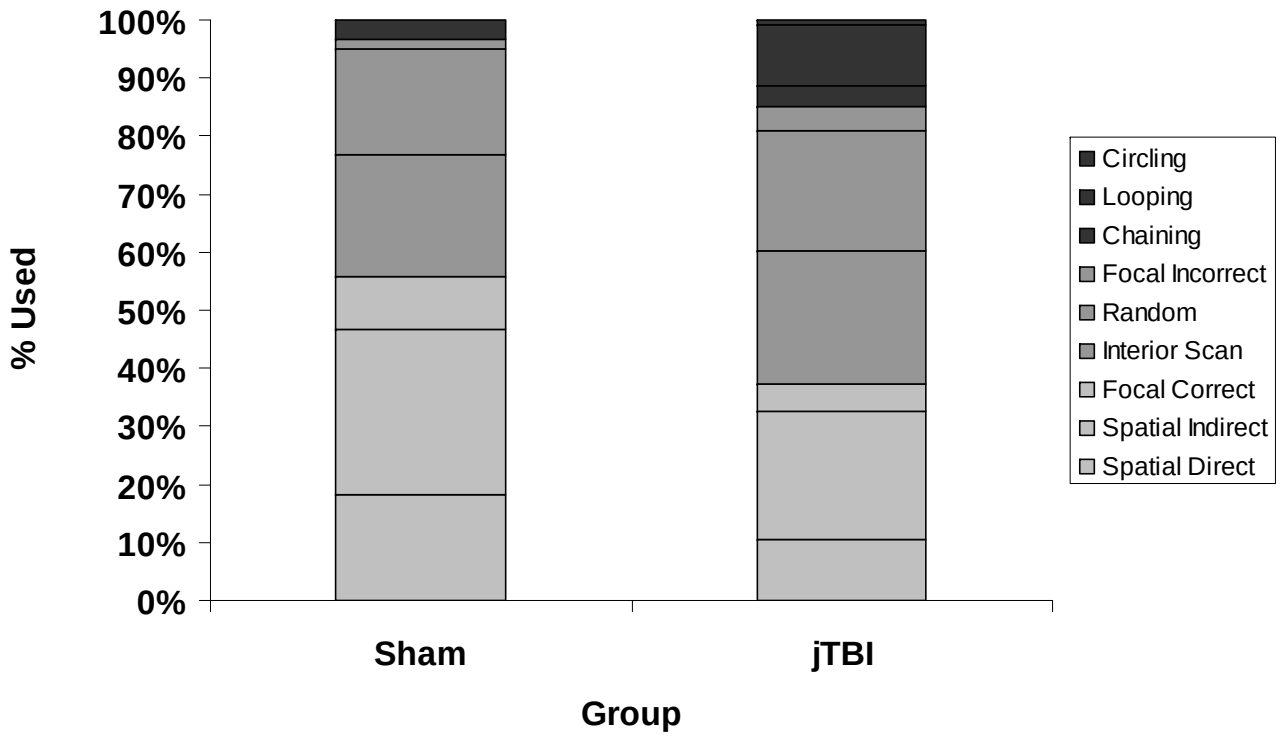

Figure 12. Water maze search strategy – 8 months post-injury

The hypothesis that search strategy would be correlated with spatial performance was partially confirmed (Tables 1-3). While TBI animals did not display a relationship between their spatial performance and search strategy, sham animals did have a significant positive correlation at 6 months post-injury, r=.75, p<.05. Neither group had a significant correlation at 8 months post-injury.

Table 2

Bivariate correlations between spatial performance and search strategy at 6 and 8 months post-injury

Measure	*1*	*2*	*3*	*4*
6 month spatial performance	-			
8 month spatial performance	*.69***	-		
6 month search strategy	*.19*	*.15*	-	
8 month search strategy	*.20*	*.04*	*.63***	-

*p<.05, **p<.001

Table 3

Bivariate correlations between spatial performance and search strategy at 6 and 8 months post-injury – sham animals

Measure	*1*	*2*	*3*	*4*
6 month spatial performance	-			
8 month spatial performance	*.48*	-		
6 month search strategy	*.75**	*.26*	-	
8 month search strategy	*.36*	*-.01*	*.51*	-

*p<.05, **p<.001

Table 4

Bivariate correlations between spatial performance and search strategy at 6 and 8 months post-injury – TBI animals

Measure	*1*	*2*	*3*	*4*
6 month spatial performance	-			
8 month spatial performance	*.744***	-		
6 month search strategy	*.14*	*.12*	-	
8 month search strategy	*.18*	*.02*	*.64***	-

*p<.05, **p<.001

Discussion

The overall results from this experiment show that there are long-term behavioral deficits following a moderate to severe brain injury in neonates. jTBI mice as a group showed persistent deficits in hyperactivity, sensorimotor, and spatial learning domains, consistent with the literature of more short-term injuries (Fujimoto, et al., 2004; Max, et al., 2004).

Specifically, TBI animals displayed persistent hyperactivity over time. These differences did not resolve with repeated exposures to the test but remained constant up to 8 months after the injury, equivalent to an adult human in terms of development. These results corroborate the clinical literature on ADHD after TBI in human patients of ages 0-18, consistent with the neonatal age used in this study (Geraldina, et al., 2003; Max, et al., 1998). Similarly, results show that younger, more severely injured animals performed worse than other TBI groups over time. Like the above TBI/sham differences, post-natal day 7 animals showed more hyperactivity than any group except P10 mild animals. These results corroborate the shorter-term data in the literature suggesting that more severe injuries will produce greater sensorimotor deficits (Li, et al., 2006; Max, et al., 2004).

TBI animals did show long-term motor deficits compared to sham animals. These differences existed at both the 6 month and 8 month timepoints, suggesting a statistical lack of "rehabilitation" over time, with motor deficits between sham and TBI animals growing larger over time due to the slower rate of adaptation to the task demonstrated by the TBI animals. This finding coincides with the findings of other studies, that have

demonstrated in the short-term that moderate or severely injured animals demonstrate a much slower rate of recovery (Hamm, et al., 1994). These findings do run contrary to other examples in the literature, which found no discernable sensorimotor deficits after 11 weeks (Fujimoto, et al., 2004; Lindner et al., 1998), and normal sensorimotor performance in 2.0mm animals 30 days after injury using identical TBI parameters (Brody, et al., 2007). However, these studies all examined TBI in older juvenile or adult animals rather than the neonatal groups used in this study.

The injury severity may explain the lack of differences seen between animals of the two injury conditions. While the literature has shown that animals injured to different degrees recover at different rates, the relatively severe injury model used in this study may demonstrate a floor effect for the lack of recovery demonstrated. Thus, moderately injured mice performed just as poorly as severely injured mice because both groups represent the lower end of functional recovery observed on this test. Although other studies have demonstrated baseline-level sensorimotor recovery anywhere between a few days after surgery to 11 weeks (Brody, et al., 2007; Hamm, et al., 1994; Lindner, et al., 1998), the younger sample used in this study may account for the floor effect observed in terms of sensorimotor performance. Clinically, younger patients do often demonstrate worse motor performance than older children or adolescents which corroborates the results obtained by our animal model (Ewing-Cobbs, et al., 1998).

TBI animals displayed lower overall anxiety-like behaviors than shams. This was opposite the hypothesized effect. The obtained result may be due to several sham animals that remained within the confines of the walled area nearly exclusively, nearly 98% of the time. Qualitatively these animals appeared calm and displayed normal

exploratory behaviors, but did not venture beyond the walled area for a large percentage of time. Statistically, these animals were not outliers and thus were not removed from analysis. TBI animals also expressed a much lower minimum score, due to some animals remaining in the lit area the vast majority of their trials. These animals also did not appear overly stressed (i.e., did not "freeze" in the open), but simply appeared unmotivated to explore. If the above results are considered valid, they contradict previously published evidence showing elevated anxiety in humans and animals following TBI (Granacher, 2003; N. C. Jones, et al., 2008; Jorge & Starkstein, 2005). However, most of the experimental animal data has used rats rather than mice. Although no published evidence comparing rats and mice on the zero maze was found, it is possible that the obtained results are indicative of a simple species difference.

TBI animals displayed no differences in depressive-like symptoms on the forced swim test. These results were similar to those observed by other studies at the 6-month timepoint (N. C. Jones, et al., 2008). Depressive effects have been seen experimentally up to 90 days after injury, and this test has been shown to be a good measure of helplessness and depression (Lahmame, et al., 1997; Milman, et al., 2005; Porsolt, et al., 1977). However, published experimental data have either not focused on TBI or not obtained results after 90 days. For these reasons, any follow-up long-term testing should include shorter timepoints (e.g. 30 or 90 days) to corroborate the injury model with previously published data.

TBI animals showed persistent spatial learning deficits on the Morris water maze compared to sham animals. These results are consistent with past literature, which has shown the water maze to be an effective discriminant between TBI and sham animals

(Brody, et al., 2007; Fujimoto, et al., 2004; Morris, 1984; Prins, et al., 2003; Zohar, et al., 2003). However, no studies have extrapolated cognitive differences following TBI out to 8 months in a juvenile model. These results are consistent with clinical literature, which has shown similar memory deficits following a TBI (Donders & Warschausky, 2007; H. S. Levin, et al., 1982). The fact that obtained results from this study showed a consistent learning deficit, rather than a timepoint-sensitive interaction, suggests that the cognitive deficits have stabilized by 8 months, and little improvement would be seen past that point.

TBI animals displayed less efficient search strategies on the Morris water maze compared to sham animals. Although both groups used more spatial-type strategies from initial testing at 6 months post-injury to the final 8 month timepoint, sham animals used more overall spatial strategies than TBI animals, suggesting a qualitative memory difference between the two groups. The fact that TBI animals used a greater percentage of less efficient non-spatial type strategies compared to shams points to a "compensation effect" whereby TBI animals circumvent their memory deficits using other means. The fact that these differences occurred even after 8 months show that TBI animals show persistent hippocampus-mediated learning and memory deficits that do not resolve over time.

While TBI animals did not demonstrate any relationship between their spatial performance and search strategy, sham animals did exhibit a relationship at 6 months post-injury. This suggests that a more efficient search strategy is associated with better spatial performance in sham animals, but not in TBI animals. The lack of a relationship between strategy and performance in TBI animals indicates that a more efficient search

strategy is not associated with an increase in actual spatial performance. Thus, although TBI animals showed improvements in their spatial abilities and search strategies over time, consistent between-group differences and the lack of a performance-strategy relationship suggest that TBI animals do not benefit from a more efficient strategy. Sham animals, however, showed a correlation between their spatial abilities and strategy use at 6 months, suggesting they were able to benefit from employing more spatial-type strategies. The lack of a relationship at 8 months in sham animals may represent a possible floor effect in strategy use, as sham animals had less room to improve strategy usage relative to their overall spatial performance, given their relatively high use (40%) of spatial strategies at 6 months.

In terms of power, appropriate N's were used for this experiment as suggested by pilot data (suggested N=48). Appropriate power (>.80) was achieved for all significant effects other than between-group differences for the activity test. However, as power analyses are more concerned with type-II error and a significant difference was found, this was less of a concern. All non-significant findings demonstrated a lack of appropriate power. This is more of a concern, and calls into question several non-significant findings, particularly the depressive findings, as between-group anxiety differences achieved a more reliable power of .62. The lack of a standardized forced swim protocol, due to its relative scarcity in the TBI literature, may account for the large within-group variability and small effect sizes noted for depressive effects. Future research would benefit from a more robust a priori power analysis incorporating all planned behavioral tests.

In summary, this study has shown that persistent, stable deficits still exist up to 8 months post-injury in neonatal mice. These results suggest that a middle-aged human who suffered a TBI as a newborn may see effects as late as middle age or beyond. Because longitudinal human studies of this nature would take an immensely long time, animal research should be used to expedite the study of long-term TBI and design therapeutic interventions. Whereas few differences between the two ages or severities existed, many TBI-sham differences were noted. These differences were persistent, suggesting long-term impairment in sensorimotor and hippocampally-mediated memory domains, and the presence of hyperactivity in TBI animals (compared to shams). Using these domains as a guide, future research can focus on specific interventions that target these domains to bring rehabilitation to the many clinical victims of TBI.

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