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A RAT MODEL OF SLEEP DEPRIVATION PRIOR TO TRAUMATIC BRAIN INJURY

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**ABSTRACT**

Traumatic Brain Injury (TBI) has been called the “signature injury” of U.S. soldiers in Iraq and Afghanistan. Soldiers undergo a variety of stressors during their tours of duty that could complicate recovery from TBI, one of which is sleep deprivation (SD). In this study, we sought to create a rat model exploring the effects of prior REM sleep deprivation (RSD) on recovery from TBI-induced sensorimotor and cognitive deficits. Rats were deprived of REM sleep before they underwent a controlled cortical impact (CCI) to mimic a TBI. Forelimb sensorimotor function, hindlimb motor function, forelimb motor function, and spatial learning were assessed using the Bilateral Tactile Stimulation (BTS) test, Ledge Tapered Beam (LTB) test, Limb-use Asymmetry Cylinder (LAC) test, and Morris Water Maze (MWM) respectively. Our hypothesis was that RSD would impede CCI recovery compared to controls that underwent CCI without prior RSD. However, rats undergoing RSD prior to CCI exhibited less impairment during the BTS and LTB tests than controls that underwent CCI without prior RSD. Additionally, control rats that underwent the condition where they spent 24 hours in the RSD chamber without RSD prior to CCI performed worse than all the other groups during the MWM task. No group differences were found in the LAC test. These findings led us to reject our hypothesis and theorize that RSD had a neuroprotective effect against TBI-related damage. Furthermore, we concluded that stress prior to TBI worsened recovery, and that SD protected against this effect.

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## CHAPTER I

### INTRODUCTION

Traumatic brain injury (TBI) has been called the signature injury of Operation Enduring Freedom and Operation Iraqi Freedom due to the high incidence of improvised explosive devices utilized in Afghanistan and Iraq. In fact, TBI accounts for 22% of wounded soldiers who come back from Afghanistan and Iraq (Wojcik, 2010). An estimated 110,392 military personnel were reported to have at least one TBI-related medical encounter, and 15,732 have been hospitalized due to TBI from 1997 to 2006. Improved armor and medical interventions have helped soldiers survive blasts in combat, but increased survival means that many more are coming back with brain injuries (Sponheim, 2011).

Some evidence has shown a higher prevalence of TBI-related cognitive deficits than expected in the soldier population (French, 2010; Howe, 2009; Luethcke, 2010). Additionally, there is an increased prevalence of PTSD and post-concussive headaches related to TBI in soldier populations (Hoge, 2008; Stulemeijer, 2006; Vargas, 2009;

Wilk, 2010). There are a variety of factors which make combat-related TBI more complex than TBI endured by the U.S. civilian population, and some speculate that the complex mechanisms of blast injuries could worsen TBI outcome. Blast-related injuries have four mechanisms that induce brain damage. These include primary effects attributed to pressurization changes from the blast wave, secondary effects caused by flying shrapnel, tertiary effects resulting from being thrown into obstacles, and quaternary effects resulting from factors such as toxins and fire (Belanger, 2009; Howe, 2009; Lippa, 2010; Luethcke, 2010).

The environment of a combat soldier includes numerous stressors which impact neurological, cognitive, and endocrine function. The impact of these stressors could also worsen recovery from TBI (French, 2010; French & Parkinson, 2008). Stress affects hormones in many ways including increasing cortisol, which suppresses the immune system and worsens its function (Zuiden, 2009), and decreasing insulin, testosterone, thyroid hormone T<sub>3</sub> and T<sub>4</sub>, and insulin-like growth factor in soldiers. They may also experience reduced nutrition and increased fatigue and sleep deprivation (SD), which may be responsible for cognitive and attention deficits. Additionally, fatigue may worsen the outcome of TBI by mediating fatigue-induced neurological changes that impact immune system regulation (Weeks, 2010).

SD, a common stressor experienced by soldiers in the combat zone (French, 2010; Van Hoof, 2008; Weeks, 2010), has been proven to be deleterious to neural and behavioral functions as evidenced in rodent models, so its effects on brain injury recovery also need to be investigated. In one example, rats that underwent 96 hours of REM sleep

deprivation (RSD) showed learning deficits in a discriminative avoidance task immediately after the RSD period (Alvarenga, 2008). In another study, rats that underwent 5 days of RSD showed impaired spatial learning in the Morris Water Maze (MWM) test. Additionally, hippocampal neuron activity was inhibited during SD, which negatively impacted memory function (Yang, 2008). SD has also been shown to decrease the metabolic rate of glucose in the prefrontal cortex, the thalamus, and the parietal lobe, which could impair higher-order cognitive function and the relaying and integration of sensory information (Tomasi, 2009). In all these SD rodent models, deficits disappeared after the rats had sufficient sleep. However, alterations in function during the SD period imply that the brain is in an altered state that may respond differently to injury.

To understand how the brain will respond to conditions co-occurring with TBI, it is important to understand what occurs when a TBI alone is present. The mechanics of TBI damage itself includes primary, direct physical damage to the brain resulting in torn and broken tissue, and secondary effects such as inflammation and swelling that arise later (Cederberg & Siesjö, 2010; Wilson, 2010). Elevated cytokines in response to TBI actually trigger cell death (Grosjean, 2007; Lenzlinger, 2001; Ziebell & Morganti-Kossmann, 2010), and excessive glutamate release also causes cell damage (Lau & Tymianski, 2010; Yi & Hazell, 2006).

Sensorimotor tests are useful in rodent models because they are indicative of the severity of TBI, (Markgraf, 2001; Yu, 2009). Sensorimotor deficits are more short-term than cognitive deficits and dissipate typically after two weeks (Shanina, 2006; Tennant &

Jones, 2009). Also, sensorimotor tests provide a means to assess and control for any motor impairments which could introduce confounding results in tests for cognitive function.

Rodent models are useful for observing how TBI impacts long-term cognitive function (Abdel-Baki, 2009). The MWM is typically used to measure spatial learning by measuring how fast a rat learns to find a hidden platform in a pool by using visual cues in the room. However, working memory can be assessed by changing the location of the platform and measuring how long it took rats to find the new locations instead of measuring how long they took to learn finding a platform in one consistent location (i.e. the traditional MWM setup). In one rat model, TBI was detrimental to working memory in a delayed match-to-place version of the MWM after a controlled cortical impact (CCI) was administered unilaterally with a 6mm diameter probe tip to the medial prefrontal cortex (mPFC). Unlike most rodent models that test for deficits a few weeks after surgery, these deficits were long-lasting, for they were exhibited four months after the injury (Hoskison, 2009). Spatial learning deficits were also found two to three weeks after rodents underwent CCI procedures above the hippocampus and the mPFC in the traditional MWM setup. Brain injured rats learned slower than the controls and there was no evidence of recovery of cognitive function (Goss, 2003; Kline, 2000; Kline, 2002; Long, 1996).

While SD alone can be deleterious to brain function, this study is focused on how initial injury severity and recovery is impacted by the SD-induced altered state, as opposed to assuming an additive effect of SD and TBI. One possibility is that prior SD

could inhibit recovery from TBI. To investigate this, we sought to study the effects of SD on TBI severity and recovery in order to more closely model a brain injury incurred under the stressors of combat. Tests of cognitive, motor, and sensory function were implemented to understand how prior SD impacts recovery from TBI. To investigate this, rats underwent 24 hours of rapid eye movement SD (RSD) via the disk-over-water method (Mendelson, 1974) immediately prior to CCI to their sensorimotor cortex. RSD was utilized instead of total SD because it is less physically stressful than total SD procedures, and it is still sufficient to impair cognitive functions in rats (Alvarenga, 2008). More details of the RSD method are specified in the methods section.

The CCI model was chosen over other models of TBI. The CCI procedure is widely accepted for emulating TBI in a laboratory setting with rodents (Dixon, 1999; Saatman, 2006). The CCI model allows localization of the injury. Also, we can dictate a more consistent degree of severity of injury by specifying the depth, width, and impact speed of the probe that induces injury (Markgraf, 2001). One frequently used TBI procedure is the Fluid Percussion Injury (FPI) model. FPI inflicts injury by applying a fluid pressure pulse to the brain through a craniotomy with a plastic cap in place. FPI is favored because it can also provides varying degrees of injury. Its drawbacks include high severity and mortality due to the technique's frequency of affecting the brain stem. It is also difficult to control the localization of the injury with this model. Other models are more localized than FPI, such as utilizing a mechanical suction force applied to the intact dura and microinjecting a fluid containing zynosan, which activates macrophages. However, these models do not cause lasting deficits, so they would not be appropriate for TBI research (Cernak, 2005; Laurer, 2000). Our primary hypothesis was that prior RSD

would exacerbate the brain's injury response and thus worsen the initial TBI and potentially impede recovery.

## CHAPTER II

### METHODS

#### Procedure

After a 7 day acclimation period, each rat was handled for five minutes daily for 5 days prior to training to increase familiarity with and decrease stress between the experimenters and the rats. This handling period occurred in the same room as where the behavioral measures were taken. At 12 weeks of age rats underwent pre-operative behavioral procedures to record baseline measures. Once baseline measures were successfully completed, rats were exposed to RSD or a control condition. After the 24-hour period, the rats underwent a CCI to model TBI. Rats had a recovery period of three days before behavioral testing. Rats were housed socially in the animal resource facility and moved to a separate room for all handling including training, behavioral measures and surgical procedures.

The rats performed four behavioral tasks. The bilateral tactile stimulation test was used to test forelimb sensorimotor function, and the beam-walking and cylinder tasks

tested hindlimb and forelimb motor function respectively. These tests are all indicative of injury to regions of the sensorimotor cortex affected with the CCI procedure.

Measures were taken on post-operative days 3, 7, 11 and 15. During a testing day, the bilateral tactile stimulation test was completed first, followed by the ledged tapered beam test and finished with the limb-use asymmetry cylinder test. The MWM procedure began on day 26 post-surgery. This test is sensitive to hippocampal damage and measures cognitive function by measuring spatial learning. During behavioral testing, the experimenter was aware of the rats' ID numbers but was naïve to experimental group. Details of each behavioral task are described in later sections.

### Subjects

Long-Evans rats were shipped at 10 weeks of age and weighing between 250 and 275 grams (Harlan Laboratories, Indianapolis, IN). This strain of rat is reliable in recording behavioral tasks and males were used to reduce variations in hormonal cycles. The rats were randomly divided into five groups: SP+CCI (Small Platform, RSD and Controlled Cortical Impact), LP+CCI (Large Platform, no SD but in RSD environment and Controlled Cortical Impact), NH+CCI (Normal Housing and Controlled Cortical Impact), SP+No Surgery (Small Platform RSD and No Surgical), and NH-Sham (Normal Housing and Sham Surgery). LP rats were kept in the same conditions as the disk-over-water RSD method, with the exception that the disk was large enough for the rat to engage in all stages of sleep including REM. The LP+CCI group was included with the purpose of controlling for the conditions in the disk-over-water method. The NH rats were kept in their standard housing condition cages. The NH+CCI group served as a control



comparison of housing conditions to the rats in the SP+CCI and LP+CCI groups. An SP+No Surgery group was implemented to control for the effects of RSD alone on behavior. Lastly, the NH+Sham group underwent a surgery but they did not sustain a brain injury, and they were also kept in standard housing. The NH+Sham group served as a control to assess the effects that surgery may have had on the rats' behavior.

There were four other possible groups that could have been added: a SP+Sham group, a LP+Sham group, a LP+No Surgery, and a NH+No Surgery group. The SP+Sham group was not included because the effects of RSD and the sham surgery are already controlled for in this study's SP Only and NH+Sham groups respectively. The LP+Sham and the LP+No Surgery groups were not included because there is already a comparison being made between the SP+CCI and LP+CCI groups to control for the condition of the environment during the RSD phase. Lastly, the NH+No Surgery group was not included because the SP+No Surgery group was expected to recover from RSD by the time that post-operative behavioral measures resumed.

		Surgery Condition		
		CCI	Sham	No Surgery
Housing Condition	SP	SP+CCI	SP+Sham	SP+No Surgery
	LP	LP+CCI	LP+Sham	LP+No Surgery
	NH	NH+CCI	NH+Sham	NH+No Surgery

Figure. 1. Experimental groups that were included and excluded.

Of the five experimental groups, the SP+CCI group was expected to exhibit the worst impairments across the four behavioral tasks because we theorized that prior RSD would worsen recovery from CCI. We also hypothesized that the LP+CCI and NH+CCI groups

would exhibit fewer deficits than the SP+CCI group because these two groups underwent CCI without prior RSD. Additionally, we hypothesized that the SP+No Surgery and NH+Sham groups would exhibit no deficits because they were not undergoing the CCI procedure. A graph roughly illustrating our hypotheses of group performances relative to each other is presented in Figure 2. The rats were organized into smaller squads to allow for manageably sized rat groups to perform experimental procedures. The number of rats in each experimental group was balanced as much as circumstances allowed (Fig. 3).

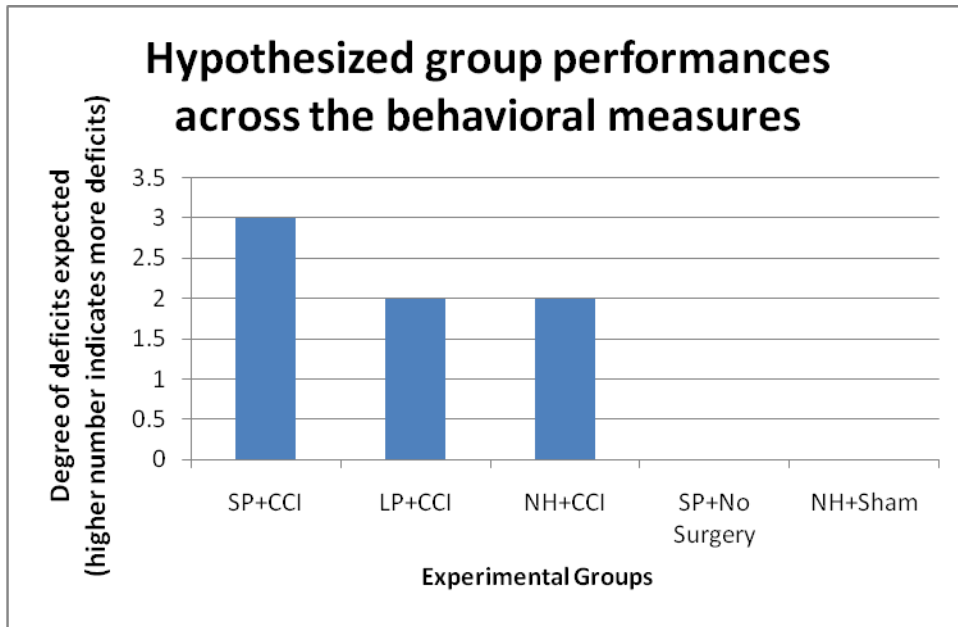


Figure 2. Hypothesized group performances across the behavioral measures

	SP+CCI	LP+CCI	NH+CCI	SP+No Surgery	NH+Sham	Total
Squad 1	1	2	1	2	0	6
Squad 2	1	2	2	3	2	10
Squad 3	3	2	2	2	2	11
Squad 4	2	2	3	3	3	13
Squad 5	3	2	2	0	3	10
Total	10	10	10	10	10	50

Figure 3. Distribution of rats among squads

#### Acclimation and Handling Procedures

After arrival, rats were housed socially for one week in a separate housing room. This room was kept on a light-dark cycle of light from 6 am to 6 pm, and dark from 6 pm to 6 am. The Monday after they arrived, they were transported by cart to the behavioral testing room for handling. The handling phase was necessary to increase the comfort level of the rats with the experimenter and the behavioral testing environment. Handling consisted of each animal being removed from the cage and gently touched and held on the experimenter's arm for five minutes before being returned to their home cage. Handling occurred daily for five days in a row, and behavioral testing began the following week.

#### Sleep Deprivation Procedure

The rats were deprived of REM sleep via the disk-over-water method proposed and first used by Mendelson, *et al.* (1974). During RSD, rats were placed in a bucket with a circular clear acrylic platform elevated four inches from the bottom of the bucket. The

diameter of the platform for rats undergoing RSD was 10 cm, and the diameter of the platform for the rats in the large platform condition was 14 cm. The bucket was filled up with water just below the level of the platform. Food and water were provided ad libitum by a water bottle mounted to the side of the bucket and food draped overhead in mesh wiring. This method deprives rats of REM sleep but not non-REM sleep because during non-REM sleep, the rat does not lose muscle tension and make contact with the water. However, muscle tension is lost during REM sleep, which causes the rat to start to fall in the water and awaken. Rats were placed on the platforms in the buckets for 24 hours. To ensure the safety and well-being of the rats, an experimenter inspected them every four hours.

#### Controlled Cortical Impact Procedure

Rats were anesthetized with 4% isoflurane in an anesthesia chamber and secured to a stereotaxic frame where their anesthesia was maintained with 2.5% isoflurane via respiration during the procedure. A midline incision was made and soft tissue was pulled back to reveal the skull. A 5 mm diameter craniotomy was centered 2 mm lateral to the midsagittal plane and -2.0 mm bregma. A 3.0 mm wide probe was then centered at -2.0 mm bregma and the CCI was set to go 2.0 mm ventral from the dorsal surface and delivered at a velocity of 3.0 m/s. This impact was located on the sensorimotor cortex of the rat immediately above the hippocampus. The incision site was sutured and secured with wound clips, and the rat was kept on a heating pad and monitored continuously, until it fully recovered from anesthesia.

### Bilateral Tactile Stimulation Test Procedure

A reliable test of sensorimotor function is the Bilateral Tactile Stimulation Test. This test is advantageous because it can compare sensorimotor function of a rodent between contralateral and ipsilateral sides relative to the CCI, thus giving insight into the severity of brain damage. Additionally, the Bilateral Tactile Stimulation Test has the ability to measure sensory functions more independently of motor functions than most other sensorimotor tests (Schallert & Woodlee, 2005). Other tests of forelimb function were considered. We did not choose the skilled reaching task because it needs a more intensive training period. Additional measures need to be taken to force rats to use their impaired limbs in this task, unlike in the BTS (Bury & Jones, 2004; Hermer-Vazquez, 2004). The vibrissae forelimb placement test involves handling the rat and observing its paw placements while being brought to the edge of a table. While this test is valuable for measuring forelimb and vestibular function, it requires a great deal of handling, and is extremely sensitive to the temperament of the rat (Schallert & Woodlee, 2005).

Each rat performed four trials of the Bilateral Tactile Stimulation Test on days 7, 6, 5, 4, and 3 pre-surgery, and on days 3, 7, 11, and 15 post-surgery. The intertrial intervals were at least five minutes. At the beginning of a trial, an adhesive paper tab was placed on the dorsal side of the relatively hairless part of each forelimb. The rat was placed back into its home cage, and measures were recorded. The times that it took for the rat to first make contact with one of the tabs, remove the first tab, and remove the second tab were recorded. The side (left or right) the rat contacted and removed the adhesive tab from

were also recorded. A trial was considered complete either when the rat removed both tabs or when two minutes had elapsed.

During the pre-surgery trials, we determined which limb the rat tended to contact first (referred to as the favored limb). To determine this, the size of the tabs placed on both of the rat's forelimbs was equal (level 0 on the adhesive ratios chart below). After four trials were completed, the favored limb was defined as the limb the rat contacted first with the highest frequency during the pre-surgery trials. Rats that underwent a CCI received the injury on the brain hemisphere contralateral to their favored limb.

For the post-surgery trials, the rat performed four trials as described above. If the rat contacted the limb ipsilateral to its injury on at least three out of the four trials, it was given additional asymmetry trials to determine the degree of severity. For these additional trials the size ratios of the tabs on the paws were altered according to the Bilateral Tactile Stimulation procedure utilized by Schallert & Woodlee (2005). On the first trial, the rat began with tabs with a level three tab size ratio (2.2:1), and the larger tab was placed on the paw that was contralateral to the injured brain hemisphere. If the rat contacted the smaller tab first, then the experimenter implemented a tab ratio two levels higher on the next trial. If the rat contacted the larger tab first, then a tab ratio one level lower was implemented on the next trial. Once the paw contacted first has reversed between two levels, the levels between which the transition occurred were averaged and recorded and the session was considered complete. For example, if the transition occurred between levels 3 and 4, the rat was given a score of 3.5.

Adhesive Ratios	
Level	Large/Small tab ratio
7	15 : 1
6	7 : 1
5	4.3 : 1
4	3 : 1
3	2.2 : 1
2	1.7 : 1
1	1.3 : 1
0	1 : 1

Figure 4. BTS Asymmetry Tab Ratios

#### Ledged Tapered Beam Test Procedure

The Ledged Tapered Beam Test (LTB) is a test of hindlimb motor function. This test measures the number of times that a rat's contralateral hindlimb relative to the side of injury slips off a balance beam. There are ledges on the sides of the beam for the rat's foot to slip onto. Other tests have to make adjustments to compensate for learning effects, such as altering width of ladder rungs in the horizontal ladder-rung test (Metz & Whishaw, 2002) and altering rotation speed on the rotorod test (Yu, 2009). However, the addition of ledges on the beam walk task is advantageous because they automatically alleviate the tendency for rats to compensate for their motor deficits. Additionally, the beam becomes narrower until it reaches the end. This makes the beam gradually more difficult and allows for separate analyses of different sections of the beam by difficulty (Schallert & Woodlee, 2005).

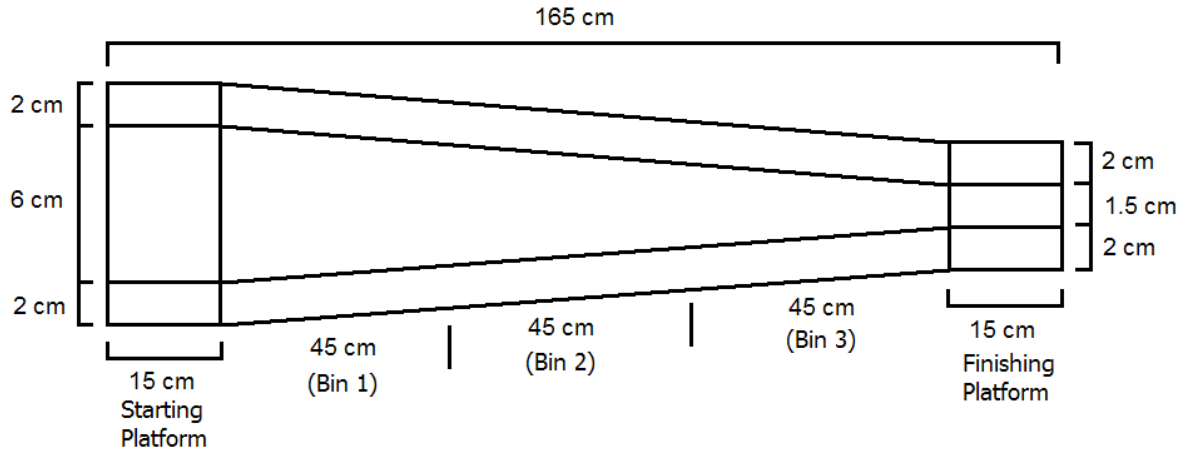


Figure 5. Ledged Tapered Beam Diagram (Above View).

The setup is an opaque beam that the rats walked across in order to reach a dark housing unit or goal box at the end. The beam is 165 cm in length, elevated at a height of 1 m, and it is tapered in such a way that it starts out 6 cm wide and gradually narrows to a width of 1.5 cm at the end. There are 2 cm wide ledges along the bottom of both sides of the beam that are 1 cm lower than the beam. A 15 x 6 cm starting platform serves as the starting point, and the 15 x 1.5 cm finishing platform at the end leads to the goal box. The tapered part of the beam was divided into three evenly spaced sections labeled as Bin 1, Bin 2, and Bin 3.

Each rat underwent this procedure on days 7, 6, 5, 4, and 3 pre-surgery in order to train the rats on the task and to obtain baseline measures. During these training days, if the rat did not leave the start platform after ten seconds, mildly adverse white noise was introduced to motivate the rat to move across the beam away from the noise. The criterion for starting to collect baseline measures was for the rat to start crossing before 10 seconds elapsed. Baseline measures were recorded three days pre-surgery. Measures



were also taken on post-operative days 3, 7, 11, and 15 in order to obtain data pertaining to the degree of motor deficits. At the start of the LTB procedure, a rat was set on the start platform. As the rat progressed down the tapered beam, it passed through the three sections labeled Bin 1, Bin 2, and Bin 3. The number of times that the rat's hindlimb slipped off the beam and touched a ledge was recorded separately for each bin and for each hindlimb. When the rat reached the ending platform that led into the goal box, it was considered one trial. Each rat completed 3 trials per day for both the pre-surgery and post-surgery days and there was a fifteen-minute rest period between each trial. Rats spent this rest period in their home cage.

#### Limb-use Asymmetry "Cylinder" Test Procedure

The Limb-use Asymmetry "Cylinder" test is widely accepted as a measure of forelimb motor function and is advantageous because it requires minimal interaction between the rat and the experimenter (Starkey, 2005; Tennant, 2009; Zhao, 2005). This test takes advantage of rodents' natural tendency to explore their environment by placing a rat into a plastic upright cylinder and observing it as it rears up on its hind legs and supports itself against the wall of the cylinder with its forelimbs. A higher ratio of ipsilateral forelimb placements relative to contralateral limb placements is indicative of greater forelimb motor impairments in this test.

A clear plastic cylinder with a height of 30 cm and a diameter of 20 cm was set upright so that it sat on its base, and the top end was open so a rat could be placed inside. To record the rat's movements, a camera was placed above the cylinder pointing downward to obtain a bird's eye view of the rat in the cylinder.

Each rat performed this task on postoperative days 3, 7, 11 and 15 post-surgery, and each rat only performed one trial of this task per day of testing. During the testing procedure, the rat was placed inside of the cylinder. With its natural curiosity, the rat explored the cylinder by rearing up on its hind legs and supporting itself against the inner wall of the cylinder with its forelimbs. The experimenter allowed the rat to rear up twenty times while exploring the cylinder, and the number of placements of its left, right, and both limbs simultaneously were recorded by analyzing the videos.

### Morris Water Maze Procedure

#### *Morris Water Maze Setup*

The MWM is widely accepted and used as a reliable indicator of spatial learning ability in laboratory rats (Long, 1996; Morris, 1982; Vorhees, 2006). Additionally, its sensitivity to hippocampal damage is also reflective of spatial learning in humans with hippocampal damage (Astur, 2002). In the MWM task, a rat was placed in a shallow pool of 21°C to 24°C water with a hidden escape platform located in one of the four quadrants. The water was rendered opaque by non-toxic white acrylic paint in order to keep the platform hidden from view. The water motivated the rat to swim around and find this escape platform to stand upon. Simple geometric visual cues surrounded the pool at four points equally spaced around the circumference of the maze for the rat to use as a reference for finding the platform. After trials, the water maze was emptied, scrubbed, and rinsed.

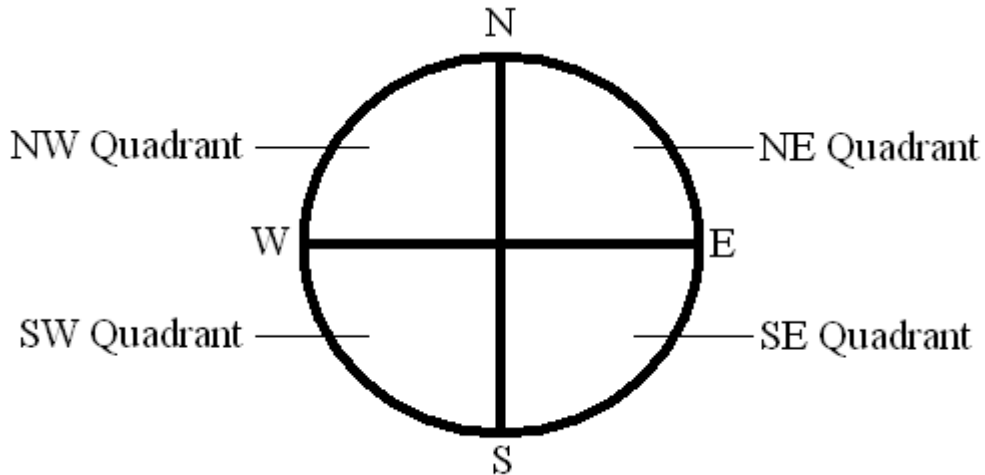


Figure 6. Morris Water Maze Diagram (Above View)

The water maze was a 213 cm diameter circular pool filled to a depth of 20 cm. The maze was divided into four equal sized quadrants by two imaginary lines drawn between the north and south point of the maze, and the east and west point of the maze. A 10 cm diameter Plexiglas platform was used as the escape platform. The escape platform stood 2 cm below the surface of the water in a constant location in quadrant IV. Geometric figures were placed on the walls of the experimental room to serve as spatial cues.

### Training Trials

Rats underwent five days of training in the MWM, which began on day 26 post surgery. On each of these five days, the rats performed four trials. Each rat was released into a different quadrant at the side of the maze for each set of trial pairs. The order of quadrants from which the rats were released was randomized. At the beginning of a trial, the rat was released into the water facing the side of the pool and allowed 120 seconds to find the escape platform. The time that it took for the rats to find the escape platform in

each trial was recorded. If 120 seconds expired before the rat found the platform, then the experimenter gently guided the rat to the platform. Once the rat was mounted on the platform, it was given 15 seconds to rest and observe spatial cues. The rat was then removed from the water maze, dried with a towel, and placed in a warming cage until it was warm and dry. The cage was set up like a normal housing cage, but a heating pad was applied to the outside of the bottom of the cage. Only rats with motor function comparable to baseline measures were included in the MWM test.

### Probe Trial

One day after the five days of training trials (day 31 post surgery), the rats underwent a probe trial. This trial was intended to help ensure that the rat learned the spatial location of the escape platform based on the spatial cues in the room as opposed to merely finding it by accident or seeing it. During the probe trial, the escape platform was removed. The rat was placed in the west point of the pool and allowed to swim for 120 seconds. Time spent in the target quadrant (SE) and the number of times that a rat crossed the former platform area were recorded.

### Cued Trial

The cued trials were performed immediately after the probe trials on post-op day 31. The rats performed the four cued trials in the same manner they performed the training trials, with the following exceptions. During the cued trial, the visual cues were removed by placing curtains around the perimeter of the pool. A white flag was placed on the escape platform in the NE quadrant to serve as an obvious visual cue. The time that it

took for the rat to find the escape platform was recorded, up to a maximum of 120 seconds. To control for rats that were not able to acquire spatial learning skills due to optical deficits, and rats that could not find the platform in the cued trials would have been evaluated as outliers for this study.

## CHAPTER III

### RESULTS

#### Bilateral Tactile Stimulation Test

In general, the results show that the sleep deprivation condition (SP+CCI) did not affect performance in the bilateral tactile stimulation test. However, significant deficits in performance were noted in the LP+CCI and NH+ CCI group compared to the SP+No Surgery group. . Thus, control groups that received CCIs without RSD had impairments, while the group that received RSD prior to CCI did not.

The SP+CCI and NH+Sham groups did not exhibit significant impairments compared to the SP+No Surgery group for latency to contact either paw (Fig. 7;  $p = 0.986$  and  $p = 0.727$ , respectively), latency to remove the first tab (Fig. 8;  $p = 0.990$  and  $p = 0.605$ , respectively), or latency to remove both tabs (Fig. 9;  $p = 0.786$  and  $p = 0.542$ , respectively). However, post-hoc analyses revealed that the LP+CCI group had significantly higher time latencies than the SP+No Surgery group for latency to remove the first tab (Fig. 8;  $p = 0.020$ ) and latency to remove both tabs (Fig. 9;  $p = 0.014$ ).

Additionally, the NH+CCI group had significantly higher time latencies than the SP+No Surgery group for the latency to remove both tabs according to post-hoc analyses (Fig. 9;  $p = 0.018$ ).

In general, overall trends revealed an effect of the CCI on performance on the Bilateral Tactile Stimulation Test across days. Latency to contact either paw increased (Fig. 7; Wilks'  $\lambda = 0.604$ ,  $F(4,37) = 6.065$ ,  $p = 0.001$ ) from baseline on each post-operative day for all groups except for the SP+No Surgery control group. Latency to remove the first tab also increased after the CCI (Fig. 8; Wilks'  $\lambda = 0.546$ ,  $F(4, 37) = 7.690$ ,  $p = 0.000$ ). Additionally, latency to remove both tabs increased after the CCI (Fig. 9; Wilks'  $\lambda = 0.367$ ,  $F(4, 37) = 16.697$ ,  $p = 0.000$ ).

For the post-trial asymmetry test, analyses of the individual days of the post-trial asymmetry tests indicated that the LP+CCI and NH+CCI groups had more severe deficits than the SP+No Surgery group (Fig. 10). One-way ANOVAs between groups were found on Post-Operative Day 3 (Fig. 10;  $F(4,41) = 4.037$ ,  $p = 0.008$ ), and post-hoc Tukey analyses indicated that the post-trial asymmetry scores of the SP+No Surgery group had smaller deficits than the LP+CCI and NH+CCI groups,  $p = 0.011$  and  $p = 0.037$ , respectively.

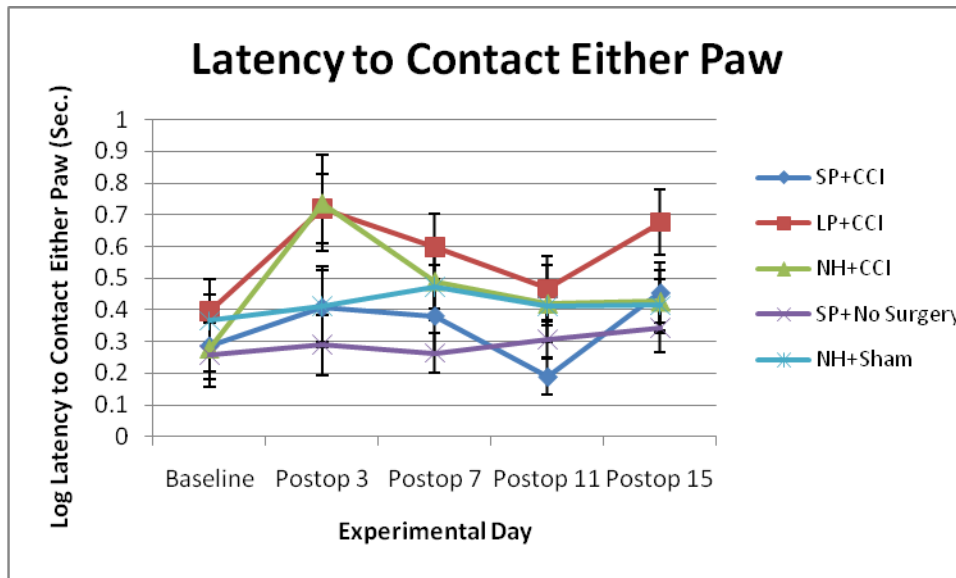


Figure 7. The latencies to contact either paw during the BTS test. The LP+CCI and NH+CCI groups exhibited greater time latencies than the SP+CCI group and the other groups. Additionally, the LP+CCI group consistently performed worse than all the other groups. Data are expressed as mean  $\pm$  S.E.M.

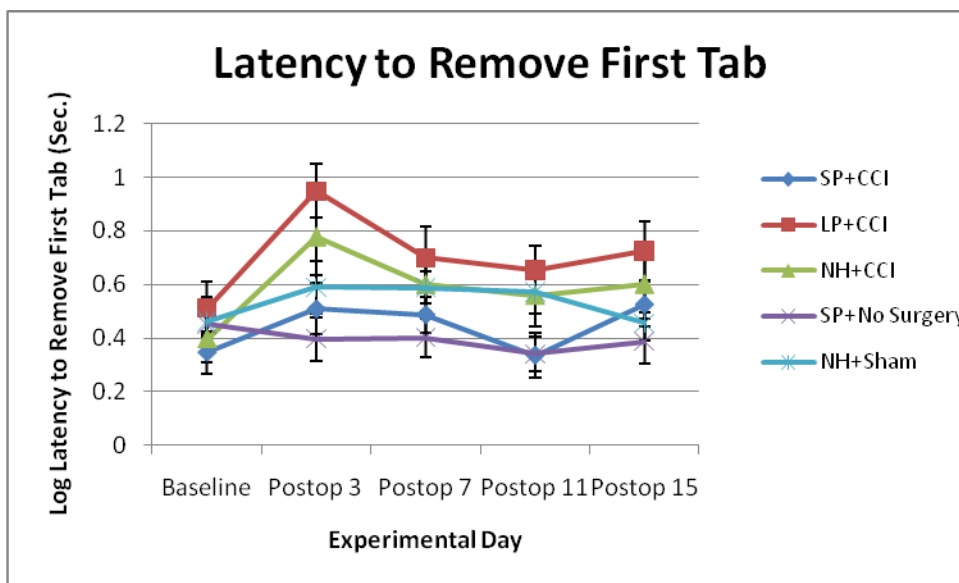


Figure 8. The latencies to remove the first tab during the BTS test. The LP+CCI group performed consistently worse on the post-operative days than the other groups, followed by the NH+CCI, NH+Sham, SP+CCI, and SP+No Surgery groups. Data are expressed as mean  $\pm$  S.E.M.



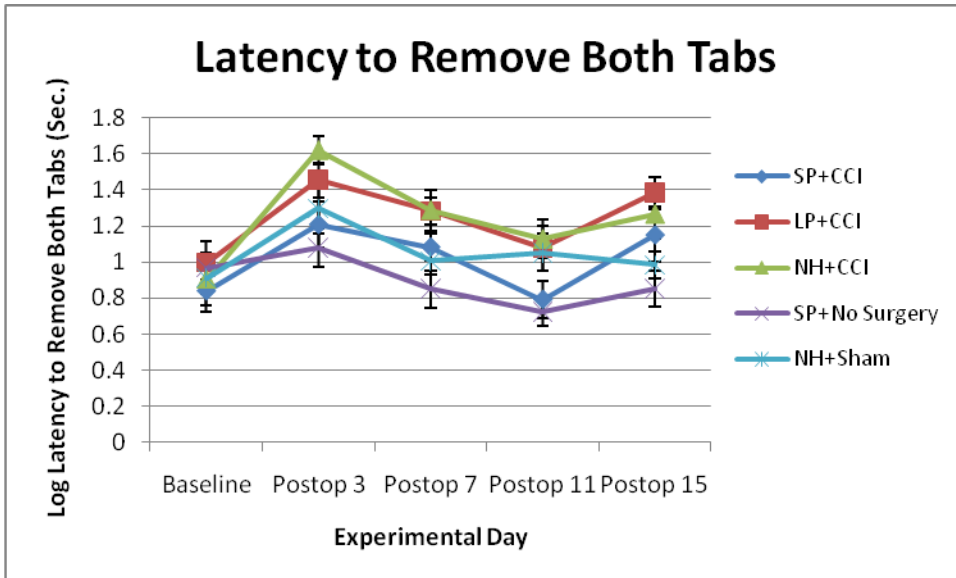


Figure 9. The latencies to remove both tabs during the BTS test. The LP+CCI group had consistently higher time latencies than the SP+CCI and other control groups across all the post-operative days. Data are expressed as mean  $\pm$  S.E.M.

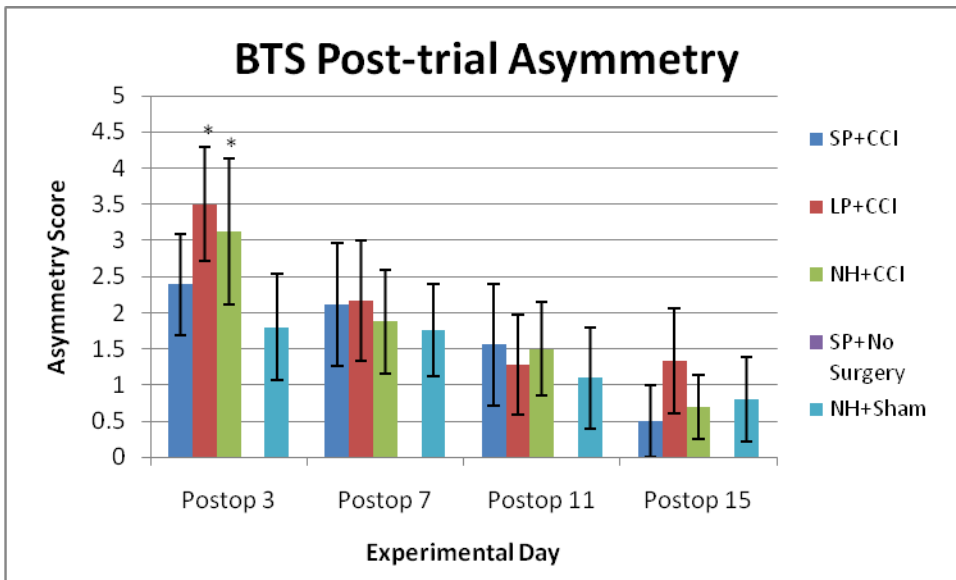


Figure 10. The post-trial asymmetry scores during the BTS test. All groups except the SP+No Surgery group exhibited deficits across the days, and deficits gradually declined over time. On post-operative day 3, the LP+CCI and NH+CCI groups exhibited higher deficits than the SP+CCI and NH+Sham groups, and the SP+No Surgery group exhibited no deficits on any day. Data are expressed as mean  $\pm$  S.E.M. (\*  $p < 0.05$  from SP+No Surgery Group).

### Ledged Tapered Beam Test

All the groups except for the SP+No Surgery group exhibited deficits during the Ledged Tapered Beam Test. However, the SP+CCI group exhibited fewer deficits than the LP+CCI and the NH+CCI groups when all these groups were compared to the SP+No Surgery group. Post-hoc analyses of the total average contralateral footslips showed that the SP+CCI and NH+Sham groups did not exhibit significant impairments compared to the SP+No Surgery group (Fig. 11;  $p = 0.188$  and  $p = 0.104$ , respectively). However, the LP+CCI and NH+CCI groups had significantly higher numbers of footslips compared to the SP+No Surgery group (Fig. 11;  $p = 0.019$  and  $p = 0.048$ , respectively).

An overall effect of CCI on performance across days was found for total average number of footslips. All groups except the SP+No Surgery group exhibited increases on post-operative day 3 that gradually decreased to the equivalent of baseline measures by post-operative day 15 (Fig. 11; Wilk's  $\lambda = F(3.046) = 47.463$ ,  $p = 0.000$ ). A similar pattern was found for Bin 1 average contralateral footslips (Fig. 12;  $F(1.509) = 15.906$ ,  $p = 0.000$ ), Bin 2 average contralateral footslips (Fig. 13;  $F(2.093) = 15.723$ ,  $p = 0.000$ ), and Bin 3 average contralateral footslips (Fig. 14; Wilk's  $\lambda = 0.246$ ,  $F(4,40) = 30.599$ ,  $p = 0.000$ ).

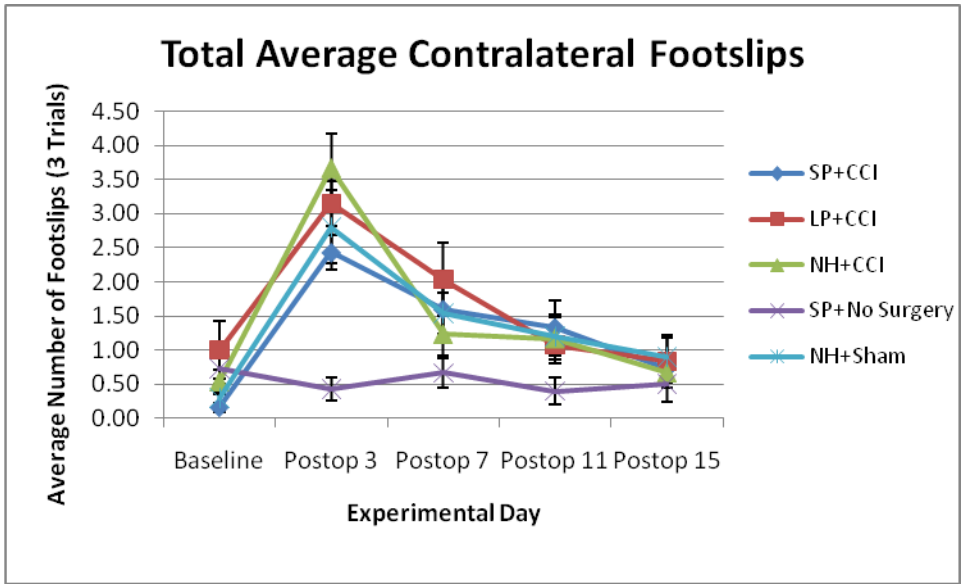


Figure 11. The average number of contralateral footslips across the entire beam during the LTB test. All groups except the SP+No Surgery group had increased footslips on post-operative day 3 that gradually decreased by post-operative day 15. On post-operative day 3, the LP+CCI and NH+CCI groups have slightly higher numbers of footslips than the SP+CCI and NH+Sham groups. Data are expressed as mean  $\pm$  S.E.M.

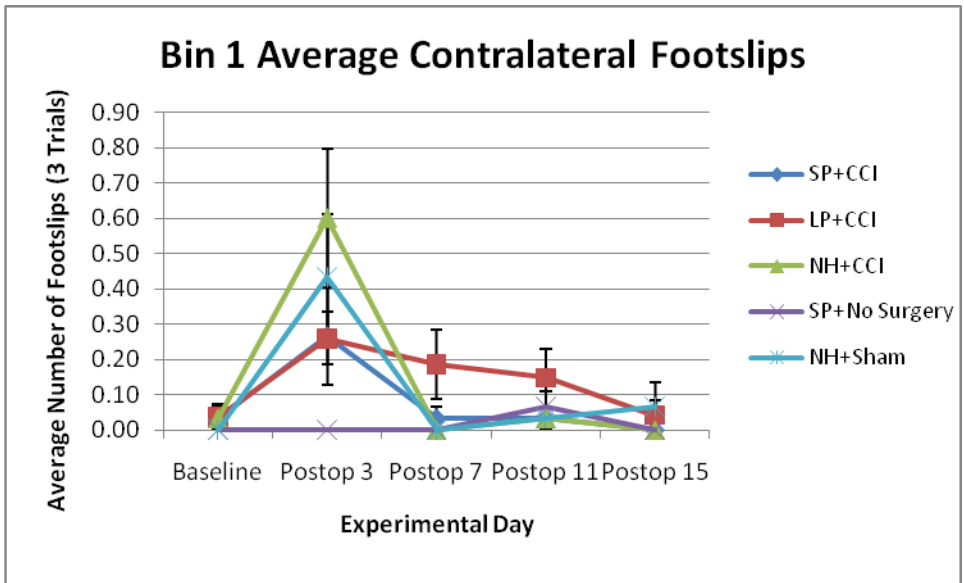


Figure 12. The average number of contralateral footslips across bin 1 during the LTB test. On post-operative day 3, there was a large increase in the NH+CCI group, followed by the NH+Sham, LP+CCI, and SP+CCI groups. Bin 1 footslips diminished by post-operative day 15, but slower for the LP+CCI group. Data are expressed as mean  $\pm$  S.E.M.

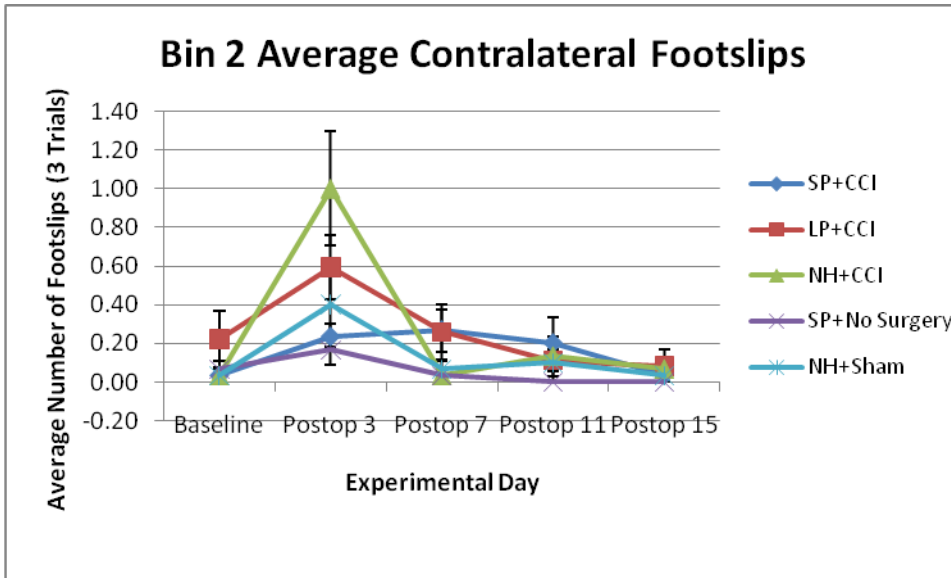


Figure 13. The average number of contralateral footslips across Bin 2 during the LTB test. On post-operative day 3, the NH+CCI and LP+CCI had the largest increases followed by the NH+Sham, SP+CCI, and SP+No Surgery groups. Data are expressed as mean  $\pm$  S.E.M.

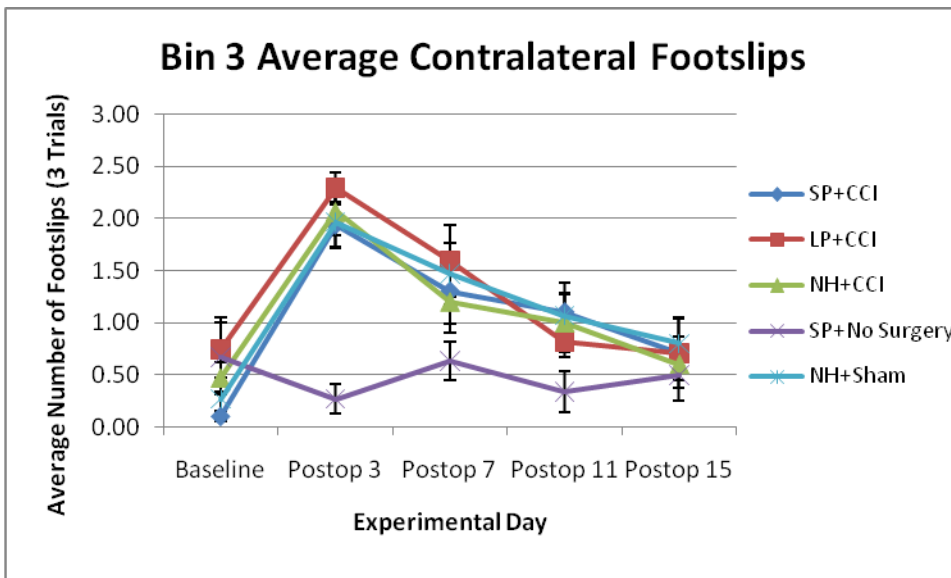


Figure 14. The average number of contralateral footslips across Bin 3 during the LTB test. All groups except for the SP+No Surgery group had large increases on post-operative day 3 that gradually decreased by post-operative day 15. Data are expressed as mean  $\pm$  S.E.M.

### Limb-use Asymmetry Cylinder Test

There were no reliable group differences found during the Limb-use Asymmetry Cylinder Test. Mauchley's Test of Sphericity was significant, so the Greenhouse-Geisser adjustment was utilized for this data. There was no interaction effect found between days and groups, and tests of between-subjects effects also indicated that there were no differences between groups,  $F(4) = 1.170$ ,  $p = 0.338$ . However, the within-subjects effects of the repeated measures ANOVA revealed that there were significant changes over days,  $F(2.393) = 3.774$ ,  $p = 0.020$ . The results of the LAC are plotted on Fig. 15.

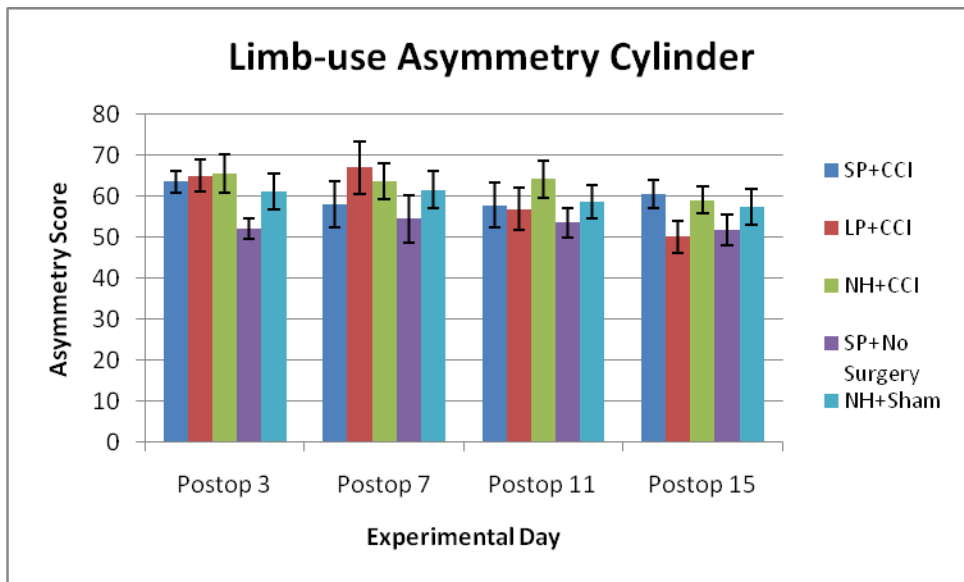


Figure 15. The asymmetry scores calculated from the LAC test data. There were no reliable group differences detected. Data are expressed as mean  $\pm$  S.E.M.

### Morris Water Maze

The LP+CCI group performed significantly worse than the SP+No Surgery group (Fig. 16;  $p = 0.001$ ), while the other groups were not statistically significant from the SP+No Surgery group (Fig. 16). There was also an effect over days found, in which all

the groups' performances improved across days (Fig. 16;  $F(2.872) = 206.342$ ,  $p = 0.000$ ). All groups showed improvement in time latencies for all the groups between days 1 and 2. However, The LP+CCI group did not show improvements between days 2 and 3 like the other control groups did. The SP+No Surgery group exhibited no improvement as this group was already performing so well that a floor effect was found where performance could not improve further. All the groups performed equally well by days 4 and 5. To analyze the times spent in target quadrant and number of crossings for the probe trials and the time latencies for the cued trials, one-way ANOVAs with post-hoc Tukey tests were utilized. No differences were found between groups during the probe and cued trials, which indicates that all rats learned the task and vision was not impaired

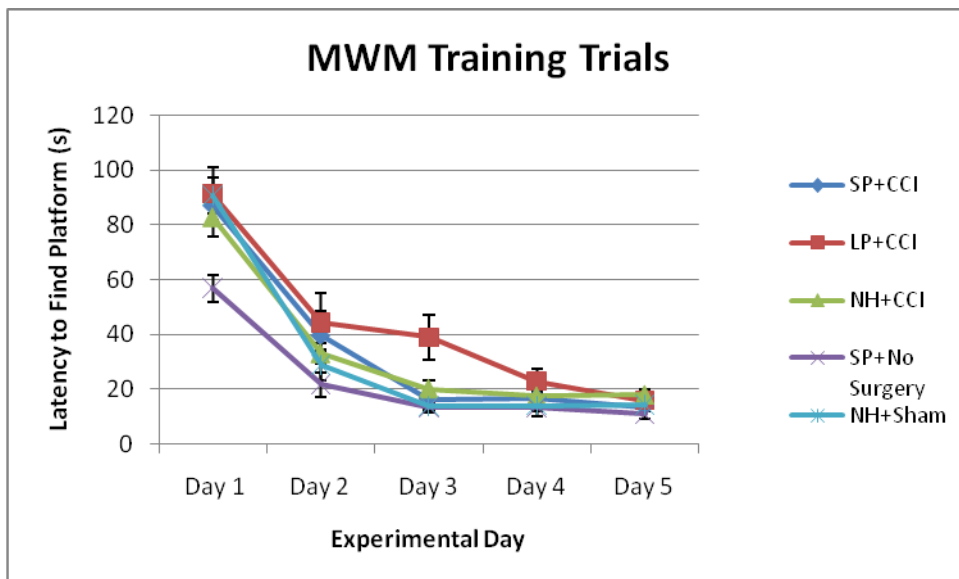


Figure 16. Time latencies during the five days of training trials of the MWM test. All the groups had improvement from day 1 to day 2 of testing. However, the LP+CCI group did not show improvement from day 2 to day 3 like the other groups. All the groups had similar performances by days 4 and 5. Data are expressed as mean  $\pm$  S.E.M.

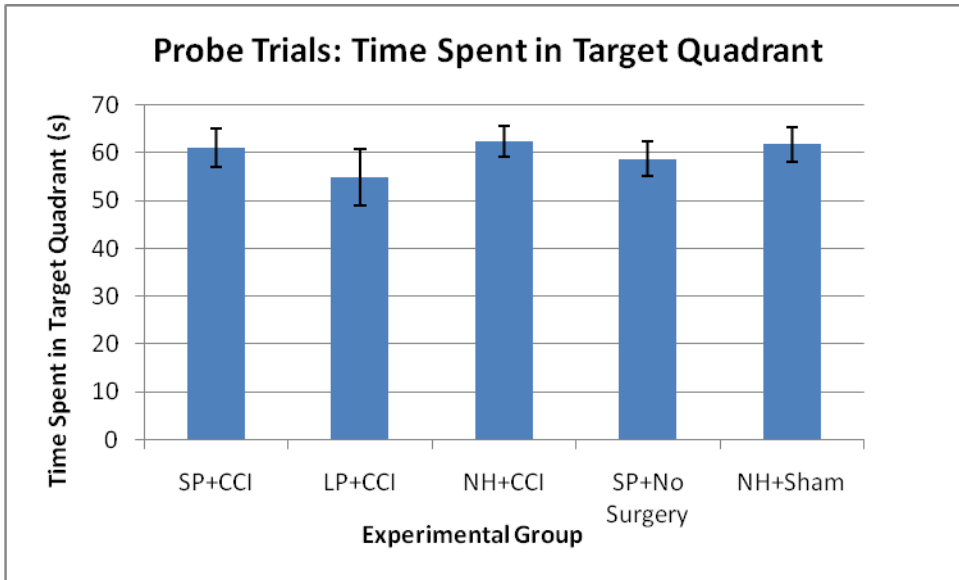


Figure 17. Time spent in the target quadrant during the probe trials of the MWM test. There were no detectable differences between groups for this measure. Data are expressed as mean  $\pm$  S.E.M.

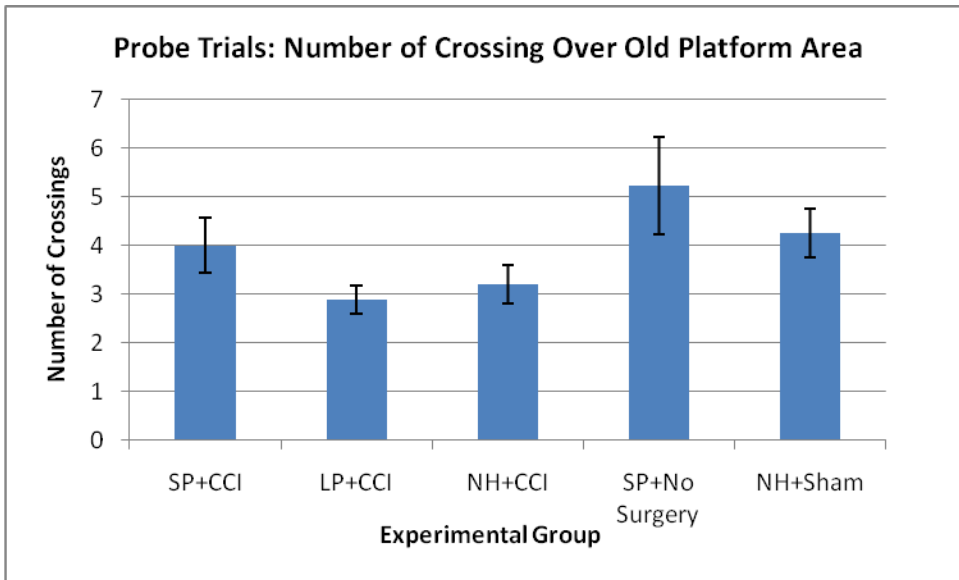


Figure 18. The number of crossings over the old platform area during the probe trials of the MWM test. There were no detectable differences between groups for this measure. Data are expressed as mean  $\pm$  S.E.M.

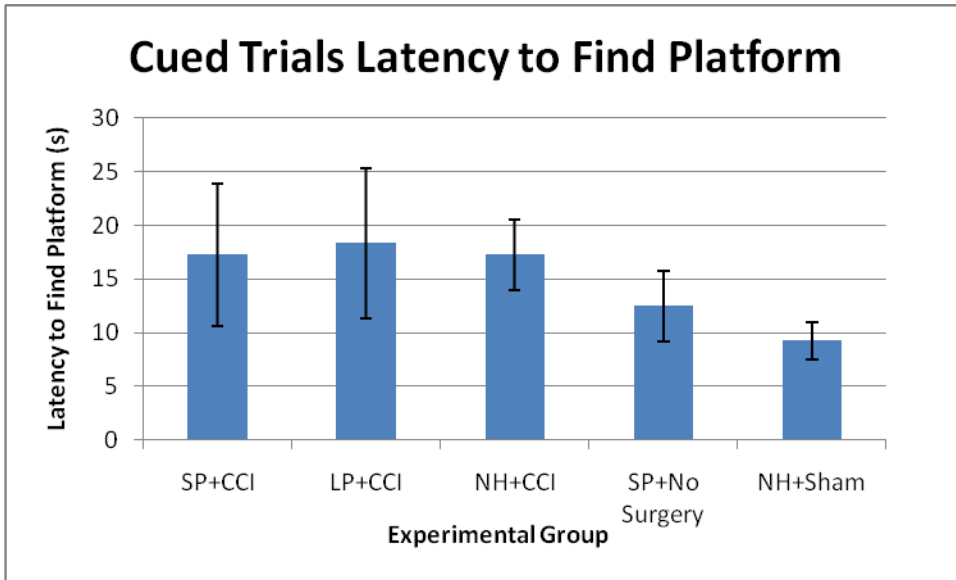


Figure 19. Time latencies during the cued trials of the MWM test. There were no detectable differences between groups for this measure. Data are expressed as mean  $\pm$  S.E.M.



## CHAPTER IV

### DISCUSSION

#### Results

The original hypothesis that RSD prior to TBI would negatively impact recovery was not supported by the results of this study. Across all four behavioral tests, the SP+CCI rats either performed just as well as or better post injury than the control non-RSD animals that received a CCI. In fact, in many of the results, the LP+CCI animals performed worse than all the other groups. We drew two main speculations from these results. First, we speculated that the stress of being in the novel environment and social isolation conditions associated with the LP could have exacerbated the injuries. The NH+CCI group was not subjected to the stressful housing conditions that the LP+CCI group was, so this could account for why the NH+CCI group did not exhibit as many deficits. Second, we theorized that RSD had a neuroprotective effect that reduced the severity of TBI because the SP+CCI group exhibited fewer deficits than the LP+CCI and NH+CCI groups. Our results were generally consistent with previous literature, as rats

that exhibited sensorimotor deficits shortly after surgery had a tendency to recover by post-operative day 15, and we also found long-term differences in cognitive deficits (Abdel-Baki, 2009; Hoskison, 2009; Kline, 2002; Shanina, 2006).

Differences in the latency to remove both tabs in the BTS test were more likely to be significant due to the unilateral nature of the injury. Rats tended to remove sticky tabs from the ipsilateral paw (unaffected) first and contralateral (affected) limb last. This led to the data for the latency to remove both tabs to be more representative of the contralateral limb, which was the limb most affected by the unilateral injury. Interestingly, we found a trend for the LP+CCI and NH+CCI groups to exhibit greater deficits than the SP+CCI group in the data for the post-trial asymmetries, which was similar to the results of the latency to remove the tabs from both paws. These results led us to suspect that RSD protected rats from CCI-related forelimb sensorimotor deficits compared to animals that underwent CCI alone.

This trend persisted in the LTB data representing hindlimb motor deficits. Rats in the LP+CCI group performed comparable to the NH+CCI group, and the SP+CCI group performed comparable to the NH+Sham group, while the SP+No Surgery group performed the best. While there was no apparent effect of RSD on CCI recovery in the data for bin 3 and total footslips, RSD appeared to alleviate hindlimb motor deficits for the SP+CCI group in the Bin 2 data. The LP+CCI group's poor performance implies that the stress of the LP+CCI housing condition may have worsened impediments of spatial learning in the MWM task. We also speculated that RSD was neuroprotective for the SP+CCI group's memory function.

## Supporting Evidence

Neuroprotective properties of SD have been found in other studies. In rodent models, ischemia, a secondary effects of TBI, Kunz (2010); Park (1999); Udomphorn (2008)) was induced immediately after SD. The sensorimotor and cognitive deficits of the SD animals were attenuated compared to injured controls without SD (Moldovan, 2010). Additionally, ischemic outcome was improved by SD as indicated by the attenuation of glial cell reaction in the rat hippocampus, and SD also attenuated hippocampal neurodegeneration (Hsu, 2003; Weil, 2009).

There are numerous neuroprotective effects that SD has that may have an impact on TBI recovery. As mentioned previously, the inflammatory response to TBI can have a neurotoxic effect on the brain. However, studies have shown that SD attenuated the expression of the pro-inflammatory cytokines IL-1 $\beta$ , and TNF- $\alpha$ , while elevating the neuroprotective cytokines IL-6 and IL-10 (Liesz, 2009; Montes-Rodríguez, 2004; Weil, 2009). SD also elevates levels of adenosine and A<sub>1</sub> adenosine receptor activation (Basheer, 2004; Basheer, 2007; Elmenhorst, 2009; Huston, 1996). Elevated adenosine levels have been known to inhibit cellular activity and consequently reduce cellular damage in cases of hypoxia and ischemia (Porkka-Heiskanen, 2002).

Elevated adenosine is known to reduce glutamate levels by activating A<sub>1</sub> receptors, which limit the influx of cellular Ca<sup>2+</sup>. This leads to a decrease in glutamate release, which is neuroprotective because increased glutamate has been indicative of worsened TBI outcome (Lau & Tymianski, 2010; Yi & Hazell, 2006). This has been verified in

other studies where glutamate levels tend to decrease after extended SD (Dash, 2009; Lopez-Rodriguez, 2009).

The worsened outcomes of the LP+CCI group can be explained by stress mechanisms that exacerbate TBI outcome, possibly by elevating cortisol which worsens the immune system (Bryan & Hernandez, 2011; Stulemeijer, 2006; Zuiden, 2009). Research has shown that the single platform RSD model is stressful for rats compared to other SD models such as the multiple platform technique (Machado, 2004). Additionally, this technique results in elevated levels of corticosterone (Coenen & van Luitelaar, 1985; Perry, 2008), which worsens the long-term outcome of TBI (Kwon, 2011). Evidence has also shown that these elevations in corticosterone are caused by social isolation (Suchecki & Tufik, 2000).

#### Limitations/Future Considerations

Conditions of the single-platform RSD procedure could induce stress effects instead of just RSD alone (Machado, 2004; McDermott, 2001; Suchecki & Tufik, 2000), so another SD technique is needed to alleviate stress's extraneous effects while investigating the effects of RSD on TBI recovery. One common alternative to the single platform technique is the multiple platform technique, which deprives rats of sleep in a similar manner to the single platform technique. However, the multiple platform technique places multiple elevated platforms in a tank and exposes multiple rats to RSD together without having to undergo the stress of social isolation (Coenen & van Luitelaar, 1985; Weil, 2009). The findings by Suchecki & Tufik (2000) indicating elevated corticosterone levels during disruption of rats' sociability further supports the use of the multiple

platform technique. Also, deficits were found in the NH+Sham group for the BTS, LTB, and MWM tasks and histological preparation of the brains revealed lesions. Thus, modifications to the current sham surgery method are needed to minimize damage to the NH+Sham group.

### Conclusions

We thought that RSD prior to TBI would worsen recovery of sensorimotor and cognitive deficits. However, RSD attenuated deficits compared to the groups that underwent CCI without RSD, which is indicative of the neuroprotective effects of RSD. Furthermore, the stress of the LP+CCI condition worsened TBI outcome, and the stress effects of the single-platform condition were alleviated by RSD. These results suggest that further understanding of the neural mechanisms of RSD and stress may lead to considerations for the treatment of TBI.

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