2009

Combined Pharmacotherapy for the Treatment of Traumatic Brain Injury Rehabilitation and Recovery of Function Following Prefrontal Cortex Controlled Cortical Impact in Rats

Abby Nicole Kyser
Cleveland State University

COMBINED PHARMACOTHERAPY FOR THE TREATMENT OF TRAUMATIC BRAIN INJURY REHABILITATION AND RECOVERY OF FUNCTION FOLLOWING PREFRONTAL CORTEX CONTROLLED CORTICAL IMPACT IN RATS

ABBY N. KYSER

Bachelor of Arts in Psychology

Mount Union College

May, 2006

submitted in partial fulfillment of requirements for the degree

MASTER OF ARTS IN PSYCHOLOGY

at the

CLEVELAND STATE UNIVERSITY

May, 2009

This thesis has been approved

for the department of PSYCHOLOGY

and the College of Graduate Studies by:

_____

Thesis Chairperson, Stephen Slane, Ph.D.

_____

Psychology Department, April 29, 2009

_____

Sherry L. Ball, Ph.D.

_____

Psychology Department, April 29, 2009

_____

Michael D. Hammonds, Ph.D.

_____

Health Sciences Department, April 29, 2009

DEDICATION

This project is dedicated to my mom and dad, who believed in me even when I was not sure I even did. To my grandmother, who is so proud of me academically and personally. This is also dedicated to Scott, who stood beside me throughout this project and brought some much needed sanity to my few hours spent at home each week, especially when I started working full-time while trying to still finish my thesis.

ACKNOWLEDGEMENT

The author would like to thank the persons who assisted in and supported the onset and completion of this project. In particular:

Psi Chi for selecting my project for partial funding in the amount of $1348.00; Dr. Sherry Ball for all of her guidance and assistance, without which this project would not have been possible; Lisa A. Franklin for her assistance with many important aspects of this project; Brett Hanzlicek for his countless hours of help in the laboratory; Jaime Schmidt for many months of help that kept us both sane. Also, a special thank you to Dr. Stephen Slane and Dr. Michael D. Hammonds for their help and assistance as members of my thesis committee.

COMBINED PHARMACOTHERAPY FOR THE TREATMENT OF TRAUMATIC BRAIN INJURY REHABILITATION AND RECOVERY OF FUNCTION FOLLOWING PREFRONTAL CORTEX CONTROLLED CORTICAL IMPACT IN RATS

ABBY N. KYSER

ABSTRACT

Traumatic brain injury (TBI) is common among military personnel, resulting from bomb blasts and explosions. The secretion of pro-inflammatory cytokines following TBI has been linked to cerebral edema and neuronal loss. The use of lovastatin for TBI has been suggested to be neuroprotective by combating cytokines and inflammation. Fluoxetine has been suggested to aid in the prevention of edema during secondary injury processes, as well as having a relationship to neural plasticity. Seventy-six Long-Evans rats were randomly assigned to CCI (controlled cortical impact) or sham-operated as well as one of the following drug conditions: no treatment, vehicle, Fluoxetine only, Lovastatin only, and combined Fluoxetine-Lovastatin. Two behavioral tasks testing motor control and somatosensory input were used to investigate recovery of function. Brain tissue was analyzed using cresyl violet stain and GFAP label. Behavioral and cell count data did not reveal significant improvements between combined Fluoxetine-Lovastatin pharmacotherapy groups and all other groups. Several methodological limitations that may account for these negative findings are discussed. Although the combination of Lovastatin and Fluoxetine did not significantly improve behavioral scores or cell counts

in the patterns expected, the possibility of utilizing a combined pharmacotherapy to treat TBI or TBI comorbid with other conditions should be investigated further.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Traumatic brain injuries (TBI) happen to approximately 1.5 million Americans a year (Rutland-Brown, Langlois, & Thomas, 2006), with the National Center for Injury Prevention and Control estimating the incidence of TBI to be 2% of the population (1999). Approximately 150,000 to 300,000 returning US soldiers have experienced some level of TBI during their tour of duty (Emery, 2007). Among Operation Iraqi Freedom and Operation Enduring Freedom veterans, 30% have experienced a TBI. Of those TBI patients, 56% have moderate to severe TBI and 44% have mild TBI (Warden, 2006). A TBI is described as an impact of the brain against the interior of the skull from accelerating-decelerating forces (Thurman, Alverson, Dunn, et al., 1999). TBI commonly occurs in motor vehicle and motorcycle accidents, accidental falls, and being struck by an object. TBI-associated costs are estimated to be over $10 billion a year in the United States alone, with the average lifetime cost per individual of nearly $2 million per person (Boswell, McErlean, & Verdile, 2002). Because of the great number of costs associated with TBI, such an injury is seriously addressed in the research and medical communities.

TBI patients have difficulty making choices, adapting to specific situations, and maintaining motivation (Powell, Al-Adawi, Morgan, & Greenwood, 1996; Schlund, 2002). Such difficulties can make life particularly difficult, especially when it comes to readjusting and trying to live the same way one did pre-injury. Many TBI sufferers have difficulty securing and holding onto jobs (Powell et al., 1996). One issue that makes readjustment particularly difficult is the appearance of sleep disturbances and insomnia post-injury (Williams, Lazic, & Ogilvie, 2008). Over time, many of the sleep disturbances will disappear, but some patients will experience persistent sleep disturbances and insomnia long after the brain injury has occurred (Williams et al., 2008). Another common issue after brain injury is memory impairment. Although memory impairment occurs with mild TBI as well, the impairments are more frequent and more pronounced in moderate to severe TBI. Memory impairments tend to show improvement within 6 months to 2 years of brain injury (Lannoo, Colardyns, Jannes, et al., 2001), but deficient learning and memory may be detected in some patients with severe TBI at 10 years post-injury (Zec, Zellers, Belman, et al., 2001). The memory impairments most likely occur because TBI leads to widespread axonal injury (Ommaya & Gennarelli, 1974) in which the frontal and temporal lobes were found to be the most vulnerable cortical areas (Adams, 1975). MRI abnormalities have been found in the medial temporal and lateral frontal lobes in addition to ventricular enlargement (Crosson, Sartor, Jenny, et al., 1993). Hippocampal atrophy has also been observed following severe TBI (Bigler, Johnson, Anderson, et al., 1996). Brain activity measured by PET scan during memory and executive function tasks showed decreased cortical metabolism in the prefrontal cortex and cingulate gyrus in patients with TBI (Fontaine, Azouvi, Remy, et

al., 1999). Patients with TBI also showed reduced activation in the frontal lobe and increased activation in the posterior brain regions during free recall (Fontaine et al., 1999). The investigators also observed additional activated areas in TBI patients as opposed to controls when engaging in a cued recall task. It is important to replicate as much as possible the spectrum of complexities associated with TBI when working with animal injury models.

Since the brain structure of a rodent is very similar to that of the human brain (Gennarelli, 1994), animal models of TBI can be used to observe the effects and test therapies. The most common area affected by TBI is the frontal cortex, especially if the injury is of an accelerating/decelerating nature. This type of injury can also result in damage to the lower portions of the brainstem (Van Reekum, Cohen, & Wong, 2000). A surgical model of frontal medial cortex (MFC) contusion has been used (Hoffman, Fulop, & Stein, 1994; Smith, Fulop, Levinsohn, et al., 2000) to simulate injuries sustained in a motor vehicle accident. The use of rodent models for the modeling of human TBI is widely accepted as a suitable choice for neurotrauma research (Cernak, 2005). One of the most frequently used models of TBI is fluid percussion injury (FPI). This model consists of a fluid pressure pulse applied to the intact dura through a craniotomy by means of a pendulum hitting a saline-filled Plexiglas cylinder cemented onto the skull over a craniotomy. Unfortunately, there are issues in reproducibility with the FPI model and it does not reflect the entire complexity of human TBI. There has also been evidence of some ipsilateral and contralateral damage in addition to the originally intended brain area (Cernak, 2005). Controlled cortical impact (CCI) is a rodent model of TBI that allows for better control over mechanical factors such as velocity of impact and depth of

the resulting deformation. The injury is delivered to the intact dura by an air-driven piston causing deformation to the underlying cortex through a craniotomy. The CCI model produces a more focused injury compared to the FPI model, as well as a replication of clinical brain injury with cortical compression (Cernak, 2005). CCI is more appropriate when used to analyze mechanisms underlying neuronal cell death and resulting neurological deficits (Cernak, 2005). There exist other direct brain deformation models of TBI, but these do not cause significant long-term deficits that would be of importance in the research of localized contusions. Marmarou's weight drop model, a closed head impact acceleration injury, causes trauma by a column of brass weights falling from a designated height through a Plexiglas tube onto a steel disc that was cemented centrally onto the skull (Foda & Marmarou, 1994; Marmarou, Foda, van den Brink, et al., 1994). The rat is placed on a deep foam bed and the impact is generated by dropping the brass weights onto the stainless steel disc. This model is popular because of cost and the ease with which the injury is inflicted (Cernak, 2005). However, the biomechanics of the impact, including velocity, are not completely controlled. This model has the possibility of a secondary drop, in which the weight rebounds from the skull of the animal resting on the foam bed (Cernak, 2005). For the purposes of this study, CCI is a good model for combining the brain injury and secondary insults seen in human TBI.

In CCI, lesions are generated using a pneumatic piston device to create the physiological and neurological changes that occur following TBI in humans (Hoffman, Stein, & Fulop, 1994; Smith et al., 2000). Using a pneumatic piston is more accurate and consistent than the weight drop procedure (Marmarou's impact acceleration model) or the

FPI model, a must for a study that requires using many animals to replicate the same brain injury model (Gennarelli, 1994).

Both primary and secondary injury processes occur in TBI. The primary injury process involves the mechanical aspects of TBI that occur at the time of injury resulting in massive initial cell death. Secretion of pro-inflammatory cytokines are involved in the development of cerebral edema and secondary neuronal loss following TBI (Chen, Hung, Chen et al., 2007). Cytokines are proteins used in cellular communication that are secreted by microglial cells, astrocytes, and leukocytes. Pro-inflammatory cytokines would be released following a brain injury and are involved in the increase in CNS injury (Wayne, 2007). The primary injury process initiates the neurological and biochemical changes of the secondary injury process (Heath & Vink, 1999). The secondary injury process includes free radical production, edema formation, and altered activity of N-methyl-D-aspartate (NMDA) receptors (Heath & Vink, 1999). In addition, a process of gliosis occurs in which astrocytes accumulate in damaged areas of the central nervous system (Pekny & Nilsson, 2005).

One proposed pharmacological intervention for a TBI is fluoxetine, a selective serotonin reuptake inhibitor (SSRI) typically prescribed for depression. Following TBI, depression is one of the most common symptoms (Rosenthal, Christense, & Ross, 1998), with manifestations of depression including fatigue, frustration, and poor concentration (Kreutzer, Seel, & Gourley, 2001). Loubinoux and colleagues have raised the possibility that serotonin agonists such as fluoxetine may enhance reorganization of motor output (Loubinoux, Pariente, Boulanour, et al., 2002). The areas of the brain where the neurotransmitter serotonin is located include the caudal raphe nucleus in the medulla near

the midline with projections to the cerebellum and spinal cord, as well as the rostral raphe nuclei in the pons that supplies the forebrain, the limbic-striatal system, hippocampus and cerebellum, and the prefrontal cortex (Kandel, 1991). In TBI, there is a shearing or tearing of the axons from the lower brainstem during acceleration/deceleration injuries, which typically includes the pons and medulla (Van Reekum et al., 2000). Past research has suggested that serotonin may enhance the production of new neurons by the activation of serotonin receptors in the brain (Breuzn & Daszuta, 1999). This seems to be of particular significance in patients with acquired brain injury because the damaged neurons are theoretically capable of regenerating new tissue in the hippocampus (Gould, 1999). Tsuiki and colleagues (1995) found deficiencies of serotonergic metabolites in the cerebral spinal fluid of patients with TBI, suggesting that an increase of serotonin in the brain and cerebral spinal fluid may be beneficial to the clinical outcome of the patients. SSRIs seem to be successful in reducing and alleviating depression, depression-related symptoms, and cognitive impairments significantly more than would be accounted for by natural recovery alone (Perino, Rago, Cicolini, et al., 2001). SSRIs have also been shown to be successful in treating other symptoms, such as emotional incontinence or pathological crying following brain injuries (Sloan, Brown, & Pentland, 1992; Seliger, Hornstein, Flax, et al., 1992). Sloan and colleagues demonstrated a positive effect of fluoxetine in the treatment of emotional incontinence, in which cases showed marked improvement within one week (Sloan et al., 1992). Fluoxetine is suggested to improve selected aspects of cognition, independent of its mood-elevating properties (Carboni, Vighini, Piubelli, et al., 2006).

Another proposed pharmacological intervention for TBI is lovastatin. Lovastatin belongs to a class of drugs referred to as statins, potent inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Endo, 2004). HMG-CoA is a key enzyme in cholesterol biosynthesis, leading to the prescribing of statins for the reduction of serum cholesterol levels. Statins have been shown to have neuroprotective effects against a variety of CNS diseases, independent of their ability to lower cholesterol (Asahi, Huang, Thomas, et al., 2005). The neuroprotective effects of statins are due to an upregulation of endothelial NO synthase (eNOS), resulting in decreased contusion volume and neurological deficits caused by ischemia and TBI (Endres et al., 1998).

There have been studies conducted on the individual pharmacotherapies of lovastatin (Asahi et al., 2005; Chen et al., 2007) and fluoxetine (Carboni et al., 2006; Singh et al., 2000) for stroke and brain injury, however there have not been any studies investigating the combination of these two existing pharmacological interventions in an attempt to treat an injury from multiple dimensions. For the treatment of soldiers who may experience a TBI, it is important to utilize a preventative treatment that will minimize secondary injury processes to the brain in combination with a pharmacological agent known for neural plasticity to aid in the repair of resulting damage. Previous research on the use of lovastatin administered prior to TBI showed the development of neuroprotective properties that decreased contusion volume in lovastatin-treated animals as compared to controls, as well as decreased cognitive deficits (Chen et al., 2007). Studies investigating fluoxetine administration after a brain injury have indicated improved cognitive abilities, including working memory, relative to increased neural plasticity (Carboni et al., 2006; Singh et al., 2000). By utilizing lovastatin as a

preventative measure prior to TBI, the goal is to combat cytokine formation and inflammation through neuroprotection. Post-TBI administration of fluoxetine as a treatment is intended to increase neural plasticity and decrease neural deficits.

The purpose of this study is to investigate the effectiveness of using a combination of existing pharmacological therapies to treat the injuries resulting from TBI. The hypothesis is that pre-injury lovastatin administration combined with post-injury fluoxetine treatment is effective as a means of combining preventative measures with post-injury treatment for TBI in personnel within a military combat situation due to the neuroprotective effects of lovastatin and the neural plasticity properties of fluoxetine.

CHAPTER II

METHODS

2.1 *Subjects*

Seventy-six male Long-Evans rats 42 to 45 days of age, weighing 150-174 grams were purchased from Harlan (Indianapolis) and housed at Louis Stokes Veterans Affairs Animal Resource Facilities (ARF). Rats were weighed and handled for 10 minutes for 5 consecutive days one day after shipment. All animals had access to food and water *ad libitum*. Animals were randomly assigned to one of ten groups: Sham Only (n=8), Sham Vehicle (n=8), Sham Fluoxetine (n=8), Sham Lovastatin (n=7), Sham Fluoxetine-Lovastatin Combination (n=7), CCI Only (n=7), CCI Vehicle (n=8), CCI Fluoxetine (n=7), CCI Lovastatin (n=8), and CCI Fluoxetine-Lovastatin Combination (n=8). A breakdown of the groups can be seen in Table I. Following CCI, surgery, animals were weighed and handled for 10 minutes daily for 7 consecutive days.

Table I. Distribution of Animals into Groups

Injury	*Treatment*	*n=*	*N=*
Sham	None	8	
Sham	Vehicle	8	
Sham	Fluoxetine	8	
Sham	Lovastatin	7	
Sham	Fluoxetine-Lovastatin Combination	7	
CCI	None	7	

CCI	Vehicle	8	
CCI	Fluoxetine	7	
CCI	Lovastatin	8	
CCI	Fluoxetine-Lovastatin Combination	8	76

Post-injury behavioral testing began on day 7 following injury (77-80 days of age) and continued until day 23 (100-103 days of age). On day 28 post-injury (approximately 105-108 days of age), animals were perfused and brains were extracted for later histological and immunohistochemical analysis.

2.2 *Controlled Cortical Impact (CCI) Surgery*

Surgery was performed at 70-73 days of age, with animals in the weight range of 250 to 350 grams. Animals were anesthetized with a mixture of ketamine and xylazine (75 mg/kg and 5 mg/kg, respectively) delivered intraperitoneally (i.p.). Once surgical plane was achieved, the incision site was prepared by shaving the animals on the dorsal side of the head using an electric trimmer followed by betadine scrub using a sterile cotton pad followed by 70% EtOH on a second sterile cotton pad. The disinfection of the scalp using betadine and 70% EtOH was alternated a total of three times. Aseptic procedures were maintained throughout surgery. Rats were placed into the stereotaxic apparatus (Stoelting, Wood Dale, IL) equipped with a heating pad and the head was held with ear bars and an incisor bar, in order to maintain the head at a consistent orientation. Animals were covered in surgical drapes before any surgical procedures were started. A midline incision was made along the dorsal side of the head to expose the cranium. The skin covering the scalp was held to the side with hemostats. The surface of the skull was wiped down using cotton-tipped applicators and sterile saline. Animals received a 5.0mm craniotomy 2.5mm lateral from bregma using a hand held drill (Ram

Micromotors, East Brunswick, NJ). The craniotomy was performed over the prefrontal cortex. The controlled cortical impact (CCI) device (myNeuroLab, St. Louis) was attached to the stereotaxic apparatus. The sterile tip of the CCI device was adjusted to A/P 3.0mm, D/V -2.2mm, and M/L +/- 2.5mm (Paxinos & Watson, 2007). The tip of the contusion device compresses the surface of the dura 2.0mm for 2.0 seconds. The impact velocity of the contusion device was adjusted to 4.0m/second. The area of the contusion will create an indentation and discoloration in the dura of the medial frontal cortex that will be evident following removal of the brain via perfusion. Bleeding was controlled using cotton wipes or surgical gauze. The incision was sutured using stainless steel wound clips and triple antibiotic ointment (E. Fougera, Melville, NY) was applied to prevent infection. Sham animals underwent all of the elements of surgical procedure up to the cortical impact. Animals were transferred to a heated post-surgical cage and monitored continuously until mobile and monitored daily for two weeks following surgery. Animals received injections of buprenorphine (0.05 mg/kg) at 12 and 24 hours post-injury as an analgesic.

Animals were given one week following injury to recover from surgery before beginning any behavioral testing (Smith et al., 2000). Beginning one day after surgery, animals were weighed and handled for 10 minutes each.

2.3 *Drug Treatment*

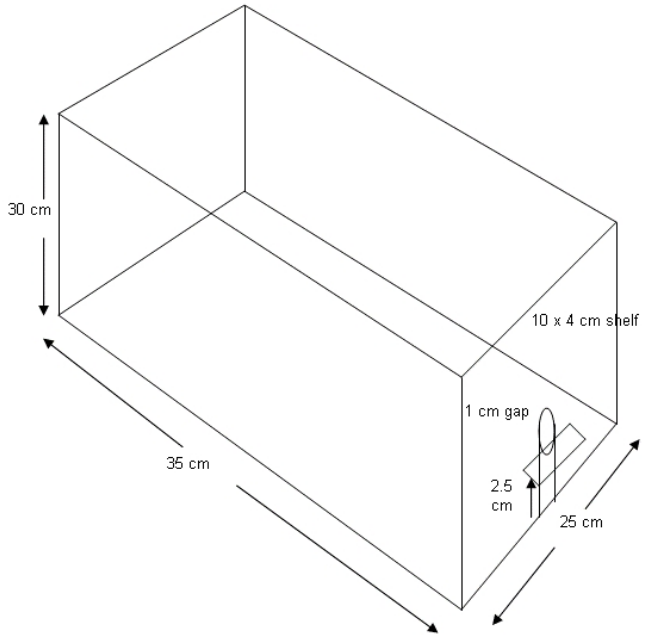
Lovastatin (Sigma Chemical Co., St. Louis, MO) diluted in sterile saline (0.9%) was administered at 4 mg/kg/day, i.p., to rodents 63-33 days of age for five days before injury. This dosage is based on previous literature by Chen and colleagues (2007).

Fluoxetine (Sigma Chemical Co., St. Louis, MO) diluted in sterile saline (0.9%) was administered at 10 mg/kg/day, i.p., to rodents 77-80 days of age for one week following surgery (Windle & Corbett, 2005; Rapp, Baader, Hu, et al., 2004). This dosage is based on previous literature by Rapp and Colleagues (2004). Fluoxetine administration at 10 mg/kg/day for 14 consecutive days is considered chronic drug exposure (Rapp et al., 2004).

2.4 *Shaping & Training: Reaching Box Task*

A reaching box task was used to test forelimb motor control. All rats were shaped prior to training for the forelimb reaching task. On the first day of shaping, animals (49 to 52 days of age) were placed in the reaching box (25 x 30 x 35 cm, with a 10 x 4 cm shelf 2.5 cm from bottom centered over a 1 cm gap for reaching) for 20 minutes along with their cage mate and quartered yogurt treat rewards (8-in-1 Pet Products, Hauppauge, NY) were placed on the floor. A diagram of the reaching box is shown in Figure 1.

Figure 1. Diagram of reaching box



Note: Diagram is not drawn to exact scale.

Day 2 was the same except that each animal was placed into the box alone. On the third day, the rewards were placed on the shelf of the box for forelimb reaching. Once animals had developed a preference for reaching (greater than 70%) with a particular forelimb and could complete at least 10 reaches in a 10 min span of time, animals began training (Hsu & Jones, 2006). All animals used in this study developed a preference for one forelimb over the other.

For training animals were placed individually inside the reaching box and trained to use the preferred limb exclusively for reaching by presentation of a yogurt treat reward to the side of the gap opposite to the preferred limb. By utilizing a single forelimb reach that was used pre-and post-surgery, investigators were looking for small differences in motor movements. Once the animal had reached a success rate of at least 40% (i.e. successful retrieval of 4 or more treats from shelf to mouth using the preferred forelimb over 10 minutes), that day was considered to be the last day of training pre-surgery (Hsu & Jones, 2006).

Following injury, animals were tested in the reaching box task for assessment of forelimb dysfunction resulting from TBI. This assessment was done for two days on days 7 and 21 post-injury (between 77-80 and 98-101 days of age, respectively). Depending on individual animals, the number of reaches ranged from 0-74 reaches.

2.5 *Bilateral Tactile Adhesive Removal Somatosensory (BTARS) Task*

At one week post-injury (approximately 79-82 days of age), animals underwent bilateral tactile adhesive removal testing. Bilaterial tactile adhesive removal somatosensory (BTARS) test assesses somatosensory dysfunction following injury. BTARS was performed for a total of 6 days on days 9, 11, 14, 16, 18, and 21 post-injury.

Each day consisted of two trials, resulting in a total of 12 trials. Stickers (Staples, Product # 297341) were placed on the top of the animal's left and right radial forelimb and the animal was returned to a clear shoebox cage with a top. The animal was timed using a stopwatch and watched carefully by the investigator. Latency of removal and the order of removal were recorded. A trial was considered over when the animal removed both stickers or when two minutes had elapsed. Whether or not the animals were able to remove the stickers, the stickers were removed from the cage to prevent confusion on the second trial. There was an intertrial interval of 5 minutes per animal.

2.6 *Histology*

At 28 days post-injury (approximately 105-108 days of age), animals were euthanized using a lethal dose of sodium pentobarbitol (75 mg/kg, i.p.) and perfused in order to obtain the tissue required for later histological analysis (Smith et al., 2000). Once animals were deeply anesthetized, they were intracardially perfused with 250 ml of 1X phosphate buffered saline (PBS, pH 7.4) followed by 500 ml of paraformaldehyde solution (PAF, pH 7.4). Tissue was extracted and postfixed in 4% PAF for storage for up to 6 months. Tissue was then transferred to 30% sucrose at least overnight (or until tissue sank to the bottom of the container) before being embedded in gelatin. The embedded tissue was fixed in a solution comprised of 25% glutaraldehyde, 1X phosphate buffer, and sucrose and rinsed for one hour before sectioning. The tissue was sectioned on a sliding microtome equipped with a freezing stage. Brains were sectioned coronally at 50µm. Approximately 96 sections from each brain were kept for mounting onto slides and used for immunohistochemistry and staining.

2.7 *Cresyl Violet Staining*

One half of the brain tissue sections were stained with cresyl violet cell body stain. Digital images were obtained using a digital camera (RT Spot, Sterling Heights, MI) attached to a light microscope. A total of 3 images from each hemisphere of the prefrontal cortex contusion area were captured and used to quantify healthy cell numbers. Images (size: 120,000 μm^2) were captured from the center of the injury, 2.5mm from the midline and 3mm from the dorsal edge of the cortex, as well as 2 images from either side of the center of the injury. Cells that were round in shape with a dark nucleus contained inside were counted as healthy cells.

2.8 *Immunohistochemistry*

The remaining brain tissue sections were labeled with mouse anti-glial fibrillary acidic protein (GFAP 1:2000, Sigma-Aldrich, Saint Louis, MO) using a free-floating protocol. The GFAP antibody labels astrocytes and Bergmann glia cells, as well as gliomas and other glial cell-derived tumors (Hicks, Hewlett, Windle, et al., 2007). Tissue sections were washed in PBS, then blocked for 1 hour with 5% normal goat serum in PBS with Triton-X at room temperature. The primary antibody was applied to sections and allowed to incubate overnight at 4 degrees Celsius. The next day, sections were washed in PBS at room temperature twice for 10 minutes each. The secondary antibody (Alexa Fluor 532 goat anti-mouse, 1:2000, Molecular Probes, Eugene, OR) was applied to sections and incubated for 2 hours at room temperature in the dark. Sections were then washed in PBS at room temperature, mounted and coverslipped using Vectashield with Dapi (Vector, Burlingame, CA) for analysis using a fluorescence microscope.

Digital images were obtained using a digital camera (RT Spot, Sterling Heights, MI) attached to a fluorescent microscope. A total of 3 images from each hemisphere of

the prefrontal cortex contusion area were captured and used to quantify GFAP-positive cells. Images (size: 120,000 μm^2) were captured from the center of the injury, 2.5mm from the midline and 3mm from the dorsal edge of the cortex, as well as 2 images from either side of the center of the injury. GFAP-positive cells fluoresced red under a green filter.

CHAPTER III

RESULTS

3.1 *Statistical Analysis*

Repeated measures analysis of variance (ANOVA) tests and one-way ANOVA tests were performed using SPSS 15.0 for Windows. For both behavioral tasks, the between-groups factor was group (Sham Only, Sham Vehicle, Sham Fluoxetine, Sham Lovastatin, Sham Combination, CCI Only, CCI Vehicle, CCI Fluoxetine, CCI Lovastatin, and CCI Combination) and the within-groups factor was day of testing. The cresyl violet and GFAP cell count data were analyzed by taking an average of stained or labeled cells in sham group brains and comparing them with the average in the CCI group brains across both hemispheres. A secondary analysis on the cell count data compared sham group brains to the injured and uninjured hemispheres of the CCI group brains. For all statistical analysis $p<.05$ was considered significant for all analyses.

Data were collected from both the reaching box task and the BTARS task. Data from the reaching box task consisted of the number of successful reaches for each session. Data collection for the BTARS task consisted of latency of adhesive removal.

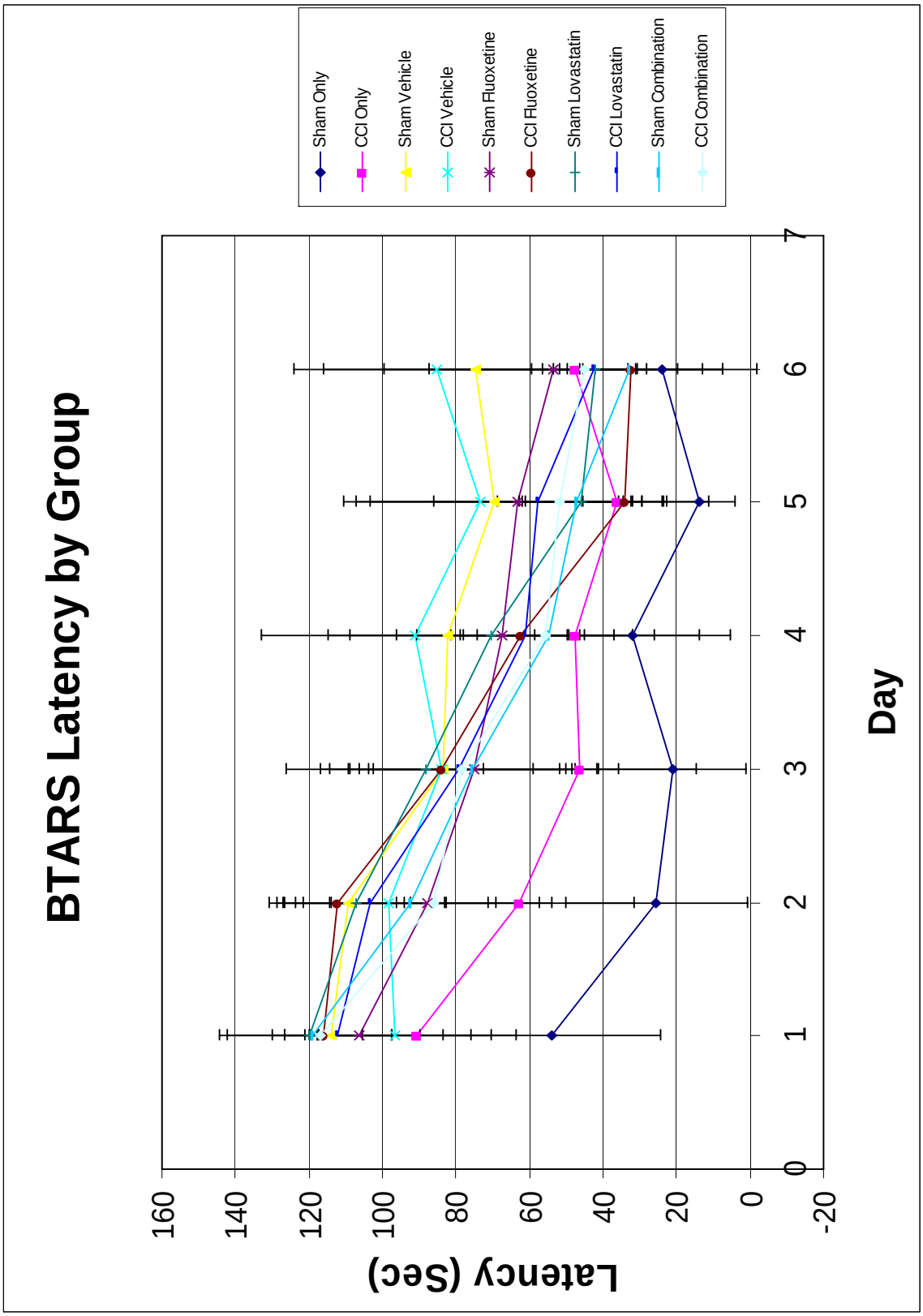
Latency was analyzed using repeated measures ANOVA. The number of reaches from the reaching box task data was also analyzed using repeated measures ANOVA.

For cell counts, brain tissue was collected from approximately A/P 4.0mm until A/P 2.0mm (distance anterior from bregma) to collect the area surrounding the injury site, located at A/P 3.0mm (Paxinos & Watson, 2007). Tissue used for cresyl violet and GFAP was selected from the center of the injury site. All images were taken from the prefrontal cortex: two images per hemisphere on either side of the injury site and one image per hemisphere at the injury site (measured from the intact cortex using a reticle).

3.2 *BTARS Data Analysis*

Latency to remove the adhesive sticker was analyzed using a repeated measures ANOVA. The factors included were group (Sham Only, Sham Vehicle, Sham Fluoxetine, Sham Lovastatin, Sham Combination, CCI Only, CCI Vehicle, CCI Fluoxetine, CCI Lovastatin and CCI Combination) and day (1-6) as the repeated measure. Rats became more successful at removing the tactile stimuli on successive trials, as the main effect of day was statistically significant $F_{(5, 330)}$=89.213, p<.001. The main effect of group was also statistically significant $F_{(9, 66)}$=6.479, p<.001, indicating that group had an effect on removing the tactile stimuli. There was a significant interaction effect of day x group $F_{(45, 330)}$=2.409, p<.001. The pattern of expected outcome did not happen as hypothesized. The expected outcome for the BTARS behavioral task was to show a similar pattern of improvement in which sham groups and drug treatment CCI groups decrease in latency over days. The pattern developed from the data collected can be seen in Figure 2.

Figure 2. BTARS Latency Scores by Group



The Sham Only and CCI Only groups followed a similar pattern in terms of decreasing latency over the first 3 days as can be seen in Figure 2. This suggests that the two groups that did not receive any injections (i.e., Sham Only and CCI Only) performed better than the animals that did receive injections regardless of whether the injection was just saline (vehicle) or a drug treatment. The animals receiving injections took longer to reach similar levels of latency than those not receiving injections. All means and standard deviations for the BTARS task are presented in Table II.

Table II. BTARS Means and Standard Deviations

	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
Group	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Sham Only	53.82	29.50	25.56	24.68	21.13	20.08	31.94	26.77	14.00	9.86	24.00	25.69
CCI Only	90.63	26.94	62.94	31.26	46.50	31.78	47.56	33.96	36.31	25.00	47.44	39.88
Sham Vehicle	113.86	16.25	109.43	17.15	83.57	33.23	82.36	32.59	69.50	37.64	74.50	41.37
CCI Vehicle	96.75	20.89	98.13	29.10	83.94	42.15	91.25	41.79	73.31	37.41	85.19	38.85
Sham Fluoxetine	106.36	36.10	87.71	33.85	75.14	39.33	67.36	41.51	63.29	39.90	53.43	46.05
CCI Fluoxetine	116.00	10.58	112.43	16.41	84.07	24.90	62.43	16.25	34.07	11.30	32.36	19.28
Sham Lovastatin	120.00	0.00	107.13	23.82	88.19	15.79	70.63	25.50	45.94	16.70	42.31	14.08
CCI Lovastatin	112.13	14.60	103.19	20.36	79.19	27.36	61.31	29.48	57.69	28.33	42.50	11.20
Sham Combination	119.21	2.08	92.57	21.35	75.43	27.16	55.00	23.18	47.14	14.65	32.64	12.96
CCI Combination	117.06	27.23	85.88	28.45	78.63	30.84	55.69	18.52	51.63	17.06	45.19	14.46

Pair-wise comparisons indicated significant group comparisons of Sham Only to all other groups except CCI Only: Sham Only v. Sham Vehicle, $p<.001$; Sham Only v. CCI Vehicle, $p<.001$; Sham Only v. Sham Fluoxetine, $p=.001$; Sham Only v. CCI Fluoxetine, $p=.001$; Sham Only v. Sham Lovastatin, $p<.001$; Sham Only v. CCI Lovastatin, $p<.001$; Sham Only v. Sham Combination, $p=.004$; Sham Only v. CCI Combination, $p=.001$. Pair-wise comparisons also showed an interaction of CCI Only with CCI Vehicle as approaching significance at $p=.054$.

3.3 *Reaching Box Data Analysis*

Number of reaches performed was analyzed by repeated measures ANOVA. The factors included were group (Sham Only, Sham Vehicle, Sham Fluoxetine, Sham Lovastatin, Sham Combination, CCI Only, CCI Vehicle, CCI Fluoxetine, CCI Lovastatin, and CCI Combination) and day (Pre-Injury, Post-Injury #1, Post-Injury #2) as the repeated measure. Rats became more successful at reaching for treats on successive trials, as the main effect of day was statistically significant $F_{(2, 132)}=10.413$, $p<.001$. The main effect of group was also statistically significant $F_{(9, 66)}=2.255$, $p=.029$, indicating that group had an effect on number of reaches performed. The group x day interaction was not statistically significant $F_{(18, 132)}=0.941$, $p=.531$. The expected pattern would have been to see the number of reaches increase for each group from Pre-Injury through Post-Injury #2, particularly for the drug-treated CCI groups. The unexpected pattern developed by the reaching box data is shown in Figure 3.

Figure 3. Reaching Box Reaches by Group

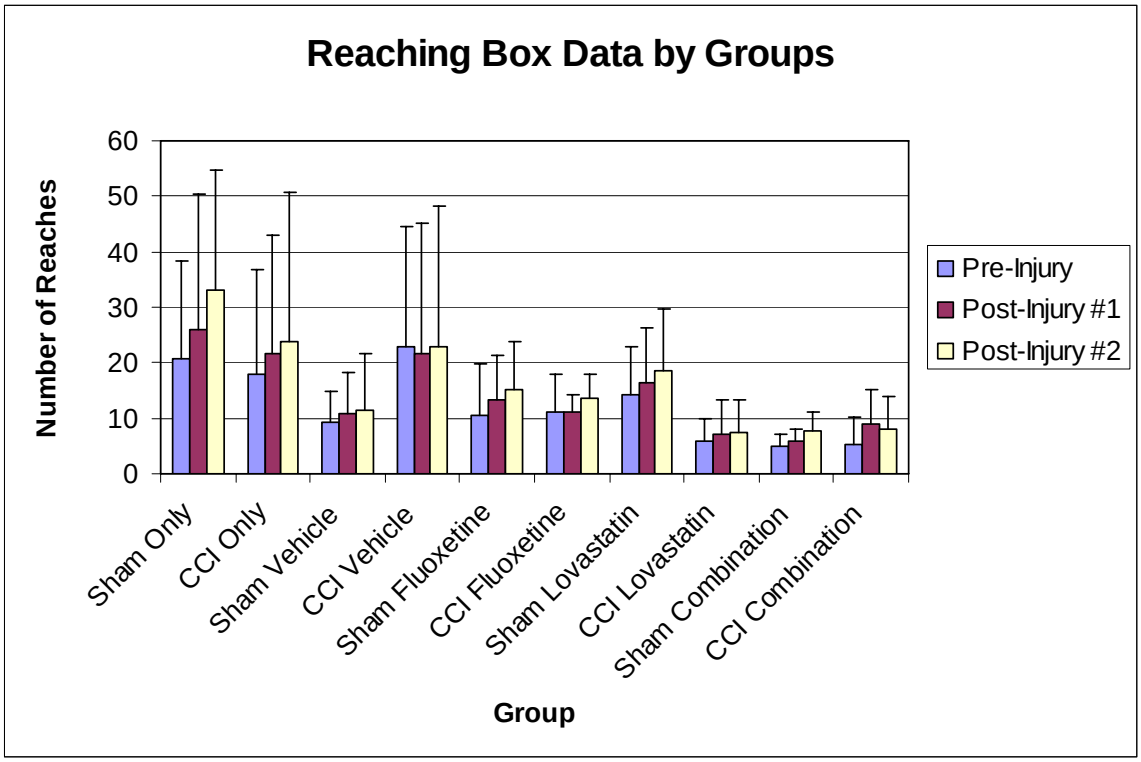


Figure 3 also shows that animals not receiving injections seemed to perform better than the animals receiving injections on the reaching box task. All means and standard deviations for the reaching box task are presented in Table III.

Table III. Reaching Box Means and Standard Deviations

	Pre-Injury		*Post-Injury #1*		*Post-Injury #2*	
Group	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Sham Only	20.63	17.73	26.00	24.41	33.13	21.67
CCI Only	18.00	18.94	21.63	21.32	23.88	26.90
Sham Vehicle	9.29	5.71	10.71	7.50	11.57	9.96
CCI Vehicle	22.75	21.84	21.63	23.51	22.75	25.55
Sham Fluoxetine	10.57	9.16	13.29	8.14	15.29	8.56
CCI Fluoxetine	11.00	6.90	11.00	3.27	13.71	4.23
Sham Lovastatin	14.13	8.73	16.50	9.70	18.50	11.15
CCI Lovastatin	5.88	4.12	7.25	5.99	7.38	5.81
Sham Combination	5.00	2.08	5.86	2.04	7.71	3.55
CCI Combination	5.38	4.72	8.88	6.22	8.00	5.88

3.4 *Cresyl Violet Cell Counts Data Analysis*

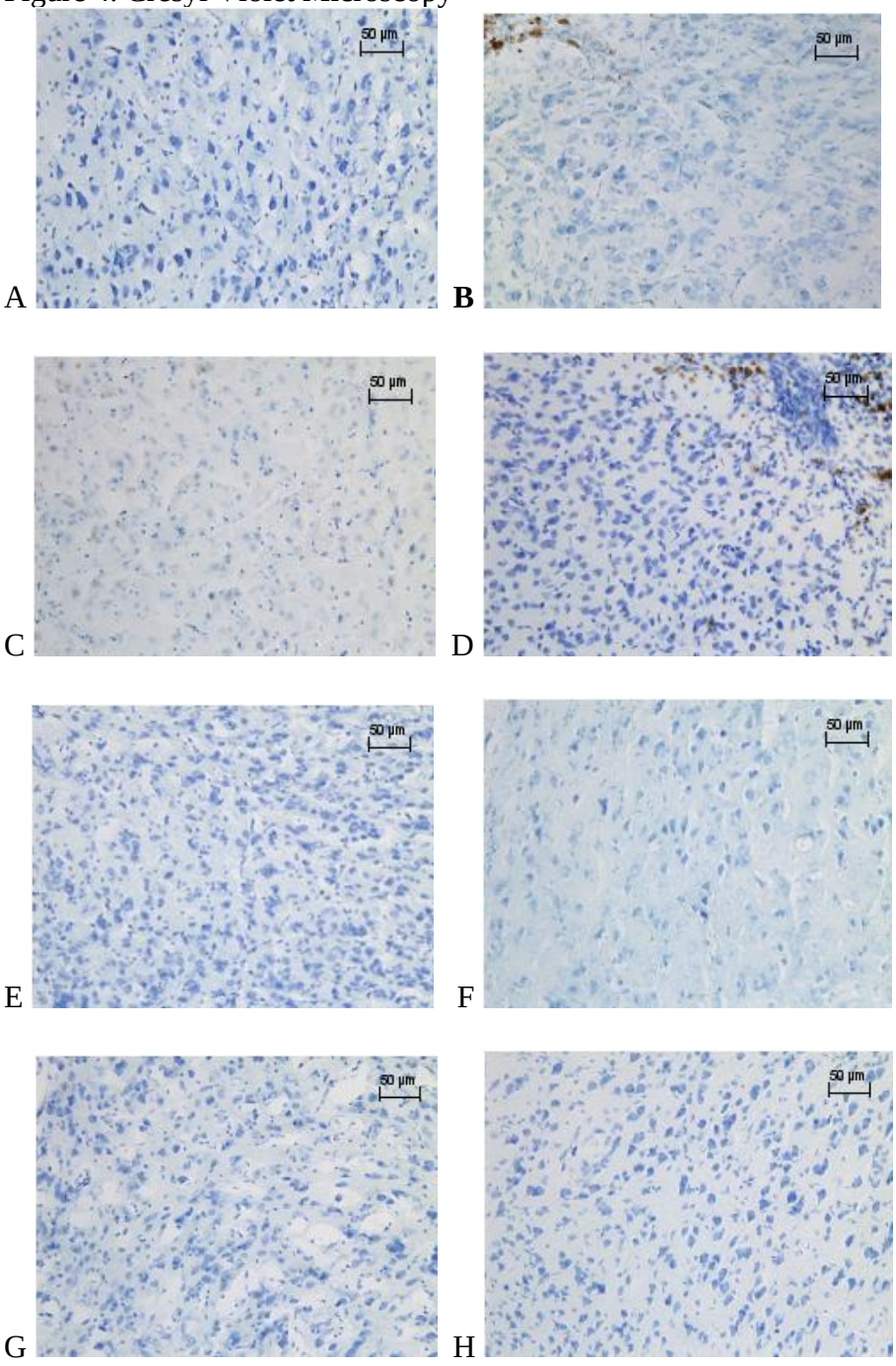
A one-way ANOVA was calculated on the average number of healthy cells counted via light microscopy of brain tissue stained with cresyl violet on the sham and CCI groups. The analysis was statistically significant, $F(14, 87) = 68.5$, $p<.001$. All means and standard deviations for the cresyl violet analysis are presented in Table IV.

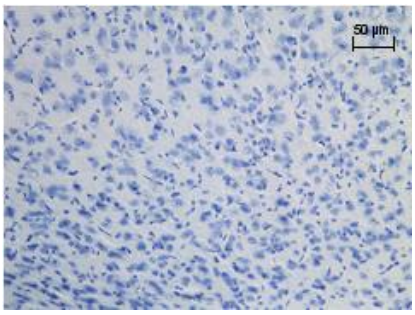
Table IV. Cresyl Violet Means and Standard Deviations by Group

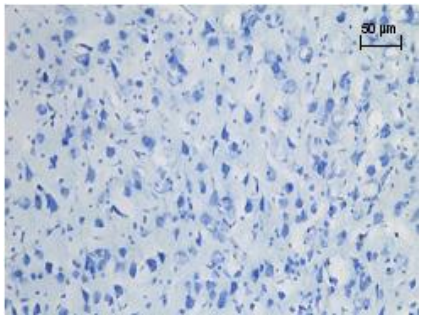
Group	*Mean*	*SD*
Sham Only	111.00	26.90
Sham Vehicle	124.00	8.96
Sham Fluoxetine	110.00	12.10
Sham Lovastatin	113.00	3.95
Sham Combination	118.00	6.95
CCI Only	74.80	1.66
CCI Vehicle	75.00	4.88
CCI Fluoxetine	58.70	3.01
CCI Lovastatin	66.30	9.23
CCI Combination	61.50	9.73

The means of each group show there is an overall greater number of healthy cells in the CCI Only and CCI Vehicle (i.e., non-drug treatment) groups as compared to the CCI drug treatment groups. A representative example of cresyl violet stained tissue taken from below the injured area from each group is shown in Figure 4.

Figure 4. Cresyl Violet Microscopy





I 

J

(A) Sham Only
(B) CCI Only
(C) Sham Vehicle
(D) CCI Vehicle
(E) Sham Fluoxetine
(F) CCI Fluoxetine
(G) Sham Lovastatin
(H) CCI Lovastatin
(I) Sham Fluoxetine-Lovastatin Combination
(J) CCI Fluoxetine-Lovastatin Combination

Bonferoni's multiple comparisons post-tests were also calculated for the cresyl violet groups. The post-tests revealed some statistically significant comparisons: Sham Only vs. all CCI groups were significant at $p<.001$, Sham Vehicle vs. all CCI groups were significant at $p<.001$, Sham Fluoxetine vs. all CCI groups were significant at $p<.001$, Sham Lovastatin vs. all CCI groups were significant at $p<.001$, and Sham Combination vs. all CCI groups were significant at $p<.001$.

A second one-way ANOVA was calculated on the average number of healthy cells counted using light microscopy of brain tissue stained with cresyl violet on the sham and each hemisphere of the CCI groups. The analysis was statistically significant, $F(9, 56) = 34.02$, $p < .001$. All means and standard deviations for the cresyl violet analysis are presented in Table V.

Table V. Cresyl Violet Means and Standard Deviations by Group and Hemisphere

Group	*Mean*	*SD*
Sham Only	111.00	26.09
Sham Vehicle	124.00	8.96

Sham Fluoxetine	110.00	12.10
Sham Lovastatin	113.00	3.95
Sham Combo	118.00	6.95
CCI Only Injured	38.71	4.88
CCI Vehicle Injured	36.25	5.61
CCI Fluoxetine Injured	38.33	2.83
CCI Lovastatin Injured	39.76	5.67
CCI Combo Injured	37.89	2.18
CCI Only Uninjured	114.00	3.09
CCI Vehicle Uninjured	113.80	7.08
CCI Fluoxetine Uninjured	79.10	5.58
CCI Lovastatin Uninjured	92.86	15.69
CCI Combination Uninjured	85.17	20.52

The means of the injured and uninjured hemispheres of the CCI groups do not show a difference among the injured hemispheres of the CCI cell counts. However, the uninjured hemispheres of the CCI cell counts showed a pattern in which the number of healthy cells was greater in the non-drug treatment groups as opposed to the drug treatment groups.

Bonferroni's multiple comparisons post-tests were also calculated for the cresyl violet groups. The post-tests revealed some statistically significant comparisons: All sham groups vs. all CCI Injured hemispheres ($p<.001$) and all CCI Injured hemispheres vs. all CCI Uninjured hemispheres ($p<.001$). All other statistically significant comparisons are presented in Table VI.

Table VI. Cresyl Violet Additional Significant Bonferroni's Multiple Comparisons

Bonferroni's Multiple Comparison Test	***p* Value**
Sham Only:	
vs. CCI Fluoxetine Uninjured	<.001
vs. CCI Combination Uninjured	<.01
Sham Vehicle:	
vs. CCI Fluoxetine Uninjured	<.001
vs. CCI Lovastatin Uninjured	<.001
vs. CCI Combination Uninjured	<.001
Sham Fluoxetine:	

vs. CCI Fluoxetine Uninjured	<.001
vs. CCI Combination Uninjured	<.05
Sham Lovastatin:	
vs. CCI Fluoxetine Uninjured	<.001
vs. CCI Combination Uninjured	<.05
Sham Combo:	
vs. CCI Fluoxetine Uninjured	<.001
vs. CCI Lovastatin Uninjured	<.05
vs. CCI Combination Uninjured	<.001
CCI Only Uninjured:	
vs. CCI Fluoxetine Uninjured	<.001
vs. CCI Combination Uninjured	<.01
CCI Vehicle Uninjured:	
vs. CCI Fluoxetine Uninjured	<.001
vs. CCI Combination Uninjured	<.001

3.5 *GFAP Cell Counts Data Analysis*

A one-way ANOVA was calculated on the average number of labeled cells counted via fluorescent microscopy of brain tissue labeled with GFAP on the sham and CCI groups. The analysis was statistically significant, $F(9, 31) = 11.34$, $p < .001$. All means and standard deviations for the GFAP cell counts are presented in Table VII.

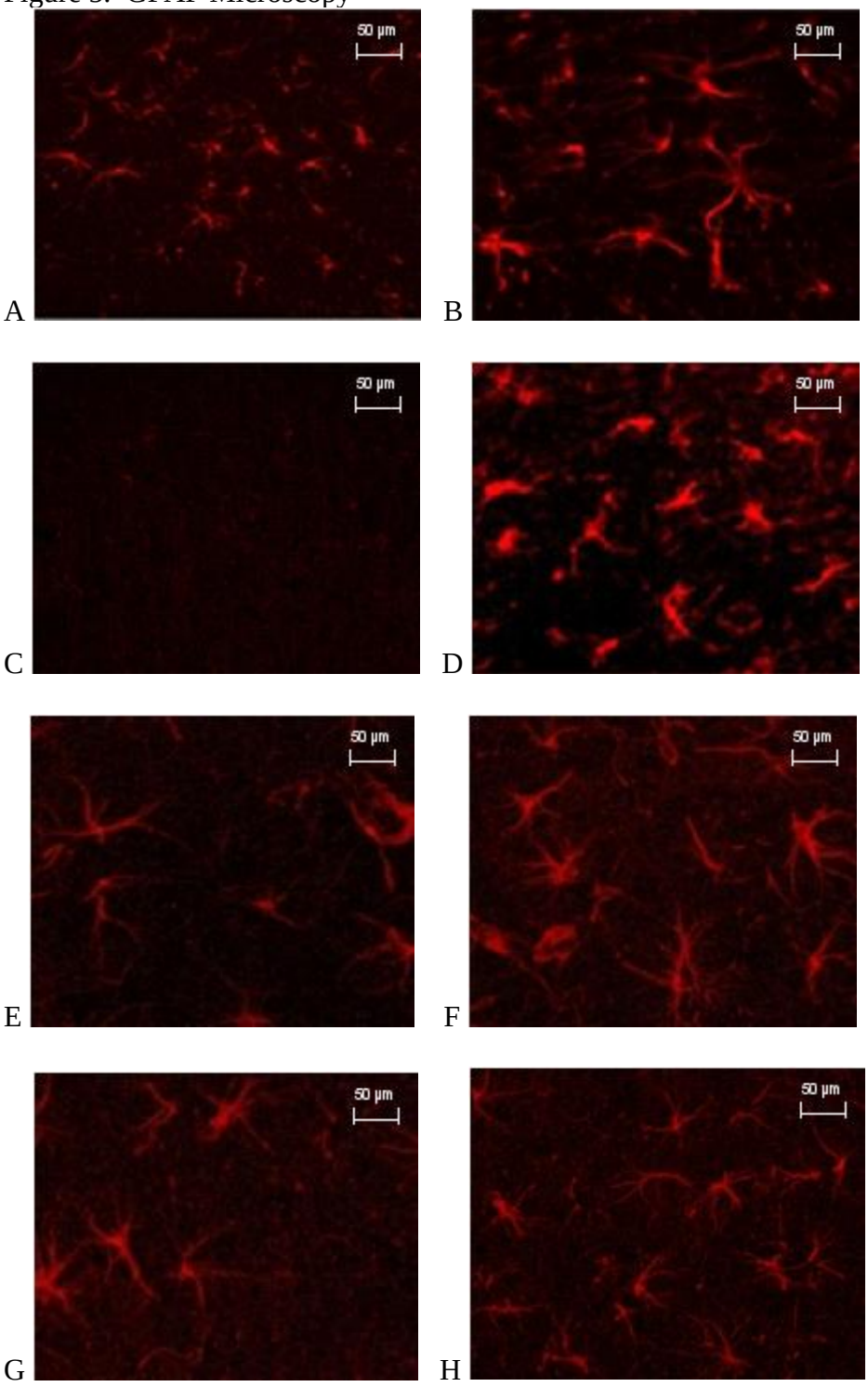
Table VII. GFAP Means and Standard Deviations by Group

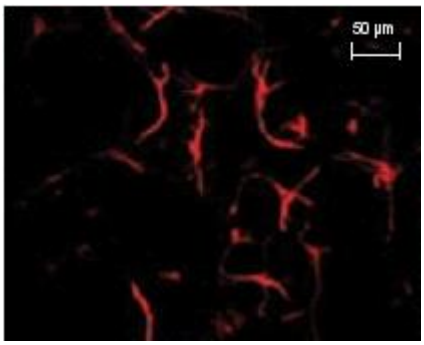
Group	*Mean*	*SD*
Sham Only	5.56	1.29
Sham Vehicle	3.94	0.20
Sham Fluoxetine	7.46	5.02
Sham Lovastatin	4.34	0.76
Sham Combination	4.27	0.61
CCI Only	11.80	9.11
CCI Vehicle	20.10	6.13
CCI Fluoxetine	14.20	0.833
CCI Lovastatin	12.80	4.68
CCI Combination	16.30	0.72

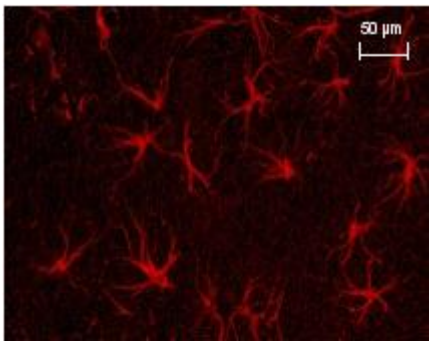
The means presented for each group show a difference in GFAP-positive cells between the sham groups and the CCI groups, with the exception of CCI only. A representative

example of GFAP-positive tissue from the area below the injury area of each group is shown in Figure 5.

Figure 5. GFAP Microscopy



I 

J 

(A) Sham Only
(B) CCI Only
(C) Sham Vehicle
(D) CCI Vehicle
(E) Sham Fluoxetine
(F) CCI Fluoxetine
(G) Sham Lovastatin
(H) CCI Lovastatin
(I) Sham Fluoxetine-Lovastatin Combination
(J) CCI Fluoxetine-Lovastatin Combination

Bonferroni's multiple comparisons post-tests were also calculated for the GFAP groups. Statistically significant comparisons are presented in Table VIII.

Table VIII. GFAP Significant Bonferroni's Multiple Comparisons

Bonferroni's Multiple Comparison Test	***p* Value**
Sham Only:	
vs. CCI Vehicle	<.001
vs. CCI Combination	<.01
Sham Vehicle:	
vs. CCI Vehicle	<.001
vs. CCI Fluoxetine	<.01
vs. CCI Combination	<.001
Sham Fluoxetine:	
vs. CCI Vehicle	<.001
vs. CCI Combination	<.01
Sham Lovastatin:	
vs. CCI Vehicle	<.001
vs. CCI Fluoxetine	<.05
vs. CCI Combination	<.01
Sham Combination:	
vs. CCI Vehicle	<.001
vs. CCI Fluoxetine	<.01
vs. CCI Lovastatin	<.05
vs. CCI Combination	<.001

A second one-way ANOVA was calculated on the average number of labeled cells counted via fluorescent microscopy of brain tissue labeled with GFAP on the sham and each hemisphere of the CCI groups. The analysis was statistically significant, $F(9, 56) = 34.02$, $p < .001$. All means and standard deviations for the GFAP analysis are presented in Table IX.

Table IX. GFAP Means and Standard Deviations by Group and Hemisphere

Group	*Mean*	*SD*
Sham Only	5.56	1.29
Sham Vehicle	3.94	0.20
Sham Fluoxetine	7.56	4.97
Sham Lovastatin	4.34	0.76
Sham Combination	4.27	0.61
CCI Only Injured	20.50	20.04
CCI Vehicle Injured	32.17	7.27
CCI Fluoxetine Injured	24.56	2.03
CCI Lovastatin Injured	10.58	12.13
CCI Combination Injured	22.34	7.67
CCI Only Uninjured	12.84	7.30
CCI Vehicle Uninjured	24.25	11.31
CCI Fluoxetine Uninjured	12.11	2.46
CCI Lovastatin Uninjured	19.67	7.35
CCI Combination Uninjured	15.35	11.01

The analysis from the injured and uninjured hemispheres shows an expected pattern of GFAP-positive cells in which the injured CCI hemispheres are greater than the uninjured CCI hemispheres. The exception from this pattern is the lovastatin injured hemisphere group compared to lovastatin uninjured hemisphere group.

Bonferroni's multiple comparisons post-tests were also calculated for the GFAP groups. The post-tests revealed that the following groups were considered statistically significant: Sham Only vs. CCI Vehicle Injured ($p<.001$), Sham Vehicle vs. CCI Vehicle Injured ($p<.001$), Sham Fluoxetine vs. CCI Vehicle Injured ($p<.001$), Sham Lovastatin vs. CCI Vehicle Injured ($p<.001$), Sham Combination vs. CCI Vehicle Injured ($p<.001$),

Sham Combination vs. CCI Fluoxetine Injured ($p<.05$), Sham Combination vs. CCI Combination Injured ($p<.05$), Sham Combination vs. CCI Vehicle Uninjured ($p<.05$), CCI Vehicle Injured vs. CCI Lovastatin Injured ($p<.05$), and CCI Vehicle Injured vs. CCI Fluoxetine Uninjured ($p<.05$).

3.6 *Further Analyses*

Two additional ANOVAs were calculated on the reaching box task using the Pre-injury reaching scores to create a baseline. The baseline was created by subtracting the individual post injury scores (i.e., Post-Injury #1 and Post-Injury #2) from the individual Pre-injury scores to see the difference in the number of reaches. The differences from the Post-Injury #1 group were analyzed against injury and treatment. The differences from the Post-Injury #2 group were also analyzed against injury and treatment. The analyses did not reveal significant differences.

A correlation was calculated across the post-injury reaching box data, the final day of the BTARS task data (i.e., Day 6), and the cresyl violet and GFAP cell counts. The correlation revealed a significant correlation of -.310 between Day 6 of the BTARS task and healthy cell counts of cresyl violet stained tissue. This relationship was statistically significant at the .05 level. The negative correlation indicates that as the latency scores on Day 6 of the BTARS task decreased, the healthy cell counts of cresyl violet stained tissue increased. No additional significant correlations were observed.

CHAPTER IV

DISCUSSION

4.1 *Current Research*

The present study demonstrated that utilizing pre-injury lovastatin, post-injury fluoxetine, or a combination of both drugs did not improve functional recovery regarding forelimb motor control. This became evident with the data from the reaching box task. Most groups saw some improvement in the number of successful reaches from Pre-Injury through Post-Injury #2, regardless of group (injury and treatment), which may suggest that an increase in the number of successful reaches could be the result of learning as opposed to a result of a specific treatment. It is important to note that the number of successful reaches were higher in groups that did not receive injections as compared to those that did receive injections. This does not follow the pattern expected by the investigator. The animals not receiving injections performing better than those that did receive injections suggests the possibility of a stress effect from receiving injections over multiple days. When post-injury scores were analyzed using additional ANOVAs calculated by creating a baseline using the pre-injury scores subtracted from post-injury scores, no significant differences were revealed.

The present study also demonstrated that utilizing pre-injury lovastatin and a combination of lovastatin and fluoxetine did not improve functional recovery regarding somatosensory dysfunction. This was demonstrated by the data from the BTARS task. The pairwise comparison of Sham Only v. CCI Fluoxetine revealed significant differences on Days 1-3, but not on days 4-6. This seems to suggest that Fluoxetine has some sort of an effect on somatosensory dysfunction in injured animals, although this effect seems to be limited. It is possible that any positive or therapeutic effect of Fluoxetine is hidden by the overall variability suggested by the correlation performed across the behavioral tasks and cell counts data.

Cell counts from the cresyl violet stain suggest that the combination therapy did not preserve healthy cells counted in the cortex of an injured (CCI) brain as compared to the number of healthy cells counted in the cortex of an uninjured (sham) brain. Healthy cells have a tendency to have a round shape with a dark nucleus contained within the cell. There were significant differences in the number of healthy cells counted between the sham groups and CCI groups, excluding CCI only. In order to suggest that the combination therapy of lovastatin and fluoxetine was a viable treatment for traumatic brain injury, the number of healthy cells counted in the CCI drug treated tissue would have been expected to be significantly greater than the CCI non-drug treated tissue. However, the means of each injured group and the group comparisons do not indicate that the CCI drug treated groups had a significantly greater number of healthy cells in the stained tissue as compared to the CCI non-drug treated groups.

The second analysis took a look at cell counts from the cresyl violet stain that looked at differences between the CCI injured and uninjured hemispheres. The analysis

of the sham brains versus the injured and uninjured hemispheres showed the greatest number of healthy cells in the sham brains, fewer healthy cells in the CCI uninjured hemispheres and the fewest healthy cells in the CCI injured hemispheres. Cell counts from the GFAP labeled tissue suggest that the drug therapy did not significantly decrease the number of labeled cells counted in the cortex of a CCI brain as compared to the number of labeled cells in the cortex of a sham brain.

There were significant differences in the number of labeled cells counted between the sham groups and CCI groups, with the exception of the CCI Only group. In order to suggest that the drug therapy of lovastatin and fluoxetine was a viable treatment for traumatic brain injury, the number of labeled cells counted in the CCI drug treated tissue would have been expected to be significantly less than the CCI non-drug treated tissue. However, the means of each injured group and the group comparisons do not indicate that the drug treated CCI groups had a significantly lesser number of labeled cells as compared to the non-drug treated CCI groups.

In the second analysis, it was revealed that the number of GFAP-positive cells counted in sham groups were fewer than those in the CCI groups. It was also shown that GFAP-positive cells were fewer in the CCI uninjured hemispheres as compared to the CCI injured hemispheres, with the exception of the CCI Lovastatin group in both hemispheres.

The cell counts data from both the cresyl violet and GFAP procedures both suggest that a drug therapy utilizing lovastatin and fluoxetine is not an effective treatment for TBI.

Previous research has indicated that both Lovastatin (Asahi et al., 2005; Chen et al., 2007) and Fluoxetine (Carboni et al., 2006; Singh et al., 2000) were effective as separate treatments for brain injuries. However, the data presented in the current study do not follow the data results of previous studies. Referring back to the correlation performed as an additional analysis, a negative correlation was seen between the final day of BTARS task testing and the cresyl violet healthy cell counts. As latency decreased on the final day of the BTARS task, the number of healthy cells counted in the cresyl violet stained tissue increased. There was no relationship demonstrated between the reaching box task and the cell counts to one another. The lack of correlation amongst the remaining items suggests that there is such a high degree of variability that any effect that does exist could be hidden by the variability itself.

4.2 *Limitations*

The BTARS latency data revealed that animals that did not receive injections performed the task better than the animals that did receive injections regardless of whether the injection was just saline (vehicle) or a drug treatment. This could suggest a negative impact of stress related to receiving injections on multiple days. One way to measure stress in individual animals would be to collect blood samples before surgery, after surgery, and before perfusion to measure levels of the hormone cortisol by means of immunoassay (Bardi, Bode, Ramirez & Brent, 2005). Another method of measuring cortisol levels would be to analyze urine from individual animals (van der Hart, de Biurrun, Czeh, et al., 2005). By analyzing cortisol levels in either blood or urine, a baseline could be created from sham and CCI animals not receiving injections and deviations from that baseline could indicate increased stress from receiving injections.

Of course, the process of measuring cortisol could also lead to unintentional stress in study animals. In order to avoid additional stress, the drugs could be administered in either food or water. The animals are still receiving the drug, but the difference is in the route of administration, completely avoiding the administration of injections altogether.

One limitation to the current study was that the entire study was conducted over several months, running 3 to 12 animals per group and oftentimes overlapping another group by about a week. This could have had an impact on later behavioral data due to investigator learning (i.e., over time being able to run the behavioral tasks more efficiently).

One possible explanation for the unexpected results of the cell count data could be in the mechanics of the injury. The CCI contusion device is equipped with autoclavable metal tips that cause the tissue deformation at the point of contact. The tip size used to create the CCI injury for this project was 5mm. It is possible that the tip or the velocity causes an injury that is too large, causing it to mask other processes that have occurred in the injured cortex. To test this possibility, it would be necessary to perform several surgeries by utilizing smaller tips and lower the velocity in different animals. When the behavioral aspects are complete, taking a look at the resulting histology would reveal whether differences exist from using smaller tips or lowering the velocity.

An acknowledged limitation to the current study is due to the nature of lovastatin as a drug. Because lovastatin was meant to be used as a preventative measure, the effects of using lovastatin prior to injury would not be applicable to an everyday situation. Any observed effects of lovastatin would most likely only apply to soldiers in a military combat situation where risk of a TBI is greater or in those already prescribed lovastatin

for cholesterol-lowering reasons who may succumb to a TBI at some point in their life while still taking the prescription. Because the side effects of lovastatin are considered mild and short-lived, prescribing the medication as a pre-injury preventative drug could be considered relatively safe for most soldiers.

4.3 *Future Research*

Although the combination of lovastatin and fluoxetine was not as hoped for the treatment of TBI, combined pharmacotherapies should still be explored. Creating the right combination of existing drugs, drugs currently being developed, or drugs that have yet to be developed could be the key to treating individuals who have experienced a TBI. Because TBI is known to have multiple mechanistic processes, it is important to target the initial massive cell death that begins the injury and to intervene before the secondary cascade leads to further and more extensive cellular damage. A combined pharmacotherapy could still be the solution to treating TBI, especially in a military combat situation.

Future research should also focus on the diagnostic procedures for TBI. A recent study by Hajszan and Colleagues (2009) illustrated that synapses in the hippocampus are able to remodel themselves during depression. Because depression is common in TBI and Post-Traumatic Stress Disorder (PTSD), there is some recognition of the possibility of comorbid characteristics of PTSD with underlying mild TBI. It is estimated that as many as 30% of soldiers returning from Operations Iraqi Freedom and Enduring Freedom suffer from PTSD. Some of the symptoms of PTSD are very similar to symptoms of TBI, including depression, memory loss, and sudden anger. In fact, a soldier with mild TBI coupled with PTSD is likely to have greater difficulties adjusting because PTSD is

exacerbating some elements of the TBI (Elsevier, 2009). By employing more accurate procedures for TBI and PTSD, nurses and physicians may have more success treating a multi-faceted injury when there are diagnostic procedures which can pinpoint both the primary and secondary elements of what a patient is suffering from.

REFERENCES

Adams, J. H. (1975). The neuropathology of head injury. In P. J. Binken & G. W. Bruyn (Eds), *Handbook of Clinical Neurology* (pp. 35-65). New York: Elsevier.

Asahi, M., Huang, Z., Thomas, S., Yoshimura, S., Sumii, T., Mori, T., Qui, J., Amin-Hanjani, S., Huang, P. L., Liao, J. K., Lo, E. H. & Moskowitz, M. A. (2005). Protective effects of statins involving both eNOS and tPA in focal cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism, 25*(6), 722-729.

Bardi, M., Bode, A. E., Ramirez, S. M., Brent, L. Y. (2005). Maternal care and development of stress responses in baboons. *American Journal of Primatology, 66*, 263-278.

Bigler, E. D., Johnson, S. C., Anderson, C. V., & Blatter, D. D. (1996). Traumatic brain injury and memory: The role of hippocampal atrophy. *Neuropsychology, 10*, 333-342.

Boswell, J. E., McErlean, M., & Verdile, V. P. (2002). Prevalence of traumatic brain injury in an ed population. *American Journal of Emergency Medicine, 20*(3), 177-180.

Breuzn, J. & Daszuta, A. (1999). Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. *Neuroscience, 89*, 999-1002.

Carboni, L., Vighini, M., Piubelli, C., Castelletti, L., Milli, A. & Domenici, E. (2006). Proteomic analysis of rat hippocampus and frontal cortex after chronic treatment with fluoxetine or putative novel antidepressants: CRF1 and NK1 receptor antagonists. *European Neuropsychopharmacology, 16*(7), 521-537.

Cernak, I. (2005). Animal models of head trauma. *NeuroRx: The Journal of the American Society for Experimental NeuroTherapeutics, 2,* 410-422.

Chen, S. F., Hung, T. H., Chen, C. C., Lin, K. H., Huang, Y. N., Tsai, H. C. & Wang, J. Y. (2007). Lovastatin improves histological and functional outcomes and reduces inflammation after experimental traumatic brain injury. *Life Sciences, 81,* 288-298.

Crosson, B., Sartor, K. J., Jenny, A. B., Nabors, N. A., & Moberg, P. J. (1993). Increased intrusions during verbal recall in traumatic and nontraumatic lesions of the temporal lobe. *Neuropsychology, 7,* 193-208.

Elsevier (2009, March 16). Stress May Cause The Brain To Become Disconnected. *ScienceDaily*. Retrieved March 16, 2009, from http://www.sciencedaily.com /releases/2009/03/090316075845.htm

Emery, E. (2007, April 16). TBI: Hidden wounds plague Iraq war veterans. *Denver Post.* Retrieved October 2, 2007, from http://www.denverpost.com/ci_5675337

Endo, A. (2004). The origin of the statins. *Atherosclerosis Supplements, 5,* 125-130.

Endres, M., Laufs, U., Huang, Z., Nakamura, T., Huang, P., Moskowitz, M. A. & Liao, J. K. (1998). Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. *Proceedings of the National Academy of Science of the United States of America, 95*(15), 8880-8885.

Foda, M. A. & Marmarou, A. (1994). A new model of diffuse brain injury in rats. Part II: Morphological characterization. *Journal of Neurosurgery, 80,* 301-313.

Fontaine, A., Azouvi, P., Remy, P., Bussel, B., & Samson, Y. (1999). Functional anatomy of neuropsychological deficits after severe traumatic brain injury. *Neurology, 53,* 1963-1968.

Gennarelli, T. A. (1994). Animate models of human head injury. *Journal of Neurotrauma, 11*(4), 357-363.

Gould, E. (1999). Serotonin and hippocampal neurogenesis. *Neuropsychopharmacology, 21*, 46S-51S.

Hajszan, T., Dow, A., Waner-Schmidt, J. L., Szigeti-Buck, K., Sallam, N. L., Parducz, A., Leranth, C., & Duman, R. S. (2009). Remodeling of hippocampal spine synapses in the rat learned helplessness model of depression. *Biological Psychiatry, 65*(5), 392-400.

Heath, D. L. & Vink, R. (1999). Optimization of magnesium therapy after severe diffuse axonal brain injury in rats. *The Journal of Pharmacology and Experimental Therapeutics, 288*(3), 1311-1316.

Hicks, A. U., Hewlett, K., Windle, V., Chernenko, G., Ploughman, M., Jokkonen, J., Weiss, S., & Corbett, D. (2007). Enriched environment enhances transplanted subventricular zone stem cell migration and functional recovery after stroke. *Neuroscience,146*(1), 31-40.

Hoffman, S. W., Fulop, Z. & Stein, D. G. (1994). Bilateral frontal cortical contusion in rats: Behavioral and anatomic consequences. *Journal of Neurotrauma, 11*(4), 417-431.

Hsu, J.E. & Jones, T.A. (2006). Contralesional neural plasticity and functional changes in the less-affected forelimb after large and small cortical infarcts in rats. *Experimental Neurology, 201*, 479-494.

Kandel, E. R. (1991). Disorder of mood: Depression, mania, and anxiety disorders. *Principles of Neuroscience*, 3rd ed. (pp. 869-883). New York: Elsevier.

Kreutzer, J. S., Seel, R. T., & Gourley, E. (2001). The prevalence and symptom rates of depression after traumatic brain injury: A comprehensive examination. *Brain Injury, 15*, 563-576.

Lannoo, E., Colardyns, F., Jannes, C., & De Soete, G. (2001). Course of neuropsychological recovery from moderate-to-severe head injury: A 2-year follow-up. *Brain Injury, 15*, 1-13.

Loubinoux, I., Pariente, J., Boulanour, K. et al. (2002). A single dose of serotonin neurotransmission agonist enhances motor output: Double-blind, placebo controlled, fMRI study in health subjects. *Neuroimage, 15*, 26-36.

Marmarou, A., Foda, M. A., van den Brink, W., Campbell, J., Kita, H., & Demetriadou, K. A. (1994). A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. *Journal of Neurosurgery, 80*, 291-300.

Marz, P., Otten, U., & Miserez, A.R. (2007). Statins induce differentiation and cell death in neurons and astroglia. *Glia, 55*, 1-12.

Nahas, Z., Arlinghaus, K. A., Kotrla, K. J., Clearman, R. R., & George, M. S. (1998). Rapid response of emotional incontinence to selective serotonin reuptake inhibitors. *Journal of Neuropsychiatry Clinical Neuroscience, 10*, 452-455.

National Center for Injury Prevention and Control. (1999). *Traumatic brain injury in the United States: A report to Congress*. Atlanta: GA, Centers for Disease Control and Prevention.

Paxinos, G. & Watson, C. (2007). *The Rat Brain in Stereotaxic Coordinates,* 6th ed. (pp. 8-19). Burlington, MA: Elsevier.

Pekny, M. & Nilsson, M. (2005). Astrocyte activation and reactive gliosis. *Glia, 50*, 427-434.

Perino, C., Rago, R., Cicolini, A. Torta, R., & Monaco, F. (2001). Mood and behavioral disorders following traumatic brain injury: Clinical evaluation and pharmacological management. *Brain Injury, 15*, 139-148.

Powell, J. H., Al-Adawi, S., Morgan, J., & Greenwood, R. J. (1996). Motivational deficits after brain injury: Effects of bromocriptine in 11 patients. *Journal of Neurology, Neurosurgery, & Psychiatry, 60*, 416-421.

Rapp, S., Baader, M., Hu, M., Jennen-Steinmetz, C., Henn, F.A., & Thome, J. (2004). Differential regulation of synaptic vesicle proteins by antidepressant drugs. *The Pharmacogenomics Journal, 4*, 110-113.

Rosenthal, M., Christense, B. K., & Ross, T. P. (1998). Depression following traumatic brain injury. *Archives of Physical Medicine and Rehabilitation, 79*, 90-103.

Rutland-Brown, W., Langlois, J. A. & Thomas, K. E. (2006). Incidence of traumatic brain injury in the United States, 2003. *Journal of Head Trauma Rehabilitation, 21*(6), 544-548.

Sbordone, R. J., Liter, J. C., & Pettler-Jennings, P. (1995). Recovery of function following severe traumatic brain injury: A retrospective 10-year follow-up. *Brain Injury, 9,* 285-299.

Schlund, M. W. (2002). The effects of brain injury on choice and sensitivity to remote consequences: Deficits in discriminating response-consequence relations. *Brain Injury, 16*(4), 347-357.

Seliger, G. M., Hornstein, A., Flax, J., Herbert, J., & Schroeder, K. (1992). Fluoxetine improves emotional incontinence. *Brain Injury, 6,* 267-270.

Singh, A., Black, S.E., Herrmann, N., Leibovitch, F.S., Ebert, P.L., Lawrence, J., & Szalai, J.P. (2000). Functional and neuroanatomic correlations in poststroke depression: The sunnybrook stroke study. *Stroke, 29,* 637-644.

Sloan, R. L., Brown, K. W., & Pentland, B. (1992). Fluoxetine as a treatment for emotional liability after brain injury. *Brain Injury, 6,* 315-319.

Smith, J. S., Fulop, Z. L., Levinsohn, S. A., Darrell, R. S., & Stein, D. G. (2000). Effects of the novel NMDA receptor antagonist gacyclidine on recovery from medial frontal cortex contusion injury in rats. *Neural Plasticity, 17*(1-2), 73-91.

Thurman, D., Alverson, C., Dunn, K., Guerrero, J., & Sneizek, J. (1999). Traumatic brain injury in the United States: A public health perspective. *Journal of Head Trauma Rehabilitation, 14*(6), 602-615.

Tsuiki, A., Takada, A., Nagahiro, S., Gridisa, M., & Diksic, M. (1995). Synthesis of serotonin in traumatized rat brain. *Journal of Neurochemistry, 64,* 1319-1325.

van der Hart, M. G., de Biurrun, G., Czeh, B., Rupniak, N.M., den Boer, J. A., & Fuchs, E. (2005). Chronic psychosocial stress in tree shrews: Effect of the substance P (NK1

receptor) antagonist L-760735 and clomipramine on endocrine and behavioral parameters. *Psychopharmacology, 181,* 207-216.

Van Reekum, R., Cohen, T., & Wong, J. (2000). Can traumatic brain injury cause psychiatric disorders? *Journal of Neuropsychiatry Clinical Neuroscience, 12,* 316-327.

Warden, D. (2006). Military TBI during the Iraq and Afghanistan wars. *Journal of Head Trauma Rehabilitation, 21*(5), 398-402.

Wayne, M. C. (2007). Reperfusion injury in stroke. *eMedicine.* Retrieved on April 6, 2009, from http://emedicine.medscape.com/article/1162437-overview

Williams, B. R., Lazic, S. E., & Ogilvie, R. D. (2008). Polysomnographic and quantitative EEG analysis of subjects with long-term insomnia complaints associated with mild traumatic brain injury. *Clinical Neurophysiology, 119,* 429-438.

Windle, V. & Corbett, D. (2005). Fluoxetine and recovery of motor function after focal ischemia in rats. *Brain Research, 1044,* 25-32.

Yoshimoto, K., Kato, B., Ueda, S., et al. (2001). Dopamine and serotonin uptake inhibitors on the release of dopamine and serotonin in the nucleus accumbens of young and aged rats. *Mechanisms of Ageing and Development, 122,* 1707-1721.

Zec, R. F., Zellers, D., Belman, J., Miller, J., Matthews, J., Ferneau-Belman, D., & Robbs, R. (2001). Long-term consequences of severe closed head injury on episodic memory. *Journal of Clinical and Experimental Neuropsychology, 23,* 671-691.