

THE EFFECT OF MILD TRAUMATIC BRAIN INJURY ON CYTOKINE EXPRESSION IN
BRAIN TISSUE

BY

RYAN MICHAEL GREENE

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Kinesiology
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2011

Urbana, Illinois

Master's Committee:

Professor Jeffrey A. Woods, Director of Research
Professor Kenneth R. Wilund

Abstract

Previous research suggests that mild traumatic brain injury (mTBI) results in significant cognitive and behavioral learning deficit, as well as long-term depressive like behavior in mice. mTBI has been identified as a major initiating event in long-term depressive-like behavior and early fatality observed in soldiers returning from conflicts in Iraq and Afghanistan, as well as athletes in amateur and professional sport. Purpose- The purpose of the proposed study was to investigate brain tissue for the presence of proinflammatory cytokines as a potential mechanism to explain long-term depressive-like behavior seen in previous mTBI studies. Methods- Male ICR mice (n=16) were randomly assigned to one of 2 treatment groups: mTBI (n=8) and Sham (n=8). Mice were anaesthetized and given mTBI by dropping a weight similar to previously measured body weight to an area located between the ear and the eye from a height of two feet. Baseline behavioral data was measured for both treatment groups for 8 days prior to treatment. Following mTBI, at the 48 and 72 hour time point, Roto-rod testing was conducted to ensure any differences in baseline behavioral deficit in either treatment group was not do to motor cortex impairment. Mice were sacrificed 7 days following treatment and tissue was tested for presence of proinflammatory cytokines detected via RtPCR. Results- There was only a significant treatment effect seen in the expression of proinflammatory cytokine TNF- α . All other proinflammatory cytokine expression had a high degree of variability between both groups. Roto-rod data confirmed there was no motor cortex deficit involved in deficit in behavioral measurements gathered following treatment. Conclusion- These circumstantial data suggest that proinflammatory cytokines may play a role in instigating long-term depressive like behavior by infiltrating neurological tissue in individuals afflicted with mTBI, but the weight drop model

used to inflict mTBI was too mild to induce large scale significant changes in pro-inflammatory cytokines or long-term behavioral depression.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Jeff Woods, for his continue support of this research, particularly in the face of many setbacks. Having you as an advisor has been a very enjoyable, challenging, and enlightening experience. I now feel much more prepared to work independently and look at things from a scientific point of view as I move on to medical school. I'd like to thank Steve Martin, Brandt Pence, and Marc Cook for their scientific knowledge and insights, as well as helping me along the way since my undergraduate career. In my illustrious six years at the University of Illinois, I have accumulated many friends, had wonderful academic experiences, and some crazy spring lab meetings, all of which I will never forget.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION.....	1
CHAPTER 2: LITERATURE REVIEW.....	4
CHAPTER 3: METHODS.....	12
CHAPTER 4: RESULTS.....	16
CHAPTER 5: DISCUSSION.....	22
CHAPTER 6: LIMITATIONS.....	24
CHAPTER 7: FUTURE DIRECTIONS.....	25
CHAPTER 8: SUMMARY AND CONCLUSION.....	27
REFERENCES.....	29

CHAPTER 1: INTRODUCTION

Mild traumatic brain injury (mTBI) is the principal result from falls, acts of war and violence, and sport-related injury (McAllister, 1992). Presently, more than 5.3 million people are survivors of traumatic brain injuries. More than 75% of injuries considered mTBI occur mainly while individuals are participating in sporting and leisure activities (McAllister, 1992). Sports-related brain injuries occur to 1.6-3.8 million individuals annually. The total direct and indirect cost for these injuries has been projected to surpass 60 billion dollars (Elder & Cristian, 2009) (Aubry, et al., 2002). In addition, a recent study has suggested a large percentage of soldiers returning of Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) have or should be diagnosed with mTBI by the time they return from deployment (Elder & Cristian, 2009) (Vanderploeg, Belanger, & Curtiss, 2009) (Koren, Norman, Cohen, Berman, & Klein, 2005). It is believed that a large percentage of veterans returning from service who have suffered mTBI will experience long-term health deficit of some type and affect their inability to return to active service or effectively assimilate back into civilian society (Elder & Cristian, 2009) (Vanderploeg, Belanger, & Curtiss, 2009) (Koren, Norman, Cohen, Berman, & Klein, 2005).

A discrepancy encountered in the current mTBI literature is lack of a universally accepted definition of mTBI. For the most part, clinicians define mTBI as any injury that manifests itself by at least one of the following: (1) any period of loss of consciousness, (2) any loss of memory for events immediately before or after the incident, (3) any alteration of mental state at the time of the accident (dizziness, disorientation, confusion), and (focal neurological deficits, which may or may not be transient, but when the severity of the accident does not exceed the following; a) loss of consciousness of 30 minutes or less; (b) after 30 minutes, an

initial Glasgow Coma Scale score of 13-15; and (c) posttraumatic amnesia not greater than 24 hours (Weighill, 1983) (Slagle, 1990). Figure 1 describes the wide variety of diagnostic criterion that contribute to this discrepancy.

Depression After Mild Traumatic Brain Injury

Table I. Assessment of Mild Traumatic Brain Injury

Authors	Assessment
Novack <i>et al.</i> , 1984	PTA < 24 h
Saran, 1985	LOC < 20 min
Atteberry-Bennett <i>et al.</i> , 1986	GCS 13-15
Diamond <i>et al.</i> , 1988	LOC < 20 min
Saran, 1988	LOC < 20 min
Schoenhuber and Gentilini, 1988	LOC 20 min, GCS 13-15
Mobayed and Dinan, 1990	LOC < 20 min
Leininger <i>et al.</i> , 1991	LOC or PTA < 20 min, GCS 13-15
O'Carroll <i>et al.</i> , 1991	GCS 13-15
Alexander, 1992	LOC 15 min, PTA < 2h
Bohnen, Jolles <i>et al.</i> , 1992	LOC 15 min, GCS 13-15, PTA < 1 h
Bohnen, Twijnstra, and Jolles, 1992	LOC 15 min, GCS 13-15, PTA < 1 h
Dinan and Mobayed, 1992	LOC < 20 min
Fedoroff <i>et al.</i> , 1992	GCS 12-15
Jorge, Robinson, Arndt <i>et al.</i> , 1993	GCS 12-15
Gfeller <i>et al.</i> , 1994	LOC < 1 h
Fann <i>et al.</i> , 1995	LOC 30 min, GCS 13-15, PTA 24 h
King, 1996	PTA < 24 h
Parker and Rosenblum, 1996	LOC < 5 min

Figure 1. (Busch & Alpern, 1998)

It is proposed that most of the impairment in mental functions experienced post-mTBI have a common biological origin in the form of inflammation within the brain. It is believed that these deficits are exacerbated in stressful conditions such as combat (Busch & Alpern, 1998). Indeed, recent research has identified a role for brain proinflammatory cytokine expression in mediating changes in behavior and cognition (Milman, Rosenberg, Weizman, & Pick, 2005). An untested but likely mechanism for this post morbid increase in behavioral and cognitive disorders is the infiltration of pro-inflammatory cytokine action in the brain. The proposed mechanism involves cytokine-induced activation of critical tryptophan (Trp) catabolizing enzyme indoleamine 2,3-dioxygenase (IDO). IDO would theoretically convert Trp to a neurotoxic

quinolinic acid (QA), thus reducing serotonin synthesis and creating an imbalance between serotonin and QA. This would result in cognitive impairment and depressive like symptoms. Such effects have been shown before with work from our lab and others following mTBI (Milman, Rosenberg, Weizman, & Pick, 2005), but confirmation of any cytokine action in neurological tissue have yet to be discovered.

Purpose

It has been widely accepted that TBI, specifically mTBI, can lead to a host of physical, cognitive, and emotional deficits both acutely following insult, as well as long term depressive like behavior. The current study embraces a multidisciplinary mechanistic approach to understanding the symptom burden experienced by people suffering from mTBI to examine if pro-inflammatory cytokine expression in brain tissue may in fact be a culprit in depressive like behavior seen in time points far removed from mTBI.

Specific Aim

To examine the effect of mTBI administered to mice and the potential expression of pro-inflammatory cytokine expression in brain tissue that may be the cause of depressive like behavior previously seen following mTBI.

Hypothesis

Following mTBI, expression levels of pro-inflammatory cytokines in brain tissue will be significantly elevated compared to non-mTBI controls.

CHAPTER 2: LITERATURE REVIEW

mTBI is a result from a direct or indirect force applied to the head that produces neuropathologic changes to brain tissue (Aubry, et al., 2002). Despite the lack of structural changes to cerebral tissue, the functional alterations in brain tissue may manifest in a variety of phenotypes (Giza & Hovda, 2001) (Fann, Katon, Uomoto, & Esselman, 1995). Those who have suffered from mTBI commonly report deficits in the ability to maintain balance and exhibit decreases in neurocognitive functioning (Broglia & Puetz, 2008).

Even mild trauma to the brain can produce neuropathologic changes similar, if not greater in quantity in some cases, to traumatic brain injuries (McBeath & Nanda, 1994). Many studies have investigated damage to the brain after concussion and recent research has contradicted previous belief in the sense that the greater amount of damage in mTBI cases is not a result of direct impact to cranial bones, but rather inertial forces on the brain (acceleration and deceleration within the cranium) as a result of an impact (Bond, 1984). Research has shown that direct impact is not necessary for significant brain damage to occur seen in many mTBI cases. Previous research has shown that uniform compressive stresses are well tolerated by neural tissue. Shearing forces can fracture and stretch axons in the brain to the point that cell bodies of axons are compromised (Cantu, 1992). This disruption of axon cell bodies can interrupt blood flow to the brain due to tearing of blood vessels leading to significantly greater brain damage (Cantu, 1992). Povlishock and Coburn (1989) speculate that diffuse axonal damage in subjects that have suffered significant head injury is the main contributor to the disconnection seen between the Central Nervous System (CNS) and many peripheral organs, including endocrine, which constitute the morphological abnormalities seen in both behavioral and typical baseline functioning following mTBI.

The impact on neurocognitive dysfunction and motor function deficit are well documented. The literature leading to present day understanding has described that a decrease in performance in either area are believed to be the result of divergent physiological mechanisms rather than similar (McAllister, 1992).

The focus of this review will outline the mechanisms by which mTBI effects cognitive and motor, behavior, and endocrine function with hopes to establish a link between mTBI related deficits and the inflammatory effects potentially present in brain tissue that may lead to previously described depressive like behavior following neurological insult such as mTBI.

mTBI and motor function deficit

The impact of mTBI on cognitive and motor function has been well documented. It has been previously established that individuals with decreased cognitive functioning also demonstrate decreased motor function, mainly seen as a deficit in locomotor and postural control (Guskiewicz, et al., 2003). The dichotomy exists in the fact that the decrease in performance in both areas have been previously described as separate processes, but increasing evidence is now suggesting that the separation of cognitive and motor function in fact share the same neural mechanisms (Guskiewicz, et al., 2003) (De Monte, 2005). The association between cognitive and motor functioning deficit due to mTBI has been strengthened by research examinations of dual task performance. Catena et al. (2007) showed that there is a profound correlation in deficit seen in motor and cognitive functioning. Catena showed that the addition of a secondary cognitive task when paired with a motor function task increased the gait instability in mTBI patients when compared to healthy controls (Catena, van Donkelaar, & Chou, 2007). This deficit

was contributed to a decrease in attentional resources towards the motor function task due to mTBI.

De Monte et al. (2005) was one of the early studies that drew a correlation between the mechanism for motor and cognitive deficit following mTBI. This study was aimed to investigate the acute effects of mTBI on a motor performance (finger tapping) and word repetition dual task in order to determine working memory impairment in mTBI (De Monte, 2005). Patients with mTBI completed fewer correct taps in 10 seconds as well as fewer correct repetitions of words asked to be memorized than patients with non-mTBI related injuries. These results confirmed the view that there may be a significant correlation between motor and cognitive functioning most notably linked following mTBI.

In 2008, Broglio et al. conducted a study to investigate whether mTBI resulted in a change in association between motor and cognitive function. This approach differed from the literature discussed previously because Broglio et al. examined motor and cognitive functioning following mTBI together rather than separately. They hypothesized that there would be a link between cognitive and motor control functioning in individuals who suffered from mTBI. In order to assess this correlation, Broglio et al. tested cognitive and motor function pre and post mTBI by examining 36 athletes prior to and 24 hours post mTBI insult. From this investigation, Broglio et al. (2008) reported there was in actuality a significant association between cognitive and motor function indices following mTBI. In the non-injured state, Broglio et al. found that motor and cognitive functionality operated independently of one another. Following cerebral insult, the functional capacity of both processes appeared strongly associated.

Based on these findings, Broglio et al. (2008) concluded that mTBI adversely affects dual task performance. This may be attributed to the belief that mTBI reduces the finite attentional resources available to perform simultaneous tasks.

mTBI and behavior

Few studies to date have examined the relationship between behavioral and psychological effects caused by mTBI (Fann, Katon, Uomoto, & Esselman, 1995). The pathological conditions that occur most often following mTBI are often grouped together and discussed under the label of Post-concussion Syndrome (PCS) (Mittenberg, Zielinski, & Fichera, 1993). Most commonly reported symptoms are differentiated into either physical, cognitive, or psychological in nature. Depression lasting 3-9 months following insult is the most frequently reported symptom following mTBI. Unfortunately, many individuals who suffer from such depressive-like behaviors or PCS often go undiagnosed for several reasons: (1) many patients are sent home from the hospital or emergency room following mTBI and are never contacted again for follow up treatment; (2) patients may be considered to be over-expressing symptoms to deceive and acquire medication and (3) many symptoms of depressed patients may be less pronounced in some mTBI individuals compared to others due to an overall personality flattening (Mittenberg, Zielinski, & Fichera, 1993) (Fann, Katon, Uomoto, & Esselman, 1995) (Catena, van Donkelaar, & Chou, 2007).

Alexander (1992) compared a group of individuals who suffered mTBI to baseline non-mTBI controls and found that individuals in the mTBI groups (59%) complained of poor concentration, memory, and continual depressed mood as well as a significant deficit in

cognitive functionality. Alexander also found that 39% of mTBI patients showed significant psychomotor retardation.

Ettlin et al. (1992), in a study of individuals suffering from mTBI following motor vehicle accidents found that 42% of mTBI patients reported symptoms of depression in overall mood, loss of energy, and a significant lack of motivation to perform routine daily activities.

Previous work has also shown that depression or depressive like symptoms following mTBI has been linked to many behavioral deficits compared to pre-mTBI functioning that can lead to disability, loss of work, guilt, anger, pain, chronic frustration, and lifestyle changes (Gfeller, Chibnall, & Duckro, 1994). A relationship between cognitive deficit and incidence of depression following mTBI has also been elucidated. This previous research has lead to the conclusion that mTBI may be a triggering event for a set of pathophysiological changes and a greater likelihood of concomitant depressive episodes in the mTBI population compared to normal individuals (Gfeller, Chibnall, & Duckro, 1994).

Researchers also hypothesize that the shearing forces that occur during a mTBI may produce a stem of anatomical, physiological, and chemical changes that may evoke behavioral pathology so that the stressors that may not ordinarily trigger a pathological state may then have the ability to do so but the mechanisms are not yet well defined. mTBI may then be able to produce a direct pathological state from stressors associated with mTBI, such as musculoskeletal pain, inflammation, and cognitive dysfunction. Stressors that did not previously have potent enough effect to have significant psychological effect may then induce depression in mTBI sufferers (Gfeller, Chibnall, & Duckro, 1994).

In 2005, Milman et al. described the effect of mTBI on persistent cognitive and behavioral deficit. Their work further reiterated the belief that deficits in learning and memory are common repercussions of mTBI in both humans and mice. His work reflected these findings by exhibiting impairments in memory and learning assessed by passive avoidance and swim T-maze tasks. Mice that suffered mTBI showed worse performance than control mice in the passive avoidance task. Interestingly, there seemed to be a delay in the onset of deficit in this memory task of nearly 30 days. In the forced swim test, behavioral abnormality could be seen as soon as 7 days and as long as 69 days following injury. These results suggest that following mTBI, there is a tendency to develop a depressive-like state, possibly leading to earlier fatigue in injured animals and significant memory deficit.

This work reflected similar preliminary findings from unpublished data from our lab, as seen in figures 2 and 3 below, regarding behavioral deficit following mTBI conducted prior to beginning the current experiment. Taste Preference Testing was used to measure actual intake of sucrose-containing liquid compared to water, which is a symptom of depressive-like behavior, called anhedonia.

Other findings found in unpublished data from our lab similar to research described previously by Millman et al. (2005) are presented in Figure 2 below to certify that the model we used to inflict mTBI in this experiment would result in cognitive deficit expressed by Morris Water Maze (MWM). Figure 3 expresses deficit in passive avoidance tasks such as the Tail Suspension Test (TST) found in unpublished data from our lab, similar to findings in Millman et al. (2005).

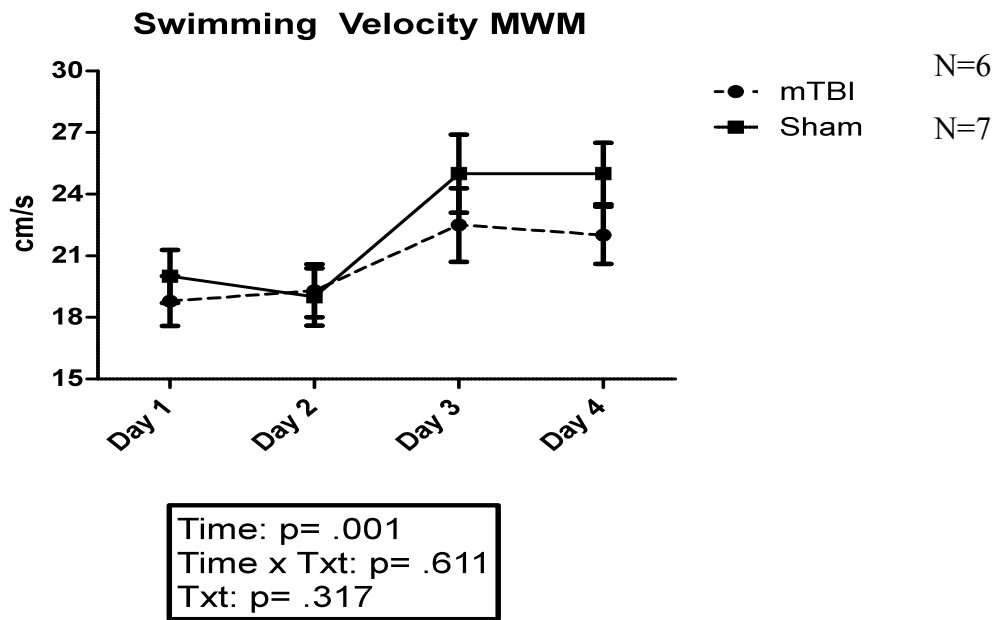


Figure 2. Morris Water Maze average swimming velocity at time points following mTBI treatment

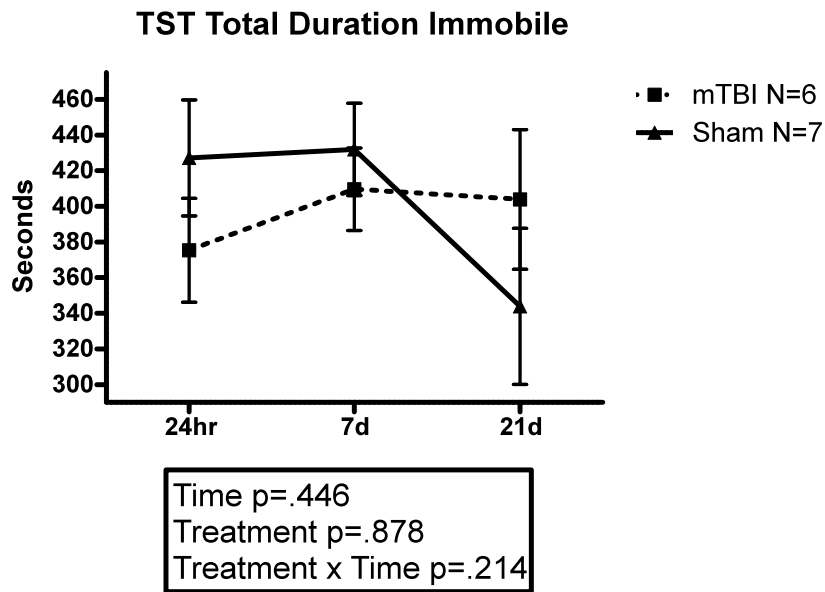


Figure 3. Tail Suspension Test duration immobile at time points following mTBI treatment

From this work by our lab and others mentioned previously, we are confident that mTBI leads to deficit in behavioral tasks and a mechanism to describe such deficit should be investigated further.

CHAPTER 3: METHODS

Study Design

This study addressed the effects of mTBI on pro-inflammatory cytokine expression in the brain and its correlation with behavioral deficits seen in previously published experimentation (Milman, Rosenberg, Weizman, & Pick, 2005). Male ICR mice (n=16), aged 8 weeks were used for this study. After approximately one week of acclimation, the mice were randomized into two groups. The groups consisted of experimental (mTBI) subjects (n=8) and control (Sham) subjects (n=8). Following acclimatization period, each mouse was implanted with a mini-mitter probe (Mini-Mitter Vital View) into the peritoneal cavity of the mouse as a tool to accurately track daily cage activity. Daily wheel running data was also collected. Following 10 days of recovery following surgical implantation and baseline cage activity data collection, mTBI was administered to each member of the mTBI group using a protocol developed by another lab (Zohar, Schreiber, Getslev, Schwartz, Mullins, & Pick, 2003), while the Sham group was led through the same procedure without mTBI insult being administered. 48 hours following mTBI, mice were subjected to Roto-rod testing to ensure that any deficit in daily cage activity was not due to motor function damage as a result of mTBI administration but rather to lack of movement motivation. Eight days following mTBI, each mouse was sacrificed. Tissues were harvested immediately after sacrifice, weighed, flash frozen and stored for later analysis or pro-inflammatory cytokine gene expression.

Animals and Diet

Male ICR mice (n=16) were purchased from Jackson Laboratories (Bar Harbor, ME). All groups were allowed to eat ad libitum and food intake and body weight were recorded once

per week throughout the duration of the study. Strict guidelines for the care and use of laboratory animals as directed by the National Institute of Health were followed and all experiments were approved by the Institutional Animal Care and Use Committee and supervised by the Division of Animal Resources at the University of Illinois at Urbana-Champaign.

Mini-mitter Implantation

Mini Mitter Vital View probes were purchased from the Mini-Mitter Company, Inc. (Bend, OR). Prior to surgery, mice were anesthetized with a sodium ketamine hydrochloride/xylazine hydrochloride solution (1mg and .1 mg/10g BW, ketamine:xylazine) injected intraperitoneally. A toe pinch was used to determine if the quality of the anesthetic was adequate. When the mice did not respond to the toe pinch, the quality of anesthetic was deemed adequate. The surgical area was then cleansed with 70% ethanol and covered with a clean drape. Surgeons wore sterile gloves, a mask, and a clean gown and used sterile instruments. Post surgical care included monitoring the mice every 15 minutes until the mice demonstrated alertness and mobility. If after six hours a mouse remained unresponsive, the mouse was euthanized by CO₂ asphyxiation.

Measurement of Daily Cage Activity and Wheel Running

Following mini-mitter implantation, mice were housed in individual cages, which were placed on receiver/energizer pads to allow tracking of their daily cage activity and wheel running via mini-mitter. Data was sent wirelessly to a computer situated in the room housing individual mouse cages. Data was collected daily to observe mouse kinetics and to ensure energizer pads and wheels were accurately collecting data.

Roto-rod Testing

To assess sensorimotor ability, mice were tested using a Roto-rod apparatus (Kinder Scientific, Poway, CA). Mice were placed on an elevated rod (7.0 mm in diameter) initially rotating at 4 rpm. Every 15 s, the rod was accelerated by 15 rpm. Fall latency was recorded by timers, which stopped when the mouse broke the photo-beams at the bottom of the chamber. Mice received three trials per day for two subsequent days.

Cytokine Gene Expression Analysis

mTBI related cytokine production of Interleukin 6 (IL-6), Interleukin 1-beta (IL-1 β), and Tumor Necrosis Factor-Alpha (TNF- α) were measured using Qiagen RNEasy mini kit. All reverse transcriptase (RT) reactions were performed using an Ambion (cat#1710) reverse transcriptase kit according to manufacturer instructions, using 1 microgram total RNA and random decamer primers for each reaction. All RNA samples were reverse transcribed simultaneously to minimize interassay variation associated with reverse transcription reaction.

Real-time RT-PCR was performed on an Applied Biosystems Prism 7900 using Taqman gene expression assays for TNF α (cat # Mm00443258_m1), IL-1 β (cat # Mm00434228_m1) and IL-6 (cat # Mm00801778_m1), purchased from Applied Biosystems. Reactions were performed in duplicate according to manufacturer instructions using 125 ng of cDNA template for each reaction. Relative quantitative measurement of target gene levels was performed using the $\Delta\Delta C_t$ method, where C_t is the threshold concentration. GAPDH was used as the endogenous housekeeping control gene.

Statistics/Data Analysis

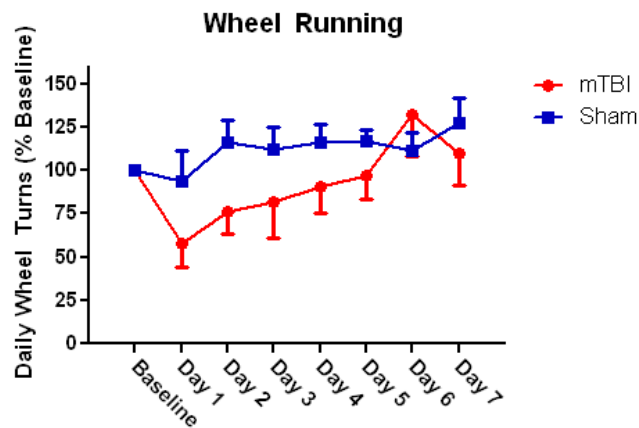
Cytokine expression levels were analyzed using a students t-test of variance; post-hoc Tukey HSD comparisons were used to determine individual group differences when a significant

F ratio was obtained. Differences in Roto-Rod testing data were determined using students t-test with post hoc Tukey comparisons where appropriate. All analyses were done using SPSS version 16.0 (Chicago, IL) and data presented as mean \pm SEM. Alpha level for main effects were set at $p \leq 0.05$.

CHAPTER 4: RESULTS

mTBI treatment induces reduced locomotor activity following treatment

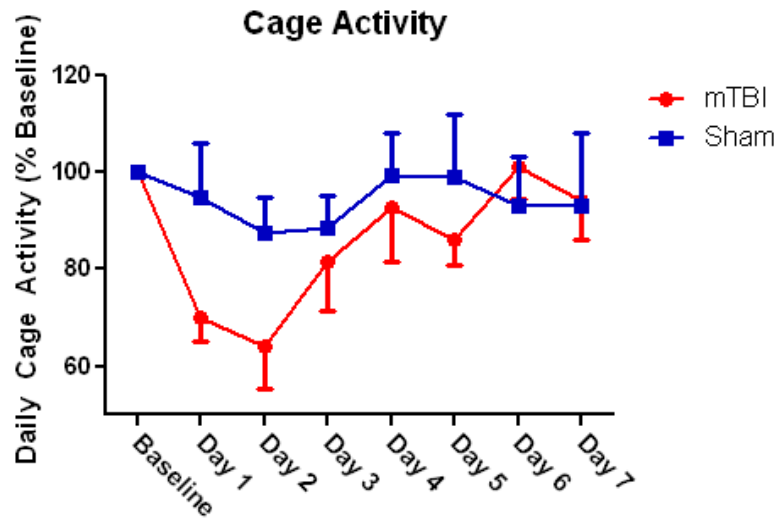
Based on data collected from mini-mitter transmission, the mTBI treatment group had a reduction in overall daily cage activity and wheel running distance (determined by percentage of baseline, pre-treatment, wheel turn values) following mTBI treatment indicating depressive-like behavior. The difference in wheel running distance ($F_{14}=6.3$) and daily cage activity ($F_{14}=5.1$) be seen graphically in Figure 4 and Figure 5 respectively.



N=8/group
Treatment Main Effect: $p=0.066$

Time $p=.011$
Treatment $=.610$
Treatment X Time $p=.290$

Figure 4. Daily wheel turns collected by treatment group for 7 days following treatment. Data expressed as a percentage of baseline daily wheel turns.



N=7/mTBI; 6/sham
Treatment Main Effect: $p=0.082$

Time $p=.446$
Treatment $p=.878$
Treatment X Time $p=.214$

Figure 5. Daily cage activity of both treatment groups collected by previously implanted mini-mitter. Data expressed as percentage of baseline cage activity collected prior to experimental treatment

Though a difference was seen between the two treatment groups, the difference was not statistically significant. Figure 6 illustrates that there was no correlation between cytokine expression and wheel running behavior at any day following treatment.

Correlations

		DeltaCTIL6	Wheelrunning	DeltaCTIL1B	DeltaCTTNFA
DeltaCTIL6	Pearson Correlation	1	.093	.686**	.751**
	Sig. (2-tailed)		.763	.003	.001
	N	16	13	16	16
Wheelrunning	Pearson Correlation	.093	1	.197	.070
	Sig. (2-tailed)	.763		.520	.820
	N	13	13	13	13
DeltaCTIL1B	Pearson Correlation	.686**	.197	1	.869**
	Sig. (2-tailed)	.003	.520		.000
	N	16	13	16	16
DeltaCTTNFA	Pearson Correlation	.751**	.070	.869**	1
	Sig. (2-tailed)	.001	.820	.000	
	N	16	13	16	16

** . Correlation is significant at the 0.01 level (2-tailed).

Figure 6. Correlation matrix comparing pro-inflammatory cytokine expression and daily wheel running values.

Reduced locomotor activity seen following mTBI treatment not due to motor-function deficit

Our data above and that of others reported that mice inflicted with mTBI demonstrated periods of significantly lower daily cage activity than non-mTBI cohorts following treatment (Milman, Rosenberg, Weizman, & Pick, 2005) (Zohar, Schreiber, Getslev, Schwartz, Mullins, & Pick, 2003). Roto-rod testing was conducted at 48 and 72 hour time periods respectively following mTBI treatment to determine if deficit in daily cage activity seen in the mTBI cohort was due to lack of desire to move similar to baseline cage activity or if mTBI treatment inflicted

physiological motor deficit that disabled the normal movement ability of the mouse. No significant difference in Roto-rod average ending speed of the rod or duration of time spent performing the task was observed between treatment groups. Data is represented graphically in Figure 7. This indicates that the mice were able to function normally and that they chose not to engage in wheel running or cage activity behavior.

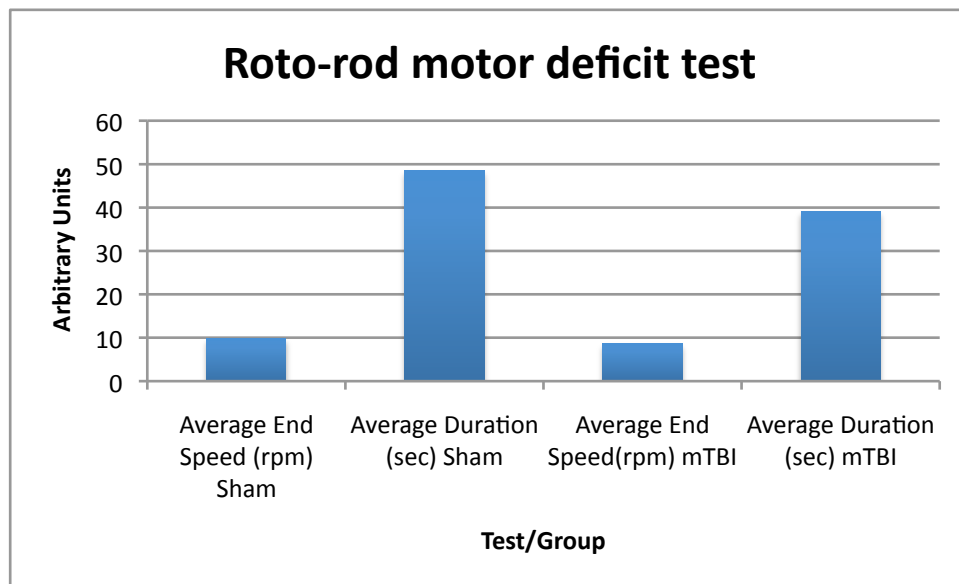
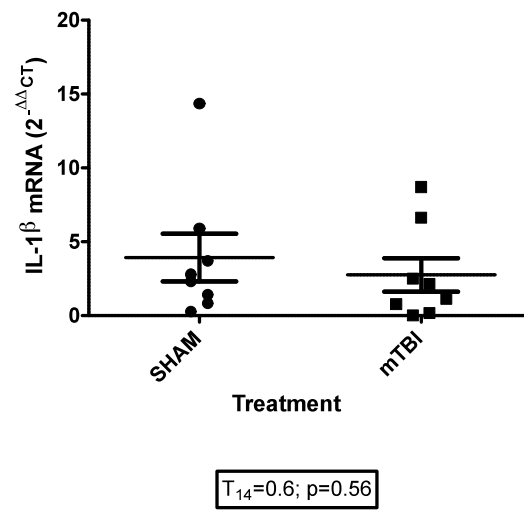


Figure 7. Average End Speed (revolutions per minute) and average Duration (seconds) before completion of Roto-rod test for each treatment group

Cytokine Expression Levels exhibit great variability between treatment groups and do not correlate with reduced activity

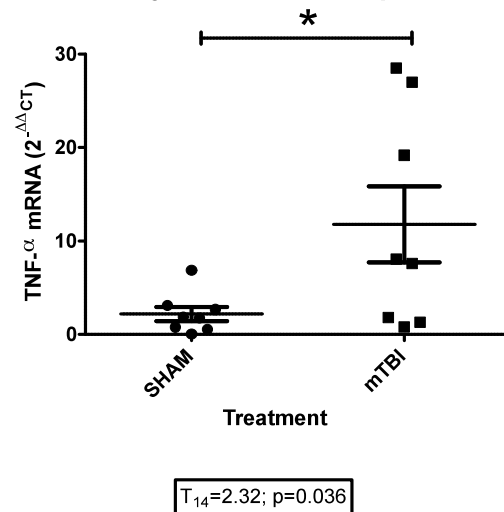
Following mTBI or SHAM treatment, only TNF- α was significantly expressed ($T_{14}=2.32$; $p=.036$) at higher levels in brain tissue in the mTBI treatment group compared to the SHAM control group. Significant variability was expressed in all groups when testing for the presence of all cytokines measured. Results for cytokine expression in the brain can be seen in Figure 8.

Effects of mTBI on IL-1 β mRNA expression in Injured Brain Hemisphere



a.

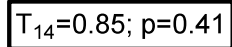
Effects of mTBI on TNF- α mRNA expression in Injured Brain Hemisphere



b.

Figure 8. a.) shows the cytokine expression level of IL1- β in brain tissue in mTBI and SHAM treated mice. b.) shows the cytokine expression of TNF- α in mTBI and SHAM treated mice. c.) shows cytokine expression of IL-6 in mTBI and SHAM treated mice.

21



21

CHAPTER 5: DISCUSSION

Findings

The most important finding of this study was that weight drop model of mTBI leads to highly variable expression of proinflammatory cytokines in brain tissue. Based on the data graphically represented in Figure 7, it is evident that there is a trend for up-regulation of pro-inflammatory cytokines in brain tissue, specifically TNF- α and IL-6, but substantial variability amongst subjects in both treatment groups only elicited significance for levels of TNF- α in brain tissue.

It has been well established that mTBI can lead to long-term behavior and cognitive deficit similar to depressive-like symptoms in mice (Zohar, Schreiber, Getslev, Schwartz, Mullins, & Pick, 2003) (Milman, Rosenberg, Weizman, & Pick, 2005). Encouragingly, we found mTBI treatment did have some effect on the test subjects, illustrated by the decrease in total wheel running behavior and daily cage activity, graphically depicted in Figure 4 and 5 respectively. Additionally, mTBI being the cause of the observed reduced motivation to run or move was further supported by Roto-rod testing, graphically depicted in Figure 6. These data further confirm the discrepancy in cage activity and total wheel running behavior was not caused by neurological motor function damage due to mTBI, but rather a chosen behavior.

Unfortunately, as can be seen in Figure 7, there was a great deal of variability in cytokine expression levels of subjects in both groups following treatment. It was expected that there might be a high degree of variability in cytokine expression levels amongst subjects in the mTBI treatment group. It was not expected to see high cytokine expression levels in the Sham

treatment group. These results indicate extenuating circumstances may have affected our results, or the proficiency of mTBI inflicted may not have been consistent or unreliable.

These data support our other data demonstrating that this mTBI model failed to induce long-term behavioral deficits like depressive-like behavior (data not shown). This was disappointing because in a published study, Milman and colleagues (2005) did demonstrate long-term depressive-like behavior (e.g. increased immobility in tail suspension and forced swim tests) in a weight drop model of mTBI. We have no explanation for the disparate findings as we fashioned our apparatus to mimic that of Milman et al.

CHAPTER 6: LIMITATIONS

There were a few limitations to this study. The primary limitation is the extensive variability of our findings in this weight drop model. It is our belief that the weight-drop model used in previous studies did not seem to reliably induce mTBI. To reliably induce mTBI, other groups have invested in hydraulic apparatus in which force and location of insult administered to the mouse can be more accurately regulated. Unfortunately, due to financial and time constraints, this more reliable model of administering mTBI was not feasible.

Another potential limitation to this study was small group numbers due to the loss of subjects in mTBI and sham experimental cohorts. Complications with mini-mitter implantation surgery or significant neurological damage inflicted during mTBI resulted in the necessity for a small number of mice to be sacrificed, thus reducing subject numbers in all groups for our purposes.

CHAPTER 7: FUTURE DIRECTIONS

If future inquiries into the mechanism tying mTBI to long-term depressive like behavior determine that pro-inflammatory cytokines do infiltrate neural tissue and lead to modulations in behavior, cognition, and memory, the next step will be to determine the mechanism by which this response can be attenuated for more rapid recovery time for individuals who have suffered mTBI.

Recent literature investigating the effects of Traumatic Brain Injury (TBI) has revealed progesterone may have a significant effect in reducing the inflammatory response following TBI (Bhagia, et al., 2010). Sex-differences in patients with TBI in pre-menopausal women show significantly lower plasma cytokine levels compared with age-matched men. It is hypothesized that these sex differences in response to TBI are attributable to higher circulating levels of progesterone in female subjects (Bhagia, et al., 2010).

Recent publications have demonstrated effectiveness of progesterone in experimental models of TBI and may be applicable to mTBI (Frank, Pape, van Griensven, Krettek, Chaudry, & Hildebrand, 2007) (Malone, Kuhls, Napolitano, McCarter, & Scalea, 2001). Research has consistently shown that post-injury administration of progesterone can attenuate the cytological, morphological, and functional behavioral and cognitive deficits caused by TBI. One reason for increased survival in progesterone treated TBI patients may lie in the ability for progesterone to substantially reduce systemic acute-phase inflammation. Frank et al. (2007) found that contusion injury to the cerebral cortex in rats induced expression of IL-1 β and TNF- α in the intestinal mucosa, followed by apoptosis of cells in the intestinal mucosa. A 5-day course treatment with P4 reduced both cytokines and cell death in the intestine, demonstrating the systemic benefits of

progesterone administration following TBI and may be as effective if supplemented following mTBI (Frank, Pape, van Griensven, Krettek, Chaudry, & Hildebrand, 2007) (Singh, 2006).

Progesterone is believed to have the ability to reduce inflammation after injury by inhibiting pro-inflammatory Th1 response and also by stimulating anti-inflammatory Th2 immune response (Stein, Wright, & Kellermann, 2008) (Kidd, 2003). These mechanisms are based on the fact that Th1/Th2 differentiation marks a decisive event very much like phase transition in the development of extended inflammation following insult to the periphery (Kidd, 2003) (Singh, 2006). This reduction in systemic inflammation may be one of the key mechanisms by which progesterone significantly increases survival after TBI in human subjects. Because TBI is essentially a massive physiological insult that leads to extreme over-activation of immune defense response, the introduction of stabilizing factors such as progesterone may allow for a reduction of this damage and increase an individuals ability to recover and may be similarly beneficial in mTBI (Stein, Wright, & Kellermann, 2008).

CHAPTER 8: SUMMARY AND CONCLUSION

Due to the increased prevalence and numerous health risks that accompany mTBI, understanding the causes and underlying physiological mechanisms that lead to long term depressive-like behavior as well as how to accurately diagnose and treat mTBI victims continues to be a high priority in current healthcare research. Currently, researchers and clinicians have yet to settle on a set of diagnostic criteria that can universally be considered sufficient to diagnose an individual who has suffered a mTBI. Recent research has identified a role for brain proinflammatory cytokine expression in mediating changes in behavior and cognition but the mechanistic pathway by which such cytokines may alter cognition and behavior has yet to be described.

It has been well established that mTBI causes deficits in cognitive functioning as well as memory and motor behavior often portrayed as depressive-like symptoms (Milman, Rosenberg, Weizman, & Pick, 2005) (Mittenberg, Zielinski, & Fichera, 1993) (Ettlin, et al., 1992) (Alexander, 1992). Previous research has lead to the conclusion that mTBI may be a triggering event for a set of pathophysiological changes and such pathophysiology can be attributed to the presence of proinflammatory cytokines in the periphery (Ettlin, et al., 1992). In addition, deficit in cognitive and behavioral learning have been identified as side effects of mTBI, likely acting through presence of proinflammatory cytokines in the periphery, similar to the effects of gram negative pathogen introduction to the physiological system (Mobayed & Dinan, 1990) (Povlishock & Coburn, 1989). Given this, investigating and trying to determine the mechanism by which long-term depressive-like behavior is produced by examining neurological tissue for the presence of proinflammatory cytokines seemed like the best course of action.

Data from this experiment suggest that mTBI does cause acute loss of motivation to perform movement 2-4 days following treatment, similar to previous unpublished data from our lab and published work by other groups. Additionally, findings from this study seem to indicate that cytokines may be up-regulated in neurological tissue based on the significant increase ($F_{14}=2.32$; $p=.036$) in TNF- α in the mTBI treatment group compared to Sham, but the variability of expression levels of all subjects, in both treatment groups, leads to unreliable findings.

If studies in the future do in fact show up-regulation of proinflammatory cytokines in neurological tissue, further examination into treatment and preventative measures against mTBI are warranted. Specifically, an area worth researching would be with the supplementation of Progesterone before or after mTBI insult. Previous research has shown that women, who naturally have higher levels of Progesterone expressed, may in fact have a protective evolutionary advantage against long-term side effects associated with mTBI.

In conclusion, mTBI has a significant effect on inducing depressive-like symptoms in subjects affecting both cognitive and behavioral learning tasks, as well as initiating depressive-like behavioral long-term following mTBI. The mechanism by which such behaviors are caused have yet to be elucidated. More research is needed to understand this process.

REFERENCES

- Alexander, M. P. (1992). Neuropsychiatric correlates of persistent postconcussive syndrome. *Journal of Head Trauma Rehabilitation* , 1, 50-69.
- Aubry, M., Cantu, R., Dvorak, J., Graf-Baumann, T., Johnston, K., Kelly, J., et al. (2002). Summary and agreement statement of the first international conference on concussion in sport. *Journal of Sports Medicine* , 36, 6-7.
- Baker, S. P., O'Neill, B., Haddon, W., & Long, W. B. (1974). A method for describing patients with multiple injuries and evaluating emergency care. *Journal of Trauma Research* , 14 (3), 187-196.
- Bhagia, V., Gilkison, C., Fitts, R. H., Zgaljardic, D. J., High Jr., W. M., Masel, B. E., et al. (2010). Effect of recombinant growth hormone replacement in a growth hormone deficient subject recovering from mild traumatic brain injury: A case report. *Brain Injury* , 24 (3), 560-567.
- Bond, M. (1984). The psychiatry of closed head injury. *Closed Head Injury: Psychological, Social, and Family consequences* , 1, 44-73.
- Broglio, S. P., & Puetz, T. W. (2008). The effect of sport concussion on neurocognitive function, self-report symptoms, and postural control: a meta-analysis. *Journal of Sports Medicine* , 42, 502-508.
- Broglio, S. P., Sosnoff, J. J., & Ferrara, M. S. (2008). Cognitive and motor function are associated following mild traumatic brain injury. *Experimental Brain Research* , 187, 563-571.
- Busch, C. R., & Alpern, H. P. (1998). Depression After Mild Traumatic Brain Injury: A Review of Current Research . *Neuropsychology Review* , 8, 95-108.
- Cantu, R. C. (1992). Cerebral concussion in sport. *Journal of Sports Medicine* , 14, 64-74.
- Catena, R. D., van Donkelaar, P., & Chou, L. S. (2007). Altered balance control following concussion is better detected with an attention test during gait. *Experimental Brain Research Review* , 176, 23-31.
- De Monte, V. E. (2005). The acute effects of mild traumatic brain injury on finger tapping with and without word repetition. *Journal of Clinical and Experimental Neuropsychology* , 27, 224-39.
- Elder, G. A., & Cristian, A. (2009). Blast-related mild traumatic brain injury: mechanisms of injury and impact on clinical care . *Mt. Sinai Journal of Medicine* , 76, 111-118.
- Ettlin, T. M., Kischka, U., Reichmann, S., Radii, E., Heim, S., Wenden, D., et al. (1992). Cerebral symptoms after whiplash injury of the neck: A prospective clinical and

neuropsychological study of whiplash injury. *Journal of neurology, Neurosurgery, and Psychiatry* , 55 (943-948).

Fann, J. R., Katon, W. J., Uomoto, J. M., & Esselman, P. C. (1995). Psychiatric disorders and functional disability in outpatients with traumatic brain injuries. *American journal of Psychiatry* , 152, 1493-1499.

Fortney, S. M. (1984). Effect of hyperosmolality on control of blood flow and sweating. *J. Appl. Physiol* , 57, 1688-1695.

Frank, M., Pape, H. C., van Griensven, M., Krettek, C., Chaudry, I. H., & Hildebrand, F. (2007). Influence of sex and age on MODS and cytokines after multiple injuries. *Shock* , 27, 151-156.

Gfeller, J. D., Chibnall, J. T., & Duckro, P. N. (1994). Postconcussion symptoms and cognitive functioning in posttraumatic headache patients. *Journal of Headache Research* , 34, 503-507.

Gibson, L., Gray, L. J., Bush, P. M., & Murphy, S. P. (2007). Progesterone for the treatment of experimental brain injury; a systematic review. *Brain* , 318-328.

Giza, C. C., & Hovda, D. A. (2001). The neurometabolic cascade of concussion. *Journal of Athletic Training* , 36, 228-235.

Grosswasser, Z., Cohen, M., & Kerren, O. (1998). Female TBI patients recover better than males. *Brain Injury* , 12, 805-808.

Guskiewicz, K. M., McCrea, M., Marshall, S. W., Cantu, R. C., Randolph, C., Barr, W., et al. (2003). Cumulative effects associated with recurrent concussion in collegiate football players: the NCAA concussion study. *JAMA* , 290, 2549-2555.

Kidd, P. (2003). Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern Med Rev* , 8, 223-246.

Koren, D., Norman, D., Cohen, A., Berman, J., & Klein, E. M. (2005). Increased PTSD risk with combat-related injury: A matched comparison study of injured and uninjured soldiers experiencing the same combat events. *American Journal of Psychiatry* , 2 (162), 276-282.

Lin, W. W., & Karin, M. (20007). A cytokine-mediated link between innate immunity, inflammation, and cancer. *Journal of Clinical Investigation* , 117, 1175-83.

Lubert, S. (1995). *Biochemistry, 4th Edition*. W.H. Freeman and Company.

M, E., Davey, S. G., Schneider, M., & Minder, C. (1997). Bias in 600 meta-analysis detected by a single graphical test. *BMJ* , 315, 629-34.

Maci, H. R., Zaslavsky, K., Teixeira, C. M., & Frankland, P. W. (2009). What is the most sensitive measure of water maze probe test performance? *Frontiers in integrative Neuroscience* (3), 1-9.

Malone, D. L., Kuhls, D., Napolitano, L. M., McCarter, R., & Scalea, T. (2001). Back to basics: validation of admission systemic inflammatory response syndrome score in predicting outcome in trauma. *J Trauma* , 51, 458-463.

- Marguiles, S., & Hicks, R. (2009). Combination Therapies for Traumatic Brain Injury Workshop Leaders. Combination therapies for traumatic brain injury: prospective considerations. *Journal of Neurotrauma* , 26, 925-939.
- McAllister, R. W. (1992). Neuropsychiatric sequelae of head injuries. *Psychiatric Clinics of North America* , 15, 395-413.
- McBeath, J. G., & Nanda, A. N. (1994). Use of dihydroergotamine in patients with postconcussion syndrome. *Journal of Headache Pathology* , 34 (148-151).
- Milman, A., Rosenberg, A., Weizman, R., & Pick, C. G. (2005). Mild traumatic brain injury induces persistent cognitive deficits and behavioral disturbances in mice. *Journal of Neurotrauma* , 22, 1038-1046.
- Mittenberg, W., Zielinski, R., & Fichera, S. (1993). Recovery from mild head injury: A treatment manual for patients. *Psychotherapy in Private Practice* , 12, 37-52.
- Mobayed, M., & Dinan, T. G. (1990). Busiprone/prolactin response in post head injury depression. *Journal of Affective Disorders* , 19, 237-241.
- Nguyen, P. W., Abel, T., Kandel, E. R., & Bourtchouladze, R. (2000). Strain-dependent differences in LTP and hippocampus dependent memory in inbred mice. *Learning and memory* (7), 170-179.
- Porsolt, R. D. (1977). Behavioral despair in mice: a primary screening test for anti-depressants. *Arch Int Pharmacodyn Ther* , 229, 327-336.
- Povlishock, J. T., & Coburn, T. H. (1989). Morphopathological change associated with mild head injury. *Mild Head Injury* , 1, 37-53.
- Roof, R. I., Dudevani, R., Braswell, L., & Stein, D. G. (1994). Progesterone facilitates cognitive recovery and reduced secondary neuronal loss caused my cortical contusion injury in male rats. *Exp Neurol* , 129, 64-69.
- Saran, A. (1985). Depression after minor closed head injury: Role of dexamethasone suppression test and antidepressants. *The Journal of Clinical Psychiatry* , 46, 335-338.
- Schumacher, M., Guennoun, R., Stein, D. G., & De Nicola, A. F. (2007). Progesterone: therapeutic opportunities for neuroprotection and myelin repair. *Pharmacological Therapy* , 116, 77-107.
- Singh, M. (2006). Progesterone-induced neuroprotection. *Endocrine* , 29, 271-274.
- Slagle, D. A. (1990). Psychiatric disorders following closed head injury: an overview of biopsychosocial factors in their etiology and management. *International Journal of Psychiatry in Medicine* , 20, 1-35.
- Stein, D. G., Wright, D. W., & Kellermann, A. L. (2008). Does progesterone have neuroprotective properties. *Ann* , 51, 164-172.

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

Vanderploeg, R. D., Belanger, H. G., & Curtiss, G. (2009). Mild traumatic brain injury and posttraumatic stress disorder and their associations with health symptoms. *Archives of Physical Medical Rehabilitation* , 7 (90), 1084-1093.

Weighill, V. E. (1983). Compensation neurosis: A review of the literature. *Journal of Psychosomatic Research* , 27, 97-104.

Zohar, O., Schreiber, S., Getslev, V., Schwartz, J., Mullins, P., & Pick, C. (2003). Closed-head minimal traumatic brain injury produces long-term cognitive deficits in mice. *Neuroscience* , 118 (4), 949-55.