

2019

The development of a novel composite score to characterize effect size of behavior and histopathology changes after a repetitive mild traumatic brain injury

<https://hdl.handle.net/2144/36169>

"Downloaded from OpenBU. Boston University's institutional repository."

BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

**THE DEVELOPMENT OF A NOVEL COMPOSITE SCORE TO
CHARACTERIZE EFFECT SIZE OF BEHAVIOR AND HISTOPATHOLOGY
CHANGES AFTER A REPETITIVE MILD TRAUMATIC BRAIN INJURY**

by

ASHLEY N. CONLEY

B.A., Colby College, 2017

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science

2019

© 2019 by
ASHLEY N. CONLEY
All rights reserved

Approved by

First Reader

Carl Franzblau, Ph.D.
Professor of Biochemistry

Second Reader

Rebekah Mannix, MD, MPH.
Associate Professor of Pediatrics and Emergency Medicine
Harvard University, School of Medicine

ACKNOWLEDGMENTS

I want to thank Dr. Rebekah Mannix, Dr. William P. Meehan III, and Dr. Jianhua Qiu for their guidance and support throughout my research. Without the assistance and technical support from Nicholas Morriss and Grace Conley, this research would not have been possible. I would also like to thank my colleagues, Sara Ospina, and Oisín Joyce.

**THE DEVELOPMENT OF A NOVEL COMPOSITE SCORE TO
CHARACTERIZE EFFECT SIZE OF BEHAVIOR AND HISTOPATHOLOGY
CHANGES AFTER A REPETITIVE MILD TRAUMATIC BRAIN INJURY**

ASHLEY N. CONLEY

ABSTRACT

In this paper, we investigate the potential for the development of a composite score investigating population-level phenotype changes in a mouse model of traumatic brain injury. Traumatic brain injuries (TBI) are a growing concern in the United States because the number of individuals impacted by TBI and associated symptoms is increasing, leading to a growing demand for research both in the clinical and preclinical setting. However, preclinical TBI modeling is complicated by the lack of inter and intra lab consistency in the assessment of behavioral and pathologic outcomes. Indeed, it remains unclear which behavior assessments are most useful in evaluating the effects of preclinical TBI. To investigate the relative contribution of various behavior tests in the assessment of preclinical TBI, three statistical models (simple linear regression, pairwise correlation, and factor analysis) were conducted on behavioral data from the Mannix-Meehan lab at Boston Children's Hospital in Boston, Massachusetts, U.S.A. from 2012-2018. In this paper, a composite metric was created from the computation analysis of the three statistical methods. The score revealed MWM and EPM as the most potent behavioral tests. The Open Field and Rotarod test had a small impact on the outcome, but only in one of the three statistical models assessed. Thus, to effectively analyze treatment

efficiencies, injury severity and long-term impairments, MWM and EPM are the best behavioral test for a mouse model. Furthermore, this method of analysis of entire populations of mice allows for more subtle phenotypic changes resultant from injury models to be revealed, and the generalizability of this model lends to widespread use.

TABLE OF CONTENTS

TITLE.....	i
COPYRIGHT PAGE.....	ii
READER APPROVAL PAGE.....	iii
ACKNOWLEDGMENTS	iv
ABSTRACT.....	v
TABLE OF CONTENTS.....	vii
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF ABBREVIATIONS.....	xi
INTRODUCTION	1
Background	1
Concussion	2
Current preclinical research	5
SPECIFIC AIMS	7
MATERIALS and METHODS.....	8
Overall Description Independent Variables Assessed for Statistical Relevance.	8
Animals	9
Repetitive Mild Traumatic Brain Injury: Drop Weight Model	9

Behavioral Tests	10
Interventions	16
RESULTS	20
Tabulations of independent variables	20
Data analyzed for consistency	25
Factor analysis of Data Sets	31
Analysis	35
DISCUSSION	38
Composite Score	39
Future work	40
APPENDIX	43
REFERENCES	50
CURRICULUM VITAE	55

LIST OF TABLES

Table	Title	Page
1	Independent variables	8
2	Number of Observations for each behavioral variable	21
3	Tabulation of condition (CHI vs. SHAM) observations	22
4	Number of observations for each treatment by condition (CHI vs. Sham)	22
5	Number of observations by the weight drop height	23
6	Number of observations by height and injury model	24
7	Number of injuries per mouse by condition	24
8	Number of observations by condition (Sham vs. CHI) for each time post-injury.	25
9	Pairwise correlation with Bonferroni adjusted significance level	26
10	Cumulated STATA generated p-values from simple linear regressions.	29
11	Pairwise correlation between conditions (Sham vs. CHI) by behavioral test	31
12	Summary table of statistically significant factors and the corresponding behavioral tests	36
13	Composite ranking table of Simple Linear Regression, pairwise correlation and factor analysis.	37

LIST OF FIGURES

Figure	Title	Page
1	Bar graph of non-injured mice by treatment for pairwise correlation significant behavioral	27
2	Simple Linear Regression Stata output example	28
3	Group breakdown for each behavioral test included in the specific data set	32
4	Cumulative Factor table rankings	35

LIST OF ABBREVIATIONS

BU.....	Boston University
CDC	Center for Disease Control and Prevention
CHI.....	Closed Head Injury
CI.....	Cognitive Impairment
CRS.....	Cognitive-related Symptoms
EPM	Elevated Plus Maze
FDA.....	Food and Drug Administration
FST.....	Forced Swim Test
ImPACT	Immediate Post-Concussion Assessment and Cognitive Testing
LD	Light-Dark Box
LOC.....	Loss of Consciousness
MRI.....	Magnetic Resonance Imaging
MWM.....	Morris Water Maze
NIH	National Institute of Health
NO.....	Novel Object
NLR.....	Novel Location
OF	Open Field
PI.....	Post Injury
rmTBI.....	Repetitive Mild Traumatic Brain Injury
TBI	Traumatic Brain Injury

INTRODUCTION

Background

Traumatic brain injuries (TBI) are the primary contributing factor towards more than 30% of all injury related deaths, and have undeniably risen in the past decade (Faul 2010). The effects of TBI can be long-term with an estimated 1.7% of the United States population, roughly 3.2 to 5.3 million individuals, living with long-term disabilities as a result of TBI (Zaloshnja 2008). According to the Center for Disease Control and Prevention, during 2013 more than 2.5 million people were diagnosed with a Traumatic Brain Injury (TBI) at emergency departments within the United States, and every day almost 45 deaths are related to TBI injuries (Taylor 2017). The National Institute of Health reported an annual cost of 76 billion dollars in medical expenses and that best form of treatment is prevention (NIH).

There are many different mechanisms of TBI, such as car accidents, traumatic exposures from a military event, or a sports related injury. Furthermore, there is a broad range of symptoms and recovery from TBI due to differences in exposure, pre-disposed health conditions, and other inter-individual differences. TBIs are generally classified as “mild”, “moderate”, or “severe” based on the initial level of consciousness (CDC). The most common type of TBI, concussion, is generally classified as a “mild” injury, though a substantial minority of patients who suffer a concussion, up to 30%, still have symptoms one month after injury (Røe 2009).

Concussion

A concussion is classified as a TBI that results in brain movement within the skull that can cause both temporary and permanent biochemical alterations and cognitive impairment (CDC: Heads Up). Currently, concussions are mostly diagnosed based on subjective symptoms such as headaches, nausea, light sensitivity, confusion and depression. In the past decade, there has been heightened concern regarding both the increased incidence of concussion as well as the long-term effects of concussion. Furthermore, sustaining multiple concussions may delay symptom resolution and return to normal activity (McAllister 2017). These concerns are amplified by recent studies that suggest that concussion and repetitive concussion are associated with increased risk of neurodegenerative and autoimmune diseases (Gardiner 2015; Graham R 2014; Marchi 2013). These studies have led to a significant public health demand to better understand the biomolecular implications of a concussion better.

Unfortunately, the individual's biological response towards a concussion is not well understood and difficult to study in the clinical setting. While current organizations and professional sports leagues have created guidelines to diagnose and treat a concussion, the majority of the tests have high degrees of false negatives and false positives (CDC). Additionally, the majority of current tests are symptom-based and as a result, these tests are extremely subjective to interpersonal variability. The establishment of baseline testing before participation in contact sport helps determine an individual's baseline brain function, which can then be compared to a test administered after a potential post-concussive event (CDC: HEADS UP). This reduces the impact of

individual variability on test reliability. However, the test requires a participant to be truthful about his or her performance, which may not be the case if an individual purposefully underperforms during the baseline test in order to prevent a diagnosis of a concussion and thus resume play. Researchers examined The Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT) and found that individuals regularly performed below his or her best effort (Higgins 2018). Therefore, there is a need to find a quantitative evaluation for concussion diagnosis less subject to variability and tampering.

In addition to the lack of reliable, objective measures to study concussion, the tissue correlates to injury remain mostly unknown. Proxy measures such as serum or imaging biomarkers suggest brain tissue changes in response to injury but tissue correlates are not available to study the effects of injury (Lipton 2013). Much of what is known about the pathophysiology of concussion is derived from animal models (Giza 2014). In the case of repetitive concussion, most data are derived from case series of deceased athletes who donated their brains for neuropathologic interrogation after death. Many of these athletes were ultimately diagnosed post-mortem with chronic traumatic encephalopathy (CTE), defined as “an abnormal perivascular accumulation of tau in neurons, astrocytes, and cell processes.”

The first post-mortem brain analysis, published by pathologist Bennet Omalu in 2005, revealed evidence of CTE in Mike Webster, a professional NFL player’s autopsy (Omalu 2005). This discovery has sparked intensive and continuing research on the impact that brain injury may have on the development of CTE and the role that the tau

proteins play within the brain. A cohort of 202 samples from American Football players postmortem was collected and analyzed with every sample having at least one diagnosed concussion throughout his lifetime (Mez 2017). In the same study, CTE was diagnosed in 87% of former players and 99% of individuals who had played professionally (Mez 2017). From the research published by the VA-BU-CLF Brain Bank, there appears to be a positive correlation between CTE, traumatic brain injuries and professional football (Mez 2017). However, CTE can only be diagnosed postmortem with neuropathological analysis, which presents distinct challenges to real-time diagnosis and treatment. Moreover, there have been significant concerns raised about selection bias in these studies.

The CTE studies have raised considerable concern about the effects of even a single concussion. Indeed, the apprehension regarding long-term sequelae of concussion has led to some advocates to call for a ban on youth football (Daneshvar 2011). The U.S. Soccer Federation included in the guidelines outlined in 2015 that children under the age of 10 years old are banned from heading a soccer ball in all activities as to mitigate the suspected neurological sequelae which may precipitate later in life (Maher 2014; Stump 2015). However, it is essential to acknowledge that the link between concussion and CTE has not been established. Not all football players that sustained a diagnosed concussion have CTE postmortem. Furthermore, there is currently no test to diagnose any individual with CTE or at risk for developing CTE (Mayo Clinic 2016). Early pathological brain changes associated with CTE had a stronger link to closed-head injury regardless of concussion symptom behavior, indicating that the severity of a concussion did not

influence the likelihood of CTE (Tagge 2018). While concussions may be a critical factor in the development of CTE, little is understood about the mechanism of either disorder or the linkage between them. Thus, there remains a demand for an understanding of closed-head injury (CHI) in regard to behavioral and histological changes that result from a concussion, both acutely and chronically after injury. The analysis may not be possible in the clinical setting where it is challenging to conduct longitudinal assessments, control for confounding variables, evaluate dose-response of injury severity and outcomes, and prospectively evaluate relevant molecular correlates.

Current preclinical research

The urgent need to better understand the short- and long-term sequelae of concussion has led many research labs to develop TBI models and investigate outcomes in these models. The Mannix-Meehan lab at Boston Children's Hospital models closed head injuries (CHI) in order to evaluate biological and behavior changes after TBI objectively. Animal models are extremely important for both animal and human health (Ericsson 2013). With an effective research paradigm and behavioral modeling, the progression, advancement, and treatment for diseases can be accurately examined. By using animal models to emulate clinical presentations of a concussion, the lab can directly study the effect of different injury severities, spacing between injuries, and explore potential therapies that may mitigate the effects of a concussion.

While preclinical models of injury offer effective ways of studying TBI, without consistency within and between research models, outcomes may differ despite identical study and treatment designs, limiting the generalizability, reproducibility, and

interpretation of findings in single studies. Also, there currently exist a broad range of metrics taken to assess the severity of the injury, many of which differ between labs and even differ across time within a single lab. Furthermore, there are many different behavioral tests currently administered across labs and also within in the Mannix-Meehan research lab. Therefore, having a standardized assessment composite score will provide a better insight into the predictive model of injury and a universal scale to detect subtle and heterogenous effects of TBI.

Thus, it is essential to have a consistent quantitative score across research that can be applied to any large group of animals, in which there is an injured and non-injured population. This allows intra- and inter-laboratory comparisons across of a number of factors and takes into account any independent variables that may differ between groups of animals. Furthermore, the population-level data offered by this type of analysis may reveal subtle effects that are lost when only comparing individual metrics. This can colloquially be called an “*index score*,” which allows for a comparison of behavioral tests within different animal groups and model paradigms, creating a composite measure of behavioral test performance across all models.

SPECIFIC AIMS

This paper's main goal is to analyze the data collected from 2012-2018 in the Mannix-Meehan lab at Boston Children's Hospital in Boston, Massachusetts, U.S.A.. To address this goal, the data is evaluated for a relationship between different injury paradigms and behavioral results to measure the influence that each behavioral test and respectively associated measure constitutes in the overall model. There were five independent variables examined in this study: height of weight drop (height), number of weight drops (number of hits), the time between injury and behavioral test (time post-injury), condition (sham vs. CHI), and treatment (received additional treatment or did not receive any treatment). Three statistical models will be evaluated in this paper. Firstly, a pairwise correlation to analyze if there is a correlation between each dependent variable and a single independent variable, providing within-group statistics. Next, a simple linear regression was applied for a single dependent variable and the five evaluated independent variables of this study listed previously. Finally, a factor analysis was performed on complete sets of data to investigate small differences that may not be significant individually, but when added together is significant for an individual behavioral test across all models. As research labs use many different behavioral tests having a standardized composite score for each behavioral test can serve as a predictive model of injury. Together, these statistical methods will provide insight into which behavioral test has the most significant power across all models and thus has the most value.

MATERIALS and METHODS

Overall Description Independent Variables Assessed for Statistical Relevance

There were five independent variables analyzed in the observational data collected from the Mannix-Meehan lab. The variables evaluated were the height of weight drop (height), number of weight drops (number of hits), the time between injury and behavioral test (time post-injury), condition (sham vs. CHI) and treatment. Each variable is described below with each category within the variable listed along with the number of observations of the given category.

Table 1. Independent variables. The five independent variables analyzed in this paper defined by the preclinical assessment that the variable addresses and the number of categories along with the number of observations per each category.

Independent Variable	Preclinical Assessment	Number of Categories and Observations
Condition	Injured versus non-injured	<ul style="list-style-type: none"> • Sham (389) • CHI (570)
Number of hits	Frequency of injury	<ul style="list-style-type: none"> • 1 hit (270) • 2 hits (38) • 4 hits (80) • 5 hits (408) • 7 hits (163)
Height of weight drop	Severity of injury	<ul style="list-style-type: none"> • 0 = shams (389) • 26'' (32) • 28'' (216) • 42'' (154) • 46'' (40) • 50'' (73) • 60'' (55)
Time post injury	Symptom retention, long-term duration	<ul style="list-style-type: none"> • 2 weeks (773) • 13 weeks (117) • 26 weeks (69)
Treatment	Each treatment is described in the Intervention section of Methods	<ul style="list-style-type: none"> • 1=Received any form of treatment (581) • 0=Did not receive any form of treatment (378)

Animals

Animals were C57BL mice purchased from Jackson Laboratory, ranging in age from eight weeks to six months. Unless otherwise indicated, mice were injured at eight weeks of age. A total number of 840 mice were analyzed at different behavioral and histological outcome variables.

Repetitive Mild Traumatic Brain Injury: Drop Weight Model

All experiments were approved by the Boston Children's Hospital Institutional Animal Care and Use Committee and complied with the NIH Guide for the Care and Use of Laboratory Animals. The weight drops repetitive mild traumatic brain injury (rmTBI) model used in the different studies analyzed in this paper have been previously described (Mannix 2013). Animals were anesthetized for 45 seconds with 4.5 % isoflurane in a 70:30 mixture of nitrous oxide and oxygen until fully unconscious. Mice were placed on a delicate task wiper (Kimwipe; Kimberly-Clark, Irving, Texas) and grasped by the tail. The mice were then placed underneath a tube of varying height such that the end of the tube was centered over bregma, approximated by centering the tube between and slightly in front of the ears, and a weight of 54 grams was dropped through the tube. The weight drop model has been extensively researched as an effective method for an animal model of repetitive traumatic brain injuries (Kalish 2016). At impact, the bolt and mouse head split the Kimwipe, allowing rotational acceleration of the head in the anterior-posterior plane. Different injury paradigms were used across the studies examined in this paper

based on varying length of guide tube and the number of injuries over a given amount of time. The weight was dropped from a height of either 26'', 28'', 42'', 46'', 50'', or 60''. The total number of injuries varied from a single injury to seven hits, and the duration of time elapsed between consecutive injuries varied from one day to one month. Controls (hereafter referred to as shams) underwent anesthesia but did not undergo concussive injury; apart from that, the paradigm of anesthesia exposure was identical to their respective injury groups. Loss of consciousness was recorded for both sham-injured and concussive-injured mice as a marker of the time for removal of anesthesia. Varying behavioral and histological data was collected based on injury paradigm.

Behavioral Tests

Assessment of spatial learning and memory

Morris Water Maze performance was used to assess spatial learning and memory and was measured at approximately three weeks post-injury (PI), three months PI, and six months PI. The apparatus consisted of a circular tank of 60 cm height and 83 cm diameter was filled to 29 cm deep with water with a temperature approximately 25°C (Meehan 2012). The activity consists of five days of trials. Days 1 through 4 are composed of two hidden trials/day, where latency to find platform was recorded as a measure of spatial learning and memory. Upon completion of the hidden trials of day 4, animals underwent a probe trial during which the platform was removed, and time spent in the quadrant formerly housing the platform was recorded as a measure of spatial memory. During the

hidden trials, a clear Plexiglas platform (10 cm in diameter) was placed at the southwest quadrant 15 cm from the wall and 0.5 cm below the surface of the water. Mice were placed facing the wall randomized to 1 of 4 starting locations (north, south, east or west). Latency to the platform was recorded as the time it took the mouse to find and mount the platform, with a maximum time of 90 seconds to perform the task. If the mice failed to find the platform, the experimenter would manually place the mice on the platform for 10 seconds. During the probe trials, the platform was removed and Noldus EthoVision ® 11.5 software (Noldus Information Technology XT) tracked the mouse's movement within an allotted 60 seconds and generated statistical data of the time spent in the target quadrant where the platform had previously been located (Liu 2017). During the visual trials, an optical red reflector cue was placed above the platform and mice were placed in the northeast quadrant facing the wall. Time was recorded similarly to hidden trials.

Assessment of exploratory activity and Impulsivity

Elevated plus maze was used to assess exploratory activity and impulsivity. The maze consists of a plus-shaped, 85 cm tall elevated platform that has two closed arms and two open arms both of 30 x 5 cm (Lafayette Instruments, Lafayette, IN). Mice were allowed to explore for five minutes after being placed in the center facing one of the closed arms and the percent time spent in each arm was tracked and calculated (Noldus Ethovision 11.5 XT). The data was analyzed as a percentage of time spent in the open arm and the percentage of time spent in the closed arm. An increase in the percentage of time in open arm reflects an increase in exploratory activity and impulsivity.

Assessment of Motor Function

Rotarod testing measured locomotor function, and involved three days of testing. Day 1 consisted of a single habituation trial, whereas days 2 and 3 were testing trials. Mice were placed in an open box that contains a 4 cm diameter rotating drum that moves at four revolutions per minute (RPM) for five minutes during day 1. On day 2 and 3, mice were placed for ten seconds to acclimate and then the rod accelerates at increased 0.1rpm/sec. Each day was composed of four separate trials (Mannix 2014). Statistical data was analyzed as the average of the four trials on day 2 and 3. A longer latency to fail is correlated with increased motor function ability.

Assessment of anxiety

The open field test was used to assess anxiety and was conducted as follows. Mice were placed in the edge facing the wall of a 45 cm diameter circular arena with plastic walls with a height of 20 cm (Mei 2018). The movement of the mouse was tracked with Noldus Ethovision 11.5 XT directly above the arena (Wageningen, the Netherlands). The arena is divided into three sections: the “wall” ring, the area closest to the wall with an inner diameter 40 cm, outer diameter 60 cm (area of 1570 cm²), the “neutral” ring, inner diameter 20 cm wide, outer diameter, 40 cm (area of 932 cm²); and then an “center” circle 20 cm in diameter (area of 314 cm²) (Mei 2018). Data was generated as the percentage of time spent in each ring during the allotted five minutes of activity exploration. An increase in the percentage of time in the wall and a decrease in the

percentage of time spent in the center ring correlates with an increase in anxious behavior, as mice have a natural aversion to brightly lighted areas.

Assessment of behavioral despair

A forced swim test was used to assess behavioral despair and was conducted in accordance with the Porsolt Forced Swim Test model (Porsolt 1977). Mice were placed individually in 40 cm tall glass cylinders with an 18 cm diameter that contained 30 cm of water at 25°C for five minutes (Liu 2017). The mouse was tracked as moving or immobile (floating), and data was presented as the percentage of time spent immobile. A higher percentage of time immobile reflects an increase in behavioral despair, a key hallmark in depressive phenotypes (Washington 2012).

Assessment of object memory

The novel location and novel object recognition tests are similar behavioral tests that assess a mouse's innate exploratory behavior (Ennacur 1988). Both behavioral tests involve the mouse being familiarized to a specific object in a specific location. The novel object consists of an open field box of 44cm by 44cm. Mice were placed in the field for five minutes for three days, to become habituated to the arena, with two different objects of roughly equal sizes, now the "familiar" objects. On the fourth day, mice were allowed to explore three identical objects, which were placed in fixed locations for 6 minutes and the movement was tracked (Noldus Ethovision XT). The activity was measured and reported as the percentage of time spent with an object in the familiar location and time

spent with an object in the novel location. For the novel object, the fourth day consisted of a novel object in the familiar location. The novel object behavioral is measured as the percentage of time spent with the novel object. For the novel object, if a mouse spends more time with the novel object, then the object memory is higher. For the novel location, if a mouse spends more time with the object in a novel location, then object location memory is higher.

Assessment of impulsivity

The light-dark box test utilizes a mouse's natural aversion to bright lights to measure impulsive behavior. In this test, mice were placed in an open field container that had brightly lit (30-50 lux) arms and non-brightly lit arms. The animal was placed in the center of the chamber, and both distances from the center and total distance traveled was recorded (Noldus Ethovision XT). The test is hypothesized to reflect anxiolytic behavior as well as anxiogenic behavior: an increase in the percentage of time spent in the light area reflects an increase in spontaneous exploratory behavior in an aversive environment and thus, increased impulsive behavior (Bourin 2003). Although the light dark box utilizes the mouse's fear of bright lights similar to the open field test and elevated plus maze, the specific method of light-dark box allows measurements of exploratory behavior rather than anxiety (Takao 2006).

Assessment of willingness to explore new environments

The Y Maze behavioral test consists of a Y shaped container that has three identical length arms at 120° angle from the adjacent arm. A mouse was placed in the center for 5 mins and the percentage of time that a mouse spent in each arm was recorded (Noldus Ethovision XT). An increase in the percentage of time spent in Y arm correlates to an increase in spontaneous alterations, which reflects exploratory behavior.

Assessment of antidepressant-like behavior

The tail suspension test consists of a mouse's behavior of holding an immobile posture when placed in a stressful situation from which it has no escape, a measure of behavioral despair similar to the aforementioned forced swim test (Cryan 2005). In this test, a mouse is suspended by the tail for 5 minutes, a stressful situation from which the mouse tries to escape. The percentage of time spent immobile is reported. An increase in immobility correlates to a decrease in effort expenditure and increased behavioral despair (Cryan 2005).

Assessment of Anhedonia

The sucrose preference test was used to measure anhedonia. Mice are placed in a cage with two sipper tubes, one tube containing normal drinking water and a second containing a 2 % sucrose solution. The water and sucrose solution levels are measured at the beginning and the end of the day and the bottles are switched each day to avoid side bias (Serchov 2016). Sucrose preference is measured as the percentage of sucrose water

intake out of the total intake consumed. An absence of sucrose preference reflects anhedonic behavior.

Interventions

Environmental Enrichment

Mice were housed randomly into two different conditions: Environmental Enrichment (EE) and Normal Cage (NC). The enrichment cage was a standardized *Marlau Environmental Enrichment Cage* (Viewpoint Behavior Technology) that consisted of a two-floor cage with a variety of enriching features such as multiple running wheels and a maze that was alternated weekly. Between 10-15 animals were housed per cage, providing social enrichment. Animals were introduced to enrichment housing three days before the injury and remained there for the duration of the experiment, being removed only for behavioral tests. Normal cages consisted of standard cages with 5 mice per cage (Liu 2017). Notably, the mice used in this study were 5-weeks-old, 3-weeks younger than mice used in any other study.

Anti-CD3

CD3 is an antigen that is partially responsible for T cell activation, and it was theorized that preventing some of this activation would mitigate a hyperactive immune response to TBI. For all anti-CD3 studies, treated animals received 0.5 micrograms (volume of 10 microliters) of anti-CD3 antibody intranasally daily, starting at 6 hours

post-injury, and extending for various durations. Control animals received intranasal saline to control for effects of handling. Groups 1 and 2 received anti-CD3 or saline for seven days at which point animals were sacrificed. Group 3 received anti-CD3 or saline daily for nine days and then thrice weekly for three months, at which point animals were sacrificed.

Flicker

Flicker treatment was theorized to restore the function of GABA-ergic neurons that may be dysfunctional after TBI. Treatment consisted of concurrent flash of light and sound at 40 interactions per second, which was theorized to restore the natural 40 Hz firing rate of these GABA-ergic neurons. Treatment lasted for one hour daily while animals were in a single housing. Light flashes were approximately 60 watts bright, and the sound was between 25 to 30 decibels.

There were three separate groups for Flicker experiments. Group 1 received one month of treatment starting three months after injury, Group 2 received one month of treatment starting immediately after the injury, and Group 3 received one month of treatment starting three months after the injury during their dark cycle, when the mice are typically awake.

Memantine

There were four different cycles of Memantine studied in this paper. In each cycle, there were three groups: CHI-Vehicle, CHI-Memantine, Sham-no treatments. Each

of the studies had an injury model of 1 injury per day for 4 days (rmTBI injury). Memantine is currently an FDA approved drug, FDA approval in October 2003, to treat confusion in Alzheimer's Disease (Cunha 2019). The goal of Memantine treatment in our lab was to target the N-methyl-d-aspartate (NMDA) receptor (NMDAR), as evidence suggest that glutamatergic toxicity may decrease and prevent rmTBI-induced neurologic deficits (Mannix, unpublished?). The mouse received Morris Water Maze, Elevated Plus Maze, Open Field, and Rotarod behavioral test to assess by motor, anxiety and impulsivity outcomes.

Cis-Tau Antibody

The "Ping" labeled data observations for the treatment group received cis-tau antibody, as it blocks apoptosis of cells as the formed cis P-tau is prevented from disrupting the mitochondrial transport and axonal networks (Lu 2016; Kondo 2015). In the Ping 6 and Ping 4-Tau 3 cohorts, both CHI cohorts received a single hit injury. The Ping 4-Tau 3 cohort received a mild injury at 26 inches, while the Ping 6 received a severe injury at 60 inches. Two additionally cohorts were also analyzed with an injury paradigm of 7 hits over nine days at 28 inches, called Ping 4-Tau 2 and the Ping Tau 3. Immunoglobulin was given to the sham treatment mice as a sham antibody as a measure to control for the existence of an antibody within the mouse model.

Tau KO

The Tau KO (TAU -/-) labeled data hypothesized that excessive tau aggregation may be linked to behavioral deficits. The transgenic tau knockout mice CHI cohort of mice received 5 hits/5 days at 42" injury model (Jackson Laboratory). These mice were homozygous in lacking the gene responsible for microtubule associated protein tau (Mapt) and as such were unable to produce microtubule associated tau. It was theorized that this would prevent potentially toxic tau buildup that may occur after injury.

Injury Spacings

In the Tau treatment paradigm, the injuries were administered at different time point depending on the group. For both the 28- and 42- inch injuries, there were five different groups. Five injuries were administered daily (1 hit per day for five days), weekly (1 hit per week for five weeks), biweekly (1 hit per 2 weeks for ten weeks), monthly (1 hit per month for five months). An additional group was aged for five months, and then administered five hits daily, to match the age of the monthly group.

RESULTS

Tabulations of independent variables

There were a total of 959 observations with five independent variables and 38 dependent variables evaluated in this paper from the statistical data collected by the Mannix-Meehan research lab from 2012-2018. Notably, not all animals received the same battery of metric tests. There were a multitude of collaborations with other laboratories and research projects conducted within the Mannix-Meehan lab, resulting in multiple variables measured for a given study, and a different set of tests administered for each study. Before analyzing the data for power and statistical relevance, the data was evaluated for the number of variables for a given behavioral outcome. Presented below is the number of data points missing by each numerical variable as calculated in StataCorp software (STATA). Additionally, due to the nature of the statistical analysis desired for this paper, three of the studies (Tau 2-Ping 4, Tau 3-Ping 4 and Ping 6) had data from the same animals on the same observations at different times. The behavioral test themselves are described above in the methods and materials section of this paper, with LOC being the loss of consciousness recorded after each injury and/or isoflurane exposure, measured as time elapsed between removal from isoflurane to spontaneous ambulation.

Table 2. Number of Observations for each behavioral variable. MWM is the Morris Water Maze behavioral test of hidden trials 1-5 (H1-H5), visual trials 1-2 (V1,V2), probe trials 1-2 (P1,P2) and probe frequency trials (P1 freq, P2 freq) listed in column 1. In column 3 is the Elevated Plus Maze (EPM) for % of time in open, % of time in closed arm, and % time making a decision (Open %, Closed %, Decision %), Open Field (OF) in % time in wall, % time in neutral and % time in center (Wall %, Neutral %, Center %). FST is force swim test over three days, NO is novel object, NLR is novel location recognition, Y maze test in Y arm, LD is a light dark test, and Sucrose is measured of 3 days after one day of habituation.

Behavioral	Number of Observations
loc1	840
loc2	695
loc3	676
loc4	676
loc5	592
loc6	208
loc7	208
mwm_h1	859
mwm_h2	859
mwm_h3	859
mwm_h4	834
mwm_h5	347
mwm_v1	835
mwm_v2	768
mwm_p1	835
mwm_p2	786
mwm_p1_freq	403
mwm_p2_freq	403

Behavioral	Number of Observations
epm_open	829
epm_closed	829
epm_decision	131
of_wall	498
of_neutral	476
of_center	476
roto_day1	666
roto_day2	666
fst_timeim~e	157
fst_timeim~2	105
fst_timeim~3	53
no_novelob~e	156
nlr_object	48
nlr_nonobj~t	48
ymaze_yarm	49
ld_light	31
tailsuspen~e	24
sucrose_day2	24
sucrose_day3	24
sucrose_day4	24

After summary statistics of the cumulative data was evaluated, a threshold of at least 500 data points for a given variable was implemented. This resulted in 17 variables being dropped with a remaining of 20 numerical variables. The remaining data was tabulated to evaluate the number of observations for five independent variables (condition, treatment, the height of injury, time post injury, and the number of hits) each listed below.

First, the condition variable was tabulated for the number of observations for CHI and Sham. Of the data analyzed almost 60% of the data points were from CHI injured mice, and 40% from Sham mice.

Table 3. Tabulation of condition (CHI vs. Sham) observations. Frequency is reported as the number of data points within the given variable and percentage is the overall percentage of a given condition.

Condition	Number of Observations	Total Percentage
SHAM	389	40.56
CHI	570	59.44
Total	959	100

Additionally, the data was analyzed by the type of treatment administered. There were nine treatments different treatment studies analyzed, and the number of observations for each type of treatment is recorded below. A treatment of “none” means that the observation was not part of a study that had any type of treatment administered.

Table 4. Number of observations for each treatment by condition (CHI vs. Sham). There was a total of 10 treatments received and the “none” treatment category includes all observation that did not receive any type of treatment.

Treatment	Sham	CHI	Total
AntiCD3	12	23	35
Cis-Tau	15	65	80
Enrichment	14	40	54
Flicker	30	30	60
IGG	16	66	82
Memantine	0	28	28
None	302	279	581
Single housing	0	12	12
Vehicle	0	27	27
Total	389	570	959

Furthermore, the variables were assessed by injury protocol based on the intensity of injury, which is indicated by the height at which the weight was dropped. A height of 60 inches reflects a severe injury, while a height under 40 is a mild injury and a height of 0 means only anesthesia exposure.

Table 5. Number of observations by the weight drop height. There were six different weight drop heights analyzed in this paper. A height of 0 indicates the number of sham observations.

Height	Number of Observation
0	389
26	32
28	216
42	154
46	40
50	73
60	55
Total	959

Additionally, the type of injury protocol was evaluated and broken down further by the height of injury. There were 11 injury protocols conducted in the Mannix-Meehan research lab over the years 2012-2018. As listed above, the injury paradigm greater than 42 inches was considered a “severe” injury was the 50 inches and 60 inches heights, and the repeated mild traumatic brain injuries (rmTBI) model were the paradigms that had multiple hits. Since 2017, the lab almost universally adopted an injury protocol of 5 hits daily over five days at 42 inches and the 7 hits over nine days at 28 inches. Within this tabulation, the number of injuries is broken down by if the drop weight model included a box and the height at which the weight was dropped.

Table 6. Number of observations by height and injury model. The injury protocol was evaluated based on the height of the given protocol. The sham observations, those that did not receive any injury, were excluded from the table listed above.

	26	28	42	46	50	60
1 hit	32	0	0	1	55	55
1 hit w/box	0	0	0	7	18	0
2 hit	0	0	0	14	0	0
2 hit w/box	0	0	0	18	0	0
4 hits/4 day	0	55	0	0	0	0
5 hits/10wks	0	12	12	0	0	0
5hits/5days	0	12	107	0	0	0
5hits/5days(aged)	0	12	11	0	0	0
5hits/5mo	0	12	12	0	0	0
5hits/5wks	0	12	12	0	0	0
7hits/9days	0	101	0	0	0	0

Furthermore, the overall number of injuries that an observation received was tabulated and compared with condition (sham vs. CHI) as it is reflective of the number of anesthesia sessions for sham and the number of anesthesia and drop weight model sessions for CHI.

Table 7. Number of injuries per mouse by the condition. Tabulation of the number of injuries versus condition (sham vs. CHI) with the total number of observations per number of injuries reported.

Number of Injuries	Sham	CHI	Total
1	102	168	270
2	6	32	38
4	25	55	80
5	194	214	408
7	62	101	163
Total	389	570	959

Another independent variable assessed in the analysis was the time post-injury that the behavioral task was administered. The goal of the difference in time post-injury

is to evaluate the behavioral changes that occurred acutely after injury (2 weeks), sub-acutely (13 weeks) and chronically (26 weeks).

Table 8. Number of observations by condition (Sham vs. CHI) for each time post injury. Tabulation of time post-injury (2 weeks, 13 weeks or 26 weeks) categorized by sham or CHI injured observations.

Time Post Injury	SHAM	CHI	Total
2	314	459	773
13	55	62	117
26	20	49	69
Total	389	570	959

Data analyzed for consistency

As multiple studies and various treatment protocols were assessed as it was essential to determine a baseline control for each behavioral. Therefore, all observations that did not receive any treatment and were not injured under any protocol were examined, a total of 302 observations. In order to examine whether these mice could be treated as a homogeneous population, a Bonferroni-adjusted significant method was applied within STATA, thus protecting from Type I errors when the null hypothesis is incorrectly rejected (Armstrong 2014). Any value that fell below the Bonferroni corrected p value was considered significant, indicating a difference between shams for that given dependent variable. This would mean that the sham group could not be treated as a homogenous population. This method is valid to use as the null hypothesis claims that treatment should not make a statistically significant difference in behavior for all observations that did not receive the weight drop injured model (shams). A pairwise

correlation for treatment (received or did not receive) for each behavioral is reported below in table 9.

Table 9. Pairwise correlation with Bonferroni adjusted significance level. The coefficients and corresponding p-values for each behavioral is reported.

Behavioral	Coefficient	P-value
loc1	-0.2152*	0.038
loc2	0.0235	1
loc3	0.071	1
loc4	0.0324	1
loc5	-0.0321	1
loc6	0.7120*	<0.001
loc7	0.0853	1
mwm_h1	-0.1473	1
mwm_h2	-0.1114	1
mwm_h3	-0.1634	1
mwm_h4	-0.1752	0.725
mwm_h5	-0.1302	1
mwm_v1	-0.0461	1
mwm_v2	-0.0181	1
mwm_p1	-0.1361	1
mwm_p2	0.2735*	<0.001
mwm_p1_freq	0.4700*	<0.001
mwm_p2_freq	0.4710*	<0.001
epm_open	-0.0116	1
epm_closed	-0.0734	1
epm_decision	0.3123	1
of_wall	0.0648	1
of_neutral	-0.1091	1
of_center	0.2181	1
roto_day1	0.062	1
roto_day2	0.0098	1
fst_timeim~e	-0.3664	1
fst_timeim~2	-0.8240*	<0.001
fst_timeim~3	0.3811	1
no_novelob~e	-0.1872	1
ld_light	-0.5586	1

If a mouse received any type of intervention, there was a statistically significant impact on behavioral outcome for the following variables: LOC1, LOC6, MWM P2-P2 freq, FST time immobile 2. Furthermore, when the listed above variables were analyzed by treatment type it was revealed the contributing factor into the statistically significant difference between shams was the Anti-CD3 treatment compared to no treatment for LOC6 and MWM P1 frequency and P2 frequency.

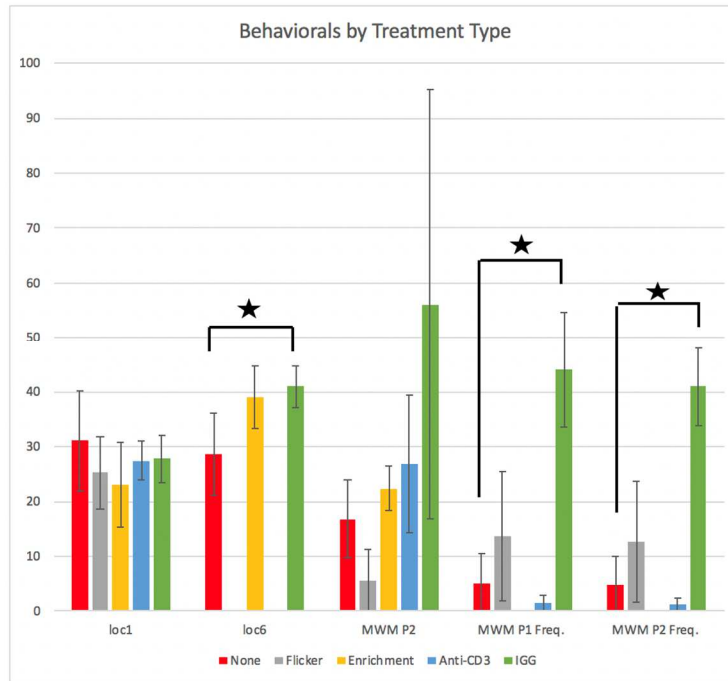


Figure 1. Bar graph of non-injured mice by treatment for pairwise correlation significant behavioral. Each statistically significant variable for the pairwise correlation was examined further by treatment type with the star representing a $p < 0.05$.

Analysis of Cumulative data between CHI and Sham

The data was examined for a difference between CHI and shams. To perform the analysis, only data that had “no treatment” was analyzed as to avoid the confounding variable that treatment may have on the results. There were 302 shams and 279 CHI that reached this threshold and were analyzed. Additionally, the following variables were dropped due to a lack of observations: tail suspension, sucrose day 2, sucrose day 3, sucrose day 4, Y-maze and novel location. First, the general model was analyzed for a significant difference between the 5 independent variables (condition (sham vs. CHI), time post-injury (2 weeks, 13 weeks, and 26 weeks), treatment (AntiCD3, Cis-Tau, Enrichment, Flicker, IGG, Memantine, None, Single-housing, Vehicle), height in inches

(0, 26, 28, 42, 46, 50, 60), and number of injuries/anesthesia (1, 2, 4, 5, 7). The sample regression was done for Morris Water Maze Hidden trial 1. Treatment was coded as a binomial variable, the observation either had a treatment (1) or no treatment (0).

Source	SS	df	MS	Number of obs	=	859
Model	44235.7489	5	8847.14977	F(5, 853)	=	19.97
Residual	377813.919	853	442.923703	Prob > F	=	0.0000
				R-squared	=	0.1048
				Adj R-squared	=	0.0996
Total	422049.667	858	491.899379	Root MSE	=	21.046

mwm_h1	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	5.990214	4.228821	1.42	0.157	-2.309899 14.29033
time_postinjury	-.7023076	.1209649	-5.81	0.000	-.9397313 -.4648839
treatment_bi	-4.421107	1.588807	-2.78	0.006	-7.539536 -1.302678
height	.070445	.1027573	0.69	0.493	-.1312418 .2721319
numhits	1.592373	.3814203	4.17	0.000	.8437409 2.341006
_cons	37.68597	2.065667	18.24	0.000	33.63159 41.74036

Figure 2. Simple Linear Regression Stata output example. The example regression displays the dependent variable (MWM H1) regressed to all 5 independent variables (condition_lchi, time_postinjury, treatment_id, height, numhits). A p>|t| output value of 0.000, represents a value that is below STATA calculation capacity and thus, is p<0.001.

The regressions were done for every dependent variable and the STATA outputs are included in Appendix A. All the P values are presented in the table below and highlighted in yellow are the values that are statistically significant with a p-value < 0.05.

Table 10. Cumulated STATA generated p-values from simple linear regressions. Each dependent variable regressed by the five independent variables previously described, with each p values reported from STATA outputs. Results with statistically significant p values ($p < 0.05$) are highlighted in yellow. Note: STATA software only generates values to the thousandths place.

Behavioral	Condition	Time Post Injury	Treatment	Height	Number of Hits
LOC1	<0.001	0.621	0.097	<0.001	0.005
LOC2	<0.001	0.089	0.002	<0.001	0.615
LOC3	<0.001	0.385	0.105	<0.001	0.928
LOC4	<0.001	0.830	0.522	<0.001	0.631
LOC5	0.002	0.812	0.162	<0.001	0.559
LOC6	0.145	0.076	<0.001	0.116	0.034
LOC7	0.045	0.595	0.400	0.038	0.923
MWM H1	0.157	<0.001	0.006	0.493	<0.001
MWM H2	<0.001	0.023	0.337	0.304	0.008
MWM H3	<0.001	0.016	0.569	0.180	0.120
MWM H4	<0.001	0.001	0.139	0.768	0.007
MWM H5	<0.001	<0.001	0.999	<0.001	0.612
MWM V1	0.534	0.807	0.591	0.002	<0.001
MWM V2	0.141	0.130	0.743	<0.001	<0.001
MWM P1	0.037	<0.001	0.198	0.001	0.696
MWM P2	<0.001	<0.001	0.001	<0.001	0.011
MWM P1 FREQ	<0.001	<0.001	<0.001	<0.001	0.001
MWM P2 FREQ	<0.001	<0.001	<0.001	<0.001	0.006
EPM OPEN	0.905	0.313	0.029	0.118	0.076
EPM CLOSED	0.456	<0.001	0.314	0.503	0.022
EPM DECISION	0.015	<0.001	0.482	0.024	0.872
OF WALL	0.532	0.070	0.494	0.786	<0.001
OF NEUTRAL	0.051	0.547	0.186	0.110	<0.001
OF CENTRAL	0.503	0.456	0.123	0.567	0.762
ROTAROD DAY 1	<0.001	0.001	0.006	0.002	0.321
ROTAROD DAY 2	<0.001	0.979	0.085	0.001	0.036
FST TIME IMMOBILE 1	0.880	0.080	0.846	0.654	<0.001
FST TIME IMMOBILE 2	0.560	0.001	0.168	0.338	<0.001
FST TIME IMMOBILE 3	0.075	<0.001	N/A	0.087	0.001
NOVEL OBJECT	0.376	<0.001	0.105	0.120	<0.001
NOVEL LOCATION OBJECT	N/A	1	N/A	0.001	N/A
NOVEL LOCATION NON-OBJECT	N/A	1	N/A	0.022	N/A
YMAZE YARM	N/A	1	N/A	0.396	N/A
LD %TIME IN LIGHT	N/A	N/A	0.010	0.444	N/A
TAIL SUSPENSION	N/A	N/A	N/A	0.461	N/A
SUCROSE DAY 2	N/A	N/A	N/A	0.767	N/A
SUCROSE DAY 3	N/A	N/A	N/A	0.110	N/A
SUCROSE DAY 4	N/A	N/A	N/A	0.148	N/A

From the simple linear regression table above, there was a significant difference between shams and CHI for the following dependent variables: LOC 1-5 & 7, MWM H2-H5, MWM P1-P2 freq, EPM decision, Rotarod Day 1 and Day 2. Furthermore, there was a significant difference between animals that received treatment and those that did not receive treatment the following dependent variables: LOC 2,6, MWM H1, P2-P2 freq, EPM Open, and Rotarod day 1 all had $p < 0.05$.

A pairwise correlation was analyzed between conditions (sham vs. CHI) controlling for treatment (none). A 5 % level of significance with Bonferroni-adjusted significant method was applied to the analysis with STATA. When examining only mice that did not receive any type of treatment were correlated the following dependent variables were statistically significant: LOC1-4, MWM H2-H4, V1, V2 and Rotarod Days 1 and 2. Mice remained unconscious significantly longer following a CHI compared to a sham injury for loss of consciousness days 1, 2, 3, and 4 at the significance level $p < 0.05$.

Table 11. Pairwise correlation between conditions (Sham vs. CHI) by behavioral test. Each dependent behavioral variable is listed with the correlation coefficient generated from STATA and the p value listed in the third column controlling for treatment.

Behavioral	Condition	P value
Time Post Injury	-0.0985	1
Number of Hits	0.0472	1
LOC 1	0.6020*	<0.001
LOC 2	0.4708*	<0.001
LOC 3	0.4528*	<0.001
LOC 4	0.3604*	<0.001
LOC 5	0.2012	0.127
LOC 6	0.3596	1
LOC 7	0.2364	1
MWM H1	0.1732	0.0589
MWM H2	0.2569*	<0.001
MWM H3	0.2448*	<0.001
MWM H4	0.3181*	<0.001
MWM H5	0.281	0.7917
MWM V1	0.1985*	0.0041
MWM V2	0.2877*	<0.001
MWM P1	-0.1538	0.3954
MWM P2	-0.1306	1
MWM P1 freq.	-0.0289	1
MWM P2 freq.	-0.0123	1
EPM Open	0.1754	0.1104
EPM Closed	-0.1635	0.3112
EPM Decision	0.1009	1
OF Wall	-0.0425	1
OF Neutral	0.0177	1
OF Center	0.0799	1
Rotarod Day 1	-0.3065*	<0.001
Rotarod Day 2	-0.2804*	0.0001
FST Time immobile 1	-0.1687	1
FST Time immobile 2	-0.0374	1
FST Time immobile 3	0.5458	1
Novel Object	-0.0636	1
LD % Time in Light	0.2165	1

Factor analysis of Data Sets

To address the presented goal of creating a composite score for behavioral test modeling of rmTBI, factor analysis in Stata software was undertaken. Factor analysis provides an exploratory method that describes unobservable latent factors that may contribute to observed behavior. The statistical method is often used in clinical neuropsychiatric studies and allows for analysis of correlated variables with independent factors (Kleinbaum 1988). To achieve the stated goal, the data had to be manipulated to

fit a format that the software could read and interpret. A complete correlation matrix was necessary for the software. Therefore, the data was condensed into observational data that contained results into four subsets outlined below.

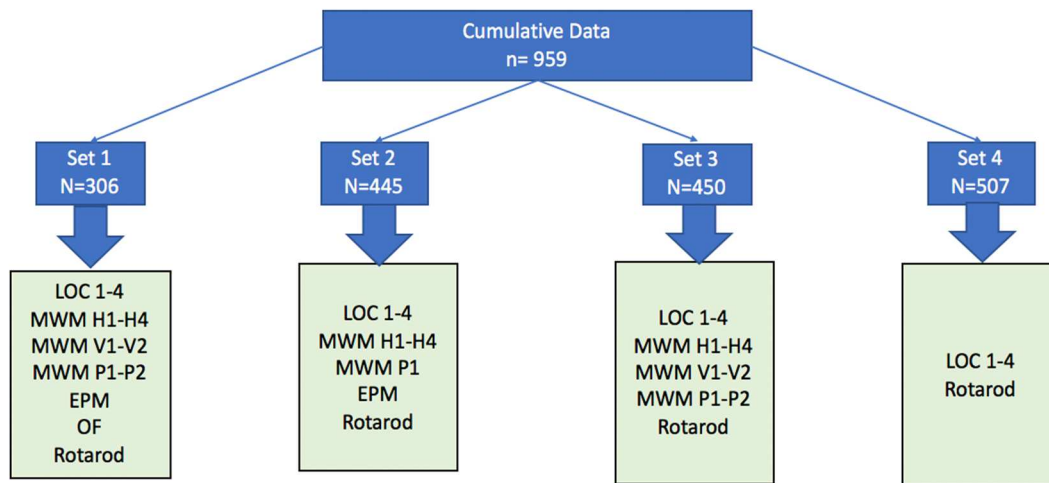


Figure 3. Group breakdown for each behavioral test included in the specific data set. Subset grouping of behavioral data with a number of observations included. Set 1 includes loss of consciousness (LOC) on days 1 thru 4, Morris Water Maze (MWM) test of hidden trials 1-4 (H1-H4), visual trials 1-2 (V1,V2), probe trials 1-2 (P1,P2), Elevated Plus Maze (EPM) data for % of time in open, % of time in closed arm, Open Field (OF) data % time in wall, % time in neutral and % time in center and Rotarod data for days 1 and 2. Set 2 contained LOC, MWM H1-H4, P1, EPM % open and % of time in open, % of time in closed arm and Rotarod on days 1 and 2. Set 3 contains LOC1-4, MWM H1-H4, V1-V2, P1-P2 and days 1 and 2 of Rotarod. Set 4 contained LOC1-4 and Rotarod data on days 1 and 2.

Factor analysis generates eigenvalues that reflect the variance of the factor. For this analysis, an arbitrary cutoff point for an eigenvalue was set to 1 for each factor (Albayram 2017). Rotated factor loadings were then applied to the factors that were above the thresholds set. An oblique rotation was applied as this method provides higher efficacy and can effectively measure both correlated and uncorrelated factors, an important distinction between orthogonal rotations being the alternative option (Osborne 2015). Additionally, recommended by Tabachnick and Fidell (2014), Samuels (2016),

Field (2013) was to apply a greater than 0.3 threshold to the factor loadings and retained factors should have at least three items with factors.

Data Set 1 Factor Analysis

Within data set 1, there were 306 observations, and all were evaluated over 19 dependent behavioral variables. There were four factor eigenvalue that were above the arbitrary threshold set as 1 (Albayram 2017). The percentage of variance included in each factor and the regression coefficient and p value are presented below in a cumulated factor data table (figure 4). Additionally, there was a high inter-factor correlation between factor 1 and factor 2 of 0.4755, but all other inter-factor values were below ± 0.20 .

Data Set 2 Factor Analysis

There were 445 observations analyzed over 13 dependent behavioral variables from data set 2. There were two factors retained, and an inter-factor correlation of 0.27 between factor 1 and 2. Only factor 2 had behavioral variable loadings of above 0.70. However, it only had two variables contributing to the factor loading, EPM_Open (0.9155) and EPM_Closed (-0.9325) on factor 2. The highest factor loading for factor 1 was MWM_H4 with 0.63. The regression coefficients are presented in the cumulative factor data table (figure 4).

Data Set 3 Factor Analysis

For data set 3, there were 450 observations and only one retained factor with 3.99524 eigenvalues. The oblique rotated factor analysis reported that 73.12% of variance was included in factor 1; the regression of the factor resulted with a 1.172 ± 0.069 coefficient, which was statistically significant at the 5 % level of significance. Only MWM_V2 satisfied the greater than 70% loading on the factor.

Data Set 4 Factor Analysis

The final data set looked specifically at rotarod data, which was the most frequently utilized behavioral test for the Mannix-Meehan lab. There were 507 observations and two retained factors with a -0.21 inter-factor correlation value. The coefficients, p values, and significant factor loading variables are presented in the cumulative factor data table (figure 4).

Cumulative Factor Data Table

The table below presents all the retained factors from the 4 data sets evaluated in this paper. Overall, the highest ranked factor was Morris Water Maze visual trial #2, EPM open and closed.

Data Set Number	Factor Number	% Variance	Regression coefficient	Regression p-value	Variables with >70% of Factor Load
1	1	42.89%	0.9898 ± 0.0932	<0.001	MWM_V1 & MWM_V2
1	2	26.30%	0.3873 ± .1074	<0.001	EPM_OPEN & EPM_CLOSED
1	3	24.48%	0.1395 ± .1154	0.227	OF_WALL & OF_NEUTRAL & OF_CENTER
1	4	14.57%	-0.6057 ± 0.096	<0.001	n/a
2	1	56.20%	1.199 ± 0.661	<0.001	n/a
2	2	39.78%	.3222 ± 0.0909	<0.001	EPM_OPEN & EPM_CLOSED
3	1	73.13%	1.17 ± 0.693	<0.001	MWM_V2
4	1	80.11%	0.94 ± 0.679	<0.001	LOC 2 & LOC 3 & LOC 4
4	2	46.74%	-0.6195 ± 0.0689	<0.001	ROTO_Day 1 & ROTO_Day 2

Figure 4. Cumulative Factor table rankings. The table reports the number of factors per data set and the variance reflected in each factor, as well as the variables that had over a 70% impact on the factor loading.

Analysis

Pairwise correlations and factor analysis were used to assess the relationship between the condition and each behavioral test. There were 31 dependent behavioral variables. These were summarized and ranked based on the measure of change as a result of rmTBI. First, the results from the simple linear regressions performed for every dependent variable and behavioral test resulted in a statistically significant difference between sham and CHI observations at the 5 % level of significance variables listed in table 8. However, when controlling for treatment, such that no observation received any treatment, the pairwise correlation revealed that there was a significant difference between CHI and sham animals in the following variables: LOC1 that LOC1-4, MWM H2-H4, V1-V2, Rotarod days 1 and 2 were statistically significant at the 5 % level of significance. Regression analysis revealed that mice that underwent a TBI remained unconscious longer than sham-injured mice.

Presented below in table 10 are the summary descriptions of each factor that was statistically significant factor.

Table 12. Summary table of statistically significant factors and the corresponding behavioral tests. Each behavioral test that had the largest factor loading impacts are reported above with additionally notes explaining keynotes with each behavioral.

Behavioral Test	Data Set	Factor	Notes
MWM	1 & 3	<ul style="list-style-type: none"> • Visual trial 2: data set 1-factor 1 (0.755) and data set 3-factor 1 (0.706) • Visual trail 1: data set 1- factor 1 (0.728) 	MWM Visual trials had the largest power, with positive loadings on factors with the highest variation in data sets 1 and 3.
EPM	1 & 2	<ul style="list-style-type: none"> • % time in Open: data set 1 (0.937) and data set 2 (0.916). • % time in closed: data set 1 (-0.8507) and data set 2 (-0.9325). 	Ranked second in data sets that included MWM V2
OF	1	<ul style="list-style-type: none"> • % of time at wall (-0.9949), % of time in neutral area (0.8651) and % of time in center (0.7243) all had large impacts on factor 3 	Factor 3 was not statistically significant, therefore does not contribute statistically significantly to factor loading.
Rotarod	4	<ul style="list-style-type: none"> • Rotarod on factor 2 Day 1 (0.752) and 	Only Rotarod data was analyzed in data set 4, as such in any data set that included other variables (not just rotarod) the Rotarod did not impact factor loading, and is not statistically significant.

From the factor analysis results, the Morris Water Maze visual trial for day 2 had the most substantial positive impact on the factor loadings in data sets 1 and data set 3.

Additionally, the behavioral test was not included in the observations examined in data set 2 and data set 4.

Also, when examining just Rotarod data with loss of consciousness data in data set 4, factor 1 had 80.1 % of data variation was explained by LOC from days 1 to days 4; while factor 2 only 46.7% of the variation was explained by the rotarod behavioral test.

Within the other data sets there was more than just rotarod behavioral data analyzed, the

behavioral test was not statistically significant on the factor loading at the 5 % level of significance.

Furthermore, the combined regression analysis with the factor analysis provided a cumulative score to assess behavioral outcomes in the mice model for rmTBI. The composite ranking table reflects each individual behavioral test ranked by the influence that the behavioral test contributed to the predictive nature of the injury condition: injury versus non-injured mice.

Table 13. Composite ranking table of Simple Linear Regression, pairwise correlation and factor analysis. Combined Factor Analysis ranking with regression analysis ranking from pairwise correlation

Behavioral	Condition	P value	Rank
MWM V2	0.2877*	<0.0001	1
MWM V1	0.1985*	0.0041	2
EPM Open	0.1754	0.1104	3
EPM Closed	-0.1635	0.3112	3
LOC 2	0.4708*	<0.0001	4
LOC 3	0.4528*	<0.0001	4
LOC 4	0.3604*	<0.0001	4
Rotarod Day 1	-0.3065*	<0.0001	5
Rotarod Day 2	-0.2804*	0.0001	5
LOC 1	0.6020*	<0.0001	N/A
MWM H2	0.2569*	<0.0001	N/A
MWM H3	0.2448*	<0.0001	N/A
MWM H4	0.3181*	<0.0001	N/A

DISCUSSION

The present study investigated the utility of an array of behavioral assessments for mice in discerning TBI mice from sham controls, through the use of varying statistical models. The primary goal of this study was to determine which of the selected behavioral tests for analysis pertain to conditions possessing adequate statistical power: injured versus non-injured mice. The outcome of this paper is the ranking of all dependent behavioral test across all models evaluated in the Mannix-Meehan research lab from 2012-2018 at Boston Children's Hospital in Boston, Massachusetts, U.S.A.. There were five independent variables measured for each observation and the corresponding clinical presentation in parenthesis: number of hits (frequency of injury), height weight dropped (severity of injury), time post-injury (symptom retention, long-term duration), treatment, and condition (injured versus non-injured). A computational analysis was conducted by three distinct statistical models: simple linear regression, pairwise correlation, and factor analysis, for a composite score of outcome assessment of a given behavioral.

Closed Head Injury Model

The research goal set forth in this paper was achieved with a closed head model that involved a weight drop injury and all behavioral tests were analyzed and ranked. Morris Water Maze and Elevated Plus Maze were the most powerful behavioral test examined, with Rotarod and Open Field having a potential impact. Each of the behavioral tests analyzed in this paper was analyzed to determine if they could detect differences between CHI and sham mice. The methods used here can be generalized to any animal

model of disease or injury and offer a powerful population-level analysis that may reveal insight into subtle phenotypic differences. Rapport (1954) stated that animal models serve as a “pragmatic device” to model a system and are effective as long as the animal study provides insight into the system (McClearn 2001).

Additionally, population-level analysis offers useful understanding into preclinical settings that can be further utilized and extrapolated into clinical domains to assess the severity of diseases/injuries, treatment efficiencies, and long-term impacts. Especially as traumatic brain injuries (TBI) continues to be prevalent across all ages and demographics and the number of individuals affected by TBI grows, the importance for preclinical models increases in a conjunctive format equitable to its translational ability. Furthermore, this method presents the ability to recognize subtle changes in animal models that may not have been captured from a single behavioral test or study.

Composite Score

The cumulative computational analysis generated a ranking of each dependent behavioral evaluated. Each behavioral was analyzed for an individual contribution in determining CHI versus sham for a given factor set, and thus the efficacy of each behavioral in assessing sham or CHI mice can be evaluated. The findings revealed that MWM V2 showed the most significant difference between sham and CHI mice, as shown by ranking 1st in the factor analysis. The Elevated plus maze proved to reliably measure a difference between CHI and Sham mice: the injury was strongly positively correlated with time spent in the open arm, and strongly negatively correlated with time spent in the closed arm of the maze. Notably, in data set 2, there were no visual trials included from

MWM. Therefore, EPM was ranked 2nd out of all the behavioral test. While all three variables of the Open Field behavioral test had a strong impact on factor 3 in data set 1, the factor was not statistically significant. This indicates that the Open Field behavioral test did not reliably measure differences between CHI and Sham animals. Therefore, it was concluded that the Rotarod behavioral was not ranked as an effective measure of the model for CHI according to the factor analysis, while it was significant for pairwise correlation and simple regression models.

In conclusion, the cumulative composite score ranks spatial learning and memory as the strongest clinical marker for rmTBI. Secondly, from EPM, exploratory activity, and impulsivity and with only a small weight on the overall composite score was a motor function. This paper suggest behavioral assessments in addition to current clinical and cognitive test prior to participating in activities that have a high chance of brain injury as well as post-injury for all brain related injuries. Visual and spatial learning test, such as the visual-spatial intelligence test (VSLT), may provide an individually tailored treatment plan (Malec 1991).

Future work

As concussion continues to be the most significant contributor to sports related injuries and the number of cases of traumatic brain injury grow each year, there remains a demand for preclinical models (Daneshvar 2011). This study revealed that the most powerful behavioral tests were the Morris Water Maze and Elevated Plus Maze in determining condition as a result of behavioral observation. While the loss of

consciousness (LOC) results from days 2, 3, 4, and Rotarod behavioral test held some power in simple linear regression and pairwise correlation; they did not play a powerful role when all variables were analyzed at once. However, there is a limitation to the factor analysis that analyzed all variables at once as a complete data set was required, which dropped a significant number of observations and dependent variables. Determining an accurate and efficient behavioral test in preclinical animal models is essential not only for clinical aspects of human patient population, but also for inter-lab comparisons. Therefore, future research labs may be able to extrapolate from this study the importance of the MWM and EPM and uses the ranked behavioral coefficients for a better interpretation and analysis of data.

Additionally, not all behavioral testing in animal models are feasible in a clinical setting or directly translatable. For example, in animal models, loss of consciousness is recorded, but when an individual sustains a head injury, it is unlikely that bystanders are recording the time the individual may be unconscious. Nor is a loss of consciousness relevant to the vast majority of mild traumatic brain injuries. Therefore, loss of consciousness may not be a behavioral change that can be reported in the clinical field. While a clinically administered test may be able to assess visual perception as well as the spatial learning and memory, similar to MWM in mice; it will be harder to administer a clinical test that assesses the exploratory activity and impulsivity behaviors assessed by EPM, though self-reported measures may potentially serve as proxies. Additionally, the specific type of motor function in the rotarod test may suggest further research in a broad range of motor skills including balance assessment that are already routinely used in the

assessment TBI. Furthermore, the severity and extent of skills is highly dependent on individual variations and specific test considerations. Overall, this method provided valuable insight into population level health of an animal model of rmTBI, and can assist in the assessment of other models of disease, and potential therapies both pre-clinically and clinically.

APPENDIX

1a. LOC1-LOC5 Simple Linear Regression outputs from Stata for all variables with p-values summarized in Table 10.

Source	SS	df	MS	Number of obs	=	840
Model	219127.494	5	43825.4987	F(5, 834)	=	153.09
Residual	238744.305	834	286.264155	Prob > F	=	0.0000
				R-squared	=	0.4786
				Adj R-squared	=	0.4755
Total	457871.799	839	545.735159	Root MSE	=	16.919

loc1	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-14.99148	3.751915	-4.00	0.000	-22.35579 -7.627178
time_postinjury	.0515047	.1042488	0.49	0.621	-.153116 .2561255
treatment_bi	-2.19875	1.322233	-1.66	0.097	-4.794047 .396546
height	1.153553	.0960058	12.02	0.000	.9651112 1.341994
numhits	-.8925123	.3163891	-2.82	0.005	-1.513525 -.2714998
_cons	34.32339	1.761034	19.49	0.000	30.86681 37.77997

Source	SS	df	MS	Number of obs	=	695
Model	46331.2698	5	9266.25396	F(5, 689)	=	50.19
Residual	127207.631	689	184.62646	Prob > F	=	0.0000
				R-squared	=	0.2670
				Adj R-squared	=	0.2617
Total	173538.901	694	250.056053	Root MSE	=	13.588

loc2	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-13.04471	3.43108	-3.80	0.000	-19.78134 -6.308084
time_postinjury	-.1452971	.0853537	-1.70	0.089	-.3128817 .0222876
treatment_bi	3.565947	1.147949	3.11	0.002	1.312049 5.819844
height	.769676	.0948177	8.12	0.000	.5835097 .9558423
numhits	-.1728892	.3440019	-0.50	0.615	-.8483071 .5025286
_cons	34.03014	2.029978	16.76	0.000	30.04446 38.01583

Source	SS	df	MS	Number of obs	=	676
Model	59159.8599	5	11831.972	F(5, 670)	=	57.70
Residual	137391.673	670	205.062198	Prob > F	=	0.0000
				R-squared	=	0.3010
				Adj R-squared	=	0.2958
Total	196551.533	675	291.187456	Root MSE	=	14.32

loc3	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-28.2707	3.779784	-7.48	0.000	-35.69235 -20.84906
time_postinjury	.0786073	.0904598	0.87	0.385	-.0990116 .2562262
treatment_bi	1.989774	1.224251	1.63	0.105	-.414056 4.393604
height	1.243784	.1064735	11.68	0.000	1.034722 1.452846
numhits	-.0348763	.3861659	-0.09	0.928	-.7931172 .7233646
_cons	35.75824	2.286992	15.64	0.000	31.26771 40.24878

Source	SS	df	MS	Number of obs	=	676
Model	35425.0024	5	7085.00047	F(5, 670)	=	35.94
Residual	132067.677	670	197.115935	Prob > F	=	0.0000
				R-squared	=	0.2115
				Adj R-squared	=	0.2056
Total	167492.679	675	248.137302	Root MSE	=	14.04

loc4	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-24.31913	3.705826	-6.56	0.000	-31.59556 -17.0427
time_postinjury	-.0190971	.0886898	-0.22	0.830	-.1932406 .1550463
treatment_bi	-.7687584	1.200296	-0.64	0.522	-3.125553 1.588036
height	1.017337	.1043902	9.75	0.000	.8123657 1.222308
numhits	.181935	.3786099	0.48	0.631	-.5614696 .9253397
_cons	36.54679	2.242243	16.30	0.000	32.14412 40.94946

Source	SS	df	MS	Number of obs	=	592
Model	57150.1877	5	11430.0375	F(5, 586)	=	10.73
Residual	624288.73	586	1065.33913	Prob > F	=	0.0000
				R-squared	=	0.0839
				Adj R-squared	=	0.0761
Total	681438.917	591	1153.02693	Root MSE	=	32.64

loc5	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-28.75671	9.08413	-3.17	0.002	-46.59813 -10.9153
time_postinjury	-.0508674	.2143253	-0.24	0.812	-.4718067 .3700718
treatment_bi	-4.118045	2.94387	-1.40	0.162	-9.899866 1.663776
height	1.254823	.2553254	4.91	0.000	.7533586 1.756287
numhits	-.5347754	.9152435	-0.58	0.559	-2.332332 1.262782
_cons	40.68351	5.551923	7.33	0.000	29.77942 51.5876

1b. LOC6-MWM H3. Simple Linear Regression outputs from Stata for all variables with p-values summarized in Table 10.

Source	SS	df	MS	Number of obs	=	208
Model	5931.82211	5	1186.36442	F(5, 202)	=	26.76
Residual	8955.69712	202	44.3351343	Prob > F	=	0.0000
				R-squared	=	0.3984
				Adj R-squared	=	0.3836
Total	14887.5192	207	71.9203828	Root MSE	=	6.6585

loc6	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-49.40248	33.77421	-1.46	0.145	-115.9977 17.19274
time_postinjury	-.0899247	.0505022	-1.78	0.076	-.1895038 .0096543
treatment_bi	9.759879	1.102686	8.85	0.000	7.585627 11.93413
height	1.932134	1.223044	1.58	0.116	-.4794359 4.343704
numhits	-.752866	.3536644	-2.13	0.034	-1.450214 -.0555184
_cons	35.16893	2.518621	13.96	0.000	30.20277 40.13509

Source	SS	df	MS	Number of obs	=	208
Model	2117.57622	5	423.515245	F(5, 202)	=	4.45
Residual	19219.3469	202	95.1452815	Prob > F	=	0.0007
				R-squared	=	0.0992
				Adj R-squared	=	0.0769
Total	21336.9231	207	103.076923	Root MSE	=	9.7542

loc7	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-99.75141	49.47716	-2.02	0.045	-197.3094 -2.193462
time_postinjury	-.0394134	.0739826	-0.53	0.595	-.1852907 .1064638
treatment_bi	1.362849	1.615368	0.84	0.400	-1.822297 4.547996
height	3.74905	1.791685	2.09	0.038	.216246 7.281853
numhits	.0501635	.5180969	0.10	0.923	-.9714083 1.071735
_cons	35.10239	3.689626	9.51	0.000	27.82727 42.37752

Source	SS	df	MS	Number of obs	=	859
Model	44235.7489	5	8847.14977	F(5, 853)	=	19.97
Residual	377813.919	853	442.923703	Prob > F	=	0.0000
				R-squared	=	0.1048
				Adj R-squared	=	0.0996
Total	422049.667	858	491.899379	Root MSE	=	21.046

mwm_h1	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	5.990214	4.228821	1.42	0.157	-2.309899 14.29033
time_postinjury	-.7023076	.1209649	-5.81	0.000	-.9397313 -.4648839
treatment_bi	-4.421107	1.588807	-2.78	0.006	-7.539536 -1.302678
height	.070445	.1027573	0.69	0.493	-.1312418 .2721319
numhits	1.592373	.3814203	4.17	0.000	.8437409 2.341006
_cons	37.68597	2.065667	18.24	0.000	33.63159 41.74036

Source	SS	df	MS	Number of obs	=	859
Model	36157.8202	5	7231.56403	F(5, 853)	=	19.01
Residual	324416.466	853	380.32411	Prob > F	=	0.0000
				R-squared	=	0.1003
				Adj R-squared	=	0.0950
Total	360574.286	858	420.249751	Root MSE	=	19.502

mwm_h2	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	16.02775	3.918607	4.09	0.000	8.336511 23.719
time_postinjury	-.2547246	.1120913	-2.27	0.023	-.4747317 -.0347176
treatment_bi	-1.415035	1.472257	-0.96	0.337	-4.304705 1.474635
height	-.0979475	.0952194	-1.03	0.304	-.2848392 .0889443
numhits	.9327087	.3534405	2.64	0.008	.2389937 1.626424
_cons	21.24385	1.914136	11.10	0.000	17.48688 25.00082

Source	SS	df	MS	Number of obs	=	859
Model	37758.4953	5	7551.69906	F(5, 853)	=	22.41
Residual	287438.375	853	336.973476	Prob > F	=	0.0000
				R-squared	=	0.1161
				Adj R-squared	=	0.1109
Total	325196.871	858	379.017332	Root MSE	=	18.357

mwm_h3	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	17.2084	3.688524	4.67	0.000	9.968757 24.44805
time_postinjury	-.253973	.1055098	-2.41	0.016	-.4610622 -.0468838
treatment_bi	.7893347	1.385812	0.57	0.569	-1.930667 3.509337
height	-.1203168	.0896285	-1.34	0.180	-.2962351 .0556015
numhits	.5171972	.3326881	1.55	0.120	-.135786 1.17018
_cons	16.75203	1.801747	9.30	0.000	13.21565 20.2884

1c. MWM H4-MWM P1. Simple Linear Regression outputs from Stata for all variables with p-values summarized in Table 10.

Source	SS	df	MS	Number of obs	=	834
Model	42438.9748	5	8487.79497	F(5, 828)	=	27.26
Residual	257782.584	828	311.331623	Prob > F	=	0.0000
				R-squared	=	0.1414
				Adj R-squared	=	0.1362
Total	300221.558	833	360.410034	Root MSE	=	17.645

mwm_h4	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	14.64984	3.728428	3.93	0.000	7.331562 21.96813
time_postinjury	-.3494795	.102112	-3.42	0.001	-.5499083 -.1490507
treatment_bi	-2.033274	1.371658	-1.48	0.139	-4.725609 .6590619
height	-.0271723	.0921581	-0.29	0.768	-.2080633 .1537187
numhits	.8791102	.3244531	2.71	0.007	.242263 1.515957
_cons	14.40248	1.7781	8.10	0.000	10.91237 17.8926

Source	SS	df	MS	Number of obs	=	347
Model	18344.1168	5	3668.82335	F(5, 341)	=	20.64
Residual	60616.8311	341	177.761968	Prob > F	=	0.0000
				R-squared	=	0.2323
				Adj R-squared	=	0.2211
Total	78960.9479	346	228.210832	Root MSE	=	13.333

mwm_h5	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	28.19181	3.596608	7.84	0.000	21.11748 35.26614
time_postinjury	-.4756724	.0906778	-5.25	0.000	-.6540306 -.2973142
treatment_bi	-.0011925	1.682443	-0.00	0.999	-3.310465 3.30808
height	-.5271013	.0905928	-5.82	0.000	-.7052925 -.3489102
numhits	-.1580824	.3109871	-0.51	0.612	-.7697768 .4536121
_cons	16.0332	1.926274	8.32	0.000	12.24433 19.82208

Source	SS	df	MS	Number of obs	=	835
Model	21259.3107	5	4251.86214	F(5, 829)	=	13.07
Residual	269734.269	829	325.373062	Prob > F	=	0.0000
				R-squared	=	0.0731
				Adj R-squared	=	0.0675
Total	290993.579	834	348.913165	Root MSE	=	18.038

mwm_v1	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-2.286498	3.675969	-0.62	0.534	-9.501798 4.928803
time_postinjury	-.0255256	.1044753	0.24	0.807	-.1795417 .2305928
treatment_bi	-.7493002	1.394327	-0.54	0.591	-3.486127 1.987527
height	.2717571	.088745	3.06	0.002	.0975658 .4459485
numhits	1.78402	.3314226	5.38	0.000	1.133494 2.434546
_cons	8.337251	1.778698	4.69	0.000	4.84597 11.82853

Source	SS	df	MS	Number of obs	=	768
Model	32475.0435	5	6495.00869	F(5, 762)	=	23.52
Residual	210389.651	762	276.101905	Prob > F	=	0.0000
				R-squared	=	0.1337
				Adj R-squared	=	0.1280
Total	242864.695	767	316.642366	Root MSE	=	16.616

mwm_v2	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-5.69761	3.867073	-1.47	0.141	-13.28899 1.893771
time_postinjury	.1503809	.0992475	1.52	0.130	-.0444501 .3452119
treatment_bi	.4461583	1.362117	0.33	0.743	-2.227789 3.120106
height	.4388259	.0977679	4.49	0.000	.2468995 .6307522
numhits	2.1147	.3207738	6.59	0.000	1.484994 2.744405
_cons	1.263192	1.782721	0.71	0.479	-2.236437 4.76282

Source	SS	df	MS	Number of obs	=	835
Model	7066.57954	5	1413.31591	F(5, 829)	=	23.48
Residual	49907.4665	829	60.2020103	Prob > F	=	0.0000
				R-squared	=	0.1240
				Adj R-squared	=	0.1187
Total	56974.0461	834	68.3142039	Root MSE	=	7.759

mwm_pl	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	3.440815	1.645568	2.09	0.037	.2108463 6.670784
time_postinjury	-.4207638	.0457537	-9.20	0.000	-.5105705 -.330957
treatment_bi	-.7661591	.5952372	-1.29	0.198	-1.934508 .4021901
height	-.1403536	.0407183	-3.45	0.001	-.2202766 -.0604305
numhits	-.0555644	.1421163	-0.39	0.696	-.3345145 .2233857
_cons	18.91643	.7759669	24.38	0.000	17.39333 20.43952

1d. MWM P2-EPM Closed. Simple Linear Regression outputs from Stata for all variables with p-values summarized in Table 10.

Source	SS	df	MS	Number of obs	=	786
Model	180006.907	5	36001.3813	F(5, 780)	=	206.48
Residual	135997.877	780	174.356252	Prob > F	=	0.0000
				R-squared	=	0.5696
				Adj R-squared	=	0.5669
Total	316004.783	785	402.553864	Root MSE	=	13.204

mwm_p2	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	17.2277	3.058384	5.63	0.000	11.22406 23.23133
time_postinjury	2.168654	.07877	27.53	0.000	2.014028 2.32328
treatment_bi	3.772569	1.079789	3.49	0.001	1.652932 5.892205
height	-.4516939	.0767672	-5.88	0.000	-.6023887 -.3009992
numhits	-.6351843	.2491816	-2.55	0.011	-1.12433 -.1460383
_cons	11.69169	1.365261	8.56	0.000	9.011665 14.37171

Source	SS	df	MS	Number of obs	=	403
Model	47578.0293	5	9515.60586	F(5, 397)	=	117.63
Residual	32115.4505	397	80.8953412	Prob > F	=	0.0000
				R-squared	=	0.5970
				Adj R-squared	=	0.5919
Total	79693.4797	402	198.242487	Root MSE	=	8.9942

mwm_pl_freq	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	35.50778	4.852376	7.32	0.000	25.96821 45.04734
time_postinjury	.7525452	.0764959	9.84	0.000	.6021575 .9029328
treatment_bi	6.13418	1.060269	5.79	0.000	4.049736 8.218624
height	-.8069252	.1089307	-7.41	0.000	-1.021078 -.5927721
numhits	-.8444423	.2432628	-3.47	0.001	-1.322687 -.366198
_cons	5.048447	1.161277	4.35	0.000	2.765427 7.331468

Source	SS	df	MS	Number of obs	=	403
Model	42446.3476	5	8489.26952	F(5, 397)	=	112.44
Residual	29973.0615	397	75.4988955	Prob > F	=	0.0000
				R-squared	=	0.5861
				Adj R-squared	=	0.5809
Total	72419.4091	402	180.147784	Root MSE	=	8.689

mwm_p2_freq	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	29.87043	4.687735	6.37	0.000	20.65455 39.08632
time_postinjury	.769713	.0739004	10.42	0.000	.624428 .914998
treatment_bi	5.432553	1.024294	5.30	0.000	3.418835 7.446272
height	-.68049	.1052347	-6.47	0.000	-.8873769 -.4736031
numhits	-.6448865	.2350089	-2.74	0.006	-1.106904 -.1828691
_cons	4.102006	1.121875	3.66	0.000	1.896448 6.307564

Source	SS	df	MS	Number of obs	=	829
Model	3940.73684	5	788.147367	F(5, 823)	=	4.61
Residual	140678.386	823	170.93364	Prob > F	=	0.0004
				R-squared	=	0.0272
				Adj R-squared	=	0.0213
Total	144619.123	828	174.660776	Root MSE	=	13.074

epm_open	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	.2912251	2.451896	0.12	0.905	-4.521481 5.103931
time_postinjury	-.0689033	.0682574	-1.01	0.313	-.2028024 .0650758
treatment_bi	-2.197629	1.00613	-2.18	0.029	-4.172512 -.2227452
height	.09517	.0607676	1.57	0.118	-.0241077 .2144476
numhits	.4466103	.2517397	1.77	0.076	-.0475171 .9407377
_cons	9.14291	1.400792	6.53	0.000	6.393364 11.89246

Source	SS	df	MS	Number of obs	=	829
Model	7506.70148	5	1501.3403	F(5, 823)	=	5.35
Residual	231158.472	823	280.872991	Prob > F	=	0.0001
				R-squared	=	0.0315
				Adj R-squared	=	0.0256
Total	238665.173	828	288.242963	Root MSE	=	16.759

epm_closed	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-2.345904	3.142992	-0.75	0.456	-8.515128 3.82332
time_postinjury	-.3154736	.0874965	-3.61	0.000	-.4872162 -.1437309
treatment_bi	1.300029	1.28972	1.01	0.314	-1.231499 3.831557
height	-.0521917	.0778956	-0.67	0.503	-.2050892 .1007057
numhits	-.7400767	.3226955	-2.29	0.022	-1.37348 -.1066737
_cons	91.74151	1.795622	51.09	0.000	88.21697 95.26605

1e. EPM Decision-Roto_Day1. Simple Linear Regression outputs from Stata for all variables with p-values summarized in Table 10.

Source	SS	df	MS	Number of obs	=	131
Model	137925.803	5	27585.1605	F(5, 125)	=	50.40
Residual	68419.686	125	547.357488	Prob > F	=	0.0000
				R-squared	=	0.6684
				Adj R-squared	=	0.6552
Total	206345.489	130	1587.27299	Root MSE	=	23.396

epm_decision	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	52.58547	21.32359	2.47	0.015	10.38343 94.7875
time_postinjury	2.342261	.3063216	7.65	0.000	1.736013 2.94851
treatment_bi	3.48457	4.943939	0.70	0.482	-6.300099 13.26924
height	-1.277122	.5589777	-2.28	0.024	-2.383408 -.1708359
numhits	-.1875205	1.163387	-0.16	0.872	-2.490008 2.114967
_cons	7.2284	7.243731	1.00	0.320	-7.107842 21.56464

Source	SS	df	MS	Number of obs	=	498
Model	4150.7605	5	830.152099	F(5, 492)	=	4.83
Residual	84627.0033	492	172.006104	Prob > F	=	0.0003
				R-squared	=	0.0468
				Adj R-squared	=	0.0371
Total	88777.7638	497	178.627291	Root MSE	=	13.115

of_wall	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-2.214211	3.538461	-0.63	0.532	-9.166569 4.738147
time_postinjury	.1568354	.0862258	1.82	0.070	-.0125809 .3262517
treatment_bi	.9455033	1.380384	0.68	0.494	-1.766671 3.657678
height	.0231296	.0849928	0.27	0.786	-.143864 .1901232
numhits	1.28088	.32456	3.95	0.000	.643185 1.918574
_cons	54.81999	1.809627	30.29	0.000	51.26444 58.37554

Source	SS	df	MS	Number of obs	=	476
Model	1754.92428	5	350.984855	F(5, 470)	=	3.16
Residual	52187.3369	470	111.036887	Prob > F	=	0.0081
				R-squared	=	0.0325
				Adj R-squared	=	0.0222
Total	53942.2611	475	113.562655	Root MSE	=	10.537

of_neutral	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	5.885746	3.009573	1.96	0.051	-.0281385 11.79963
time_postinjury	.0560037	.0928437	0.60	0.547	-.1264365 .2384439
treatment_bi	-1.486974	1.122382	-1.32	0.186	-3.692483 .7185333
height	-.114887	.0717214	-1.60	0.110	-.2558213 .0260473
numhits	-.968701	.2721968	-3.56	0.000	-1.503574 -.4338278
_cons	35.28029	1.566821	22.52	0.000	32.20145 38.35913

Source	SS	df	MS	Number of obs	=	476
Model	150.843998	5	30.1687997	F(5, 470)	=	1.13
Residual	12586.7175	470	26.78025	Prob > F	=	0.3453
				R-squared	=	0.0118
				Adj R-squared	=	0.0013
Total	12737.5615	475	26.8159189	Root MSE	=	5.175

of_center	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-.9908894	1.478014	-0.67	0.503	-3.895223 1.913444
time_postinjury	.034014	.0455959	0.75	0.456	-.0555832 .1236111
treatment_bi	.8521004	.5512068	1.55	0.123	-.2310343 1.935235
height	.0201784	.0352227	0.57	0.567	-.049035 .0893918
numhits	-.0404668	.133677	-0.30	0.762	-.3031453 .2222117
_cons	7.772182	.7694725	10.10	0.000	6.26015 9.284214

Source	SS	df	MS	Number of obs	=	667
Model	60774.272	5	12154.8544	F(5, 661)	=	20.07
Residual	400261.567	661	605.539436	Prob > F	=	0.0000
				R-squared	=	0.1318
				Adj R-squared	=	0.1253
Total	461035.839	666	692.246005	Root MSE	=	24.608

roto_day1	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-32.3232	5.890185	-5.49	0.000	-43.88893 -20.75747
time_postinjury	.6123769	.1867229	3.28	0.001	.2457354 .9790185
treatment_bi	5.605206	2.045202	2.74	0.006	1.58933 9.621082
height	.4444743	.1405484	3.16	0.002	.1684991 .7204495
numhits	-.5534258	.5571016	-0.99	0.321	-1.647328 .5404762
_cons	84.61233	3.240838	26.11	0.000	78.24875 90.97591

1f. Roto_day2-NO_noveloject. Simple Linear Regression outputs from Stata for all variables with p-values summarized in Table 10.

Source	SS	df	MS	Number of obs	=	667
Model	69197.8908	5	13839.5782	F(5, 661)	=	14.44
Residual	633711.398	661	958.716184	Prob > F	=	0.0000
				R-squared	=	0.0984
				Adj R-squared	=	0.0916
Total	702909.289	666	1055.41935	Root MSE	=	30.963

roto_day2	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-39.36648	7.411442	-5.31	0.000	-53.91928 -24.81367
time_postinjury	.0063002	.2349478	0.03	0.979	-.4550338 .4676342
treatment_bi	4.443832	2.573417	1.73	0.085	-.6092243 9.496888
height	.5753765	.1768479	3.25	0.001	.2281252 .9226278
numhits	-1.468983	.7009842	-2.10	0.036	-2.845407 -.0925593
_cons	110.0306	4.077849	26.98	0.000	102.0235 118.0377

Source	SS	df	MS	Number of obs	=	157
Model	15948.8959	5	3189.77919	F(5, 151)	=	18.28
Residual	26355.9462	151	174.54269	Prob > F	=	0.0000
				R-squared	=	0.3770
				Adj R-squared	=	0.3564
Total	42304.8421	156	271.184885	Root MSE	=	13.211

fst_timeimmob-e	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	1.846541	12.25252	0.15	0.880	-22.36198 26.05506
time_postinjury	.4369586	.247616	1.76	0.080	-.0522808 .926198
treatment_bi	-.5205066	2.673644	-0.19	0.846	-5.80309 4.762077
height	-.1233113	.2743664	-0.45	0.654	-.6654041 .4187815
numhits	-4.850768	.9379905	-5.17	0.000	-6.704049 -2.997487
_cons	66.58677	2.94235	22.63	0.000	60.77327 72.40026

Source	SS	df	MS	Number of obs	=	105
Model	43669.924	5	8733.98481	F(5, 99)	=	43.18
Residual	20022.4133	99	202.246599	Prob > F	=	0.0000
				R-squared	=	0.6856
				Adj R-squared	=	0.6698
Total	63692.3374	104	612.426321	Root MSE	=	14.221

fst_timeimmob-2	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	8.393927	14.36582	0.58	0.560	-20.11097 36.89882
time_postinjury	-1.27512	.3693008	-3.45	0.001	-2.007893 -.5423468
treatment_bi	6.472882	4.656866	1.39	0.168	-2.767351 15.71311
height	-.3096529	.3214825	-0.96	0.338	-.9475439 .3282382
numhits	-10.67505	1.289946	-8.28	0.000	-13.23458 -8.11552
_cons	92.0882	3.754517	24.53	0.000	84.63842 99.53797

note: treatment_bi omitted because of collinearity

Source	SS	df	MS	Number of obs	=	53
Model	4343.38189	4	1085.84547	F(4, 48)	=	9.19
Residual	5673.10813	48	118.189753	Prob > F	=	0.0000
				R-squared	=	0.4336
				Adj R-squared	=	0.3864
Total	10016.49	52	192.624808	Root MSE	=	10.872

fst_timeimmob-3	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	165.4672	90.84182	1.82	0.075	-17.1825 348.1169
time_postinjury	-1.824927	.3883302	-4.70	0.000	-2.605717 -1.044137
treatment_bi	0	(omitted)			
height	-5.823859	3.335513	-1.75	0.087	-12.53036 .882639
numhits	3.635021	.9748621	3.73	0.001	1.674929 5.595113
_cons	16.76483	5.226402	3.21	0.002	6.256447 27.27322

Source	SS	df	MS	Number of obs	=	156
Model	71948.1047	5	14389.6209	F(5, 150)	=	30.22
Residual	71432.3177	150	476.215451	Prob > F	=	0.0000
				R-squared	=	0.5018
				Adj R-squared	=	0.4852
Total	143380.422	155	925.034983	Root MSE	=	21.822

no_noveloject-e	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	-9.809131	11.04022	-0.89	0.376	-31.62356 12.0053
time_postinjury	4.498919	.4278311	10.52	0.000	3.653565 5.344273
treatment_bi	-6.133707	3.761798	-1.63	0.105	-13.56666 1.299249
height	.4213479	.2695091	1.56	0.120	-.1111765 .9538723
numhits	-4.8821	.7980518	-6.12	0.000	-6.458975 -3.305225
_cons	41.7577	4.644136	8.99	0.000	32.58132 50.93407

note: condition_lchi omitted because of collinearity
note: treatment_bi omitted because of collinearity
note: numhits omitted because of collinearity

1g. LD_light-Sucrose_day4. Simple Linear Regression outputs from Stata for all variables with p-values summarized in Table 10.

Source	SS	df	MS	Number of obs	=	48
Model	1071.27461	2	535.637306	F(2, 45)	=	6.52
Residual	3697.32586	45	82.162797	Prob > F	=	0.0033
				R-squared	=	0.2247
				Adj R-squared	=	0.1902
				Root MSE	=	9.0644
Total	4768.60048	47	101.459585			

ld_light	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	0 (omitted)				
time_postinjury	0 (omitted)				
treatment_bi	-10.19264	3.700804	-2.75	0.010	-17.7734 -2.61189
height	.0944525	.1217475	0.78	0.444	-.154936 .343841
numhits	0 (omitted)				
_cons	35.57956	3.756459	9.47	0.000	27.88481 43.27432

note: condition_lchi omitted because of collinearity
 note: time_postinjury omitted because of collinearity
 note: treatment_bi omitted because of collinearity
 note: numhits omitted because of collinearity

Source	SS	df	MS	Number of obs	=	24
Model	20.9357811	1	20.9357811	F(1, 22)	=	0.56
Residual	819.597995	22	37.2544543	Prob > F	=	0.4614
				R-squared	=	0.0249
				Adj R-squared	=	-0.0194
				Root MSE	=	6.1036
Total	840.533776	23	36.5449468			

tailsuspensio-e	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	0 (omitted)				
time_postinjury	0 (omitted)				
treatment_bi	0 (omitted)				
height	-.0770336	.1027602	-0.75	0.461	-.2901452 .1360779
numhits	0 (omitted)				
_cons	60.88106	2.491802	24.43	0.000	55.71338 66.04874

note: condition_lchi omitted because of collinearity
 note: time_postinjury omitted because of collinearity
 note: treatment_bi omitted because of collinearity
 note: numhits omitted because of collinearity

Source	SS	df	MS	Number of obs	=	24
Model	93.0157542	1	93.0157542	F(1, 22)	=	0.09
Residual	22757.4612	22	1034.43005	Prob > F	=	0.7671
				R-squared	=	0.0041
				Adj R-squared	=	-0.0412
				Root MSE	=	32.163
Total	22850.4769	23	993.498997			

sucrose_day2	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	0 (omitted)				
time_postinjury	0 (omitted)				
treatment_bi	0 (omitted)				
height	-.1623731	.5414848	-0.30	0.767	-1.285344 .9605977
numhits	0 (omitted)				
_cons	50.37997	13.13031	3.84	0.001	23.14937 77.61056

note: condition_lchi omitted because of collinearity
 note: time_postinjury omitted because of collinearity
 note: treatment_bi omitted because of collinearity
 note: numhits omitted because of collinearity

Source	SS	df	MS	Number of obs	=	24
Model	1433.3971	1	1433.3971	F(1, 22)	=	2.77
Residual	11364.475	22	516.567045	Prob > F	=	0.1099
				R-squared	=	0.1120
				Adj R-squared	=	0.0716
				Root MSE	=	22.728
Total	12797.8721	23	556.429221			

sucrose_day3	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	0 (omitted)				
time_postinjury	0 (omitted)				
treatment_bi	0 (omitted)				
height	.6374101	.3826477	1.67	0.110	-.1561525 1.430973
numhits	0 (omitted)				
_cons	30.66111	9.278713	3.30	0.003	11.41824 49.90398

note: condition_lchi omitted because of collinearity
 note: time_postinjury omitted because of collinearity
 note: treatment_bi omitted because of collinearity
 note: numhits omitted because of collinearity

Source	SS	df	MS	Number of obs	=	24
Model	1147.07515	1	1147.07515	F(1, 22)	=	2.25
Residual	11229.4999	22	510.431813	Prob > F	=	0.1481
				R-squared	=	0.0927
				Adj R-squared	=	0.0514
				Root MSE	=	22.593
Total	12376.575	23	538.111958			

sucrose_day4	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
condition_lchi	0 (omitted)				
time_postinjury	0 (omitted)				
treatment_bi	0 (omitted)				
height	.5702058	.3803685	1.50	0.148	-.2186302 1.359042
numhits	0 (omitted)				
_cons	33.84966	9.223447	3.67	0.001	14.72141 52.97792

REFERENCES

- Albayram O et al. (2017). *Cis P-Tau* is induced in clinical and preclinical brain injury and contributes to post-injury sequelae. *Nature Communication*, 8: 1000.
- Armstrong, RA. (2014). When to use the Bonferroni Correction. *Ophthalmic and Physiological Optics*, 34 (5) 502-508.
- Bourin M, Hascoet M. (2003). The Mouse Light/Dark Box Test. *European Journal of Pharmacology*, 463: 55-65.
- Brooks B, Silverberg N, Maxwell B, Mannix R, Zafonte R, Berkner P, Iverson G. (2018). Investigating Effects of Sex Differences and Prior Concussions on Symptom Reporting and Cognition Among Adolescent Soccer Players. *The American Journal of Sports Medicine*, 46(4), 961-968.
- Centers for Disease Control and Prevention. HEADS UP. Retrieved April 19, 2018, from https://www.cdc.gov/headsup/basics/concussion_what.html.
- Concussion Legacy Foundation. What is CTE? *Concussion Legacy Foundation*.
- Cryan JF, Mombereau C, Vassout A. (2009). The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. *Neuroscience & Biobehavioral Reviews*, 29 (4-5): 571-625.
- Cunha JP. (2019). Memantine. *RxList Inc*. Web.
- Daneshvar DH, Riley RO, Nowinski CJ, Mckee AC, Stern RA, Cantu RC. (2011). Long Term Consequences: Effects on Normal Development Profile after Concussion. *Physical Medicine and Rehabilitation Clinics of North America*. 22 (4): 683-700.
- Ennaceur A , Delacour J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1:Behavioral data. *Behavioural Brain Research*, 31: 47-59.
- Ericsson AC, Crim MJ, Franklin CL. (2013). A brief history of animal modeling. *Missouri Medicine*, 110 (3): 201-205.
- Faul M, Xu L, Wald MM, Coronado VG. (2010). Traumatic brain injury in the United States: Emergency department visits, hospitalizations and deaths 2002-2006. *Centers of Disease Control and Prevention, National Center for Injury Prevention and Control*.
- Field A. (2013). *Discovering Statistics using IBM SPSS Statistics, 4th edition*, SAGE Publications Ltd.

- Gardner RC, Yaffe K (2015). Epidemiology of mild traumatic brain injury and neurodegenerative disease. *Molecular Cell Neuroscience*, 66 (B): 75-80.
- Giza CC, Hovda DA. (2014). The New Neurometabolic Cascade of Concussion. *Neurosurgery*, 75 (4): 24-33.
- Graham R, Rivara FP, Ford MA, et al. (2014). Sports-Related Concussions in Youth: Improving the Science, Changing the Culture Committee on Sports-Related Concussions in Youth, Board on Children, Youth, and Families, Institute of Medicine, *National Academies Press (US)*.
- Higgins KL, Caze T, Maerlender A. (2018). Validity and Reliability of Baseline Testing in a Standardized Environment. *Archived Clinical Neuropsychology*, 33 (4):437-443.
- Holmes L, Tworig J, Casini J, et al. (2016). Implication of Socio-Demographics on Cognitive-Related Symptoms in Sports Concussion Among Children. *Sports Medicine – Open*, 2, 38.
- Howell, D. R., O'Brien, M. J., Beasley, M. A., Mannix, R. C., & Meehan, W. P. (2016). Initial somatic symptoms are associated with prolonged symptom duration following concussion in adolescents. *Acta Paediatrica Oslo, Norway : 1992*, 105(9), e426–e432.
- Iqbal K, Liu F, Gong C-X, Grundke-Iqbal I. (2010). Tau in Alzheimer Disease and Related Tauopathies. *Current Alzheimer research*, 7(8), 656-664.
- Kalish BT, Whalen MJ (2016). Weight Drop Models in Traumatic Brain Injury. *Methods in Molecular Biology*, 1462:193-209.
- Kleinbaum D, Kupper L, Muller K. (1988). Variable reduction and factor analysis. *Applied Regression Analysis and Other Multivariable Methods*. Nelson Education, 595-640.
- Kondo A, et al. (2015). Antibody against early driver of neurodegeneration cis P-tau blocks brain injury and tauopathy. *Nature*, 523 (7561): 431-436.
- Lipton ML, et al. (2013). Soccer heading is associated with white matter microstructural and cognitive abnormalities. *Radiology*, 268 (3): 850-857.
- Liu X, Qui J, Alcon S, Hashim J, Meehan WP, Mannix R. (2017). Environmental Enrichment Mitigates Deficits after repetitive Mild A Traumatic Brain Injury. *Journal of Neurotrauma*, 34: 2445-2455.

- Maher ME, et al. (2014). Concussions and heading in soccer: a review of the evidence of incidence, mechanisms, biomarkers and neurocognitive outcomes. *Brain Injury*, 28 (3): 271-285.
- Malec JF, Ivnik RJ, Hinkeldey NS. (1991). Visual Spatial Learning Test. *Psychological Assessment*, 3 (1): 82-88.
- Mannix RC, Meehan WP, Mandeville J, et al. (2013). Clinical correlates in an experimental model of repetitive mild brain injury. *Annals of Neurology*, 74 (1).
- Mannix RC, Berglass J, Berkner J, Moleus P, Qiu J, Andrews N, Gunner G, Berglass L, Jantzie LL, Robinson S, Meehan WP. (2014). Chronic gliosis and behavioral deficits in mice following repetitive mild traumatic brain injury. *Journal of Neurosurgery*, 121: 1342–1350.
- Mannix R, Berkner J, Mei Z, Alcon S, Hashim J, Robinson S, Jantzie L, Meehan WP, Qiu J. (2016). Adolescent Mice Demonstrate a Distinct Pattern of Injury after Repetitive Mild Traumatic Brain Injury. *Journal of Neurotrauma*, 34(2):495-504.
- Marchi N, et al. (2013). Consequences of Repeated Blood-Brain Barrier Disruption in Football Players. *Plos One*, 8 (3): e56805.
- Mayo Clinic. (2016). Chronic Traumatic Encephalopathy. *Mayo Foundation for Medical Education and Research*.
- McAllister T, McCrea M (2017). Long-Term Cognitive and Neuropsychiatric Consequences of Repetitive Concussion and Head-Impact Exposure. *Journal of Athletic Training*: March 2017, Vol. 52, No. 3, pp. 309-317.
- McClearn GE. (2001). The relevance of animal models for human populations. *Cells and Surveys: Should Biological Measures be Included in Social Science Research?* 9.
- McKee AC, Stein TD, Nowinski CJ, et al. (2013). The spectrum of disease in chronic traumatic encephalopathy. *Brain*, 136(1), 43-64.
- McKee AC, Cantu RC, Nowinski CJ, Hedley-Whyte ET, Gavett BE, Budson AE, Santini VE, Lee H-Y, Kubilus CA, Stern RA. (2009). Chronic Traumatic Encephalopathy in Athletes: Progressive Tauopathy following Repetitive Head Injury. *Journal of Neuropathology & Experimental Neurology*, 68(7), 709-735.
- Meehan WP, Mannix RC, O'Brien MJ, Collins MW. (2013). The Prevalence of Undiagnosed Concussions in Athletes. *Clinical journal of sport medicine: official journal of the Canadian Academy of Sport Medicine*, 23(5), 339-342.

- Meehan WP, Zhang J, Mannix RC, Whalen MJ. (2012) Increasing recovery time between injuries improves cognitive outcome after repetitive mild concussive brain injuries in mice. *Neurosurgery*, 71 (4): 885-892.
- Mei Z, Qiu J, Alcon S, Hashim J, Rotenberg A, Sun Y, Meehan WP, Mannix R. (2018). Memantine improves outcomes after repetitive traumatic brain injury. *Behavioural Brain Research*, 340: 195-204.
- Mez J, Daneshvar DH, Kiernan PT, et al. (2017). Clinicopathological Evaluation of Chronic Traumatic Encephalopathy in Players of American Football. *Journal of the American Medical Association*, 318(4), 360–370.
- NIH, National Institute of Neurological Disorders and Stroke. Traumatic Brain Injury: Hope Through Research. *National Institutes of Health (NIH)*.
- Omalu BI, DeKosky ST, Minster RL, Kamboh MI, Hamilton RL, Wecht CH. (2005). Chronic traumatic encephalopathy in a National Football League player. *Neurosurgery*, 57(1), 128–134.
- Osborne JW. (2015). What is Rotating in Exploratory Factor Analysis? *Practical Assessment, Research and Evaluation*, 20 (2).
- Papa L, Edwards D, Ramia M. Exploring Serum Biomarkers for Mild Traumatic Brain Injury. *Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects*, 22.
- Papa L, Mendes ME, Braga CF. (2012). Mild Traumatic Brain Injury among the Geriatric Population. *Current translational geriatrics and experimental gerontology reports*, 1(3), 135-142.
- Peters R. (2006). Ageing and the brain. *Postgraduate Medical Journal*, 82(964):84-88.
- Lu KP, et al. (2016). Potential of the Antibody Against cis-Phosphorylated Tau in the Early Diagnosis, Treatment, and Prevention of Alzheimer Disease and Brain Injury. *Clinical Implications of Basic Neuroscience Research*, 73 (11): 1356-1362.
- Porsolt RD, Bertin A, Jalfre M. (1977). Behavioral despair in mice: a primary screening test for antidepressants. *Archives Internationales de pharmacodynamie et de therapie*, 229: 327–336.
- Rapaport A (1954). Operational Philosophy: Integrating Knowledge and Action. *Harper and Brothers Publishers*.

- Røe C, Sveen U, Alvsåker K, Bautz-Holter E. (2009) Post-concussion symptoms after mild traumatic brain injury: influence of demographic factors and injury severity in a 1-year cohort study, *Disability and Rehabilitation*, 31(15):1235-1243.
- StataCorp. (2017). *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC
- Stump S. (2015). No more heading: US Soccer out with new guidelines for youth soccer. *Today News: NBC Universal 2019*.
- Serchov T, Calker D, Biber K. (2016). Sucrose preference test to measure anhedonic behaviour in mice. *Bio-protocol*, 6:(19).
- Tabachnick BG, Fidell LS. (2014). *Using Multivariate Statistics*, 6th edition, *Pearson*.
- Takao K, Miyakawa T. (2006). Light/Dark Transition Test for Mice. *Journal of Visualized Experiments*, 1: 104.
- Tagge CA, et al. (2018). Concussion, microvascular injury and early tauopathy in young athletes after impact head injury and an impact concussion mouse model. *Brain: A Journal of Neurology*, 141 (2): 422-458.
- Taylor CA, Bell JM, Breiding MJ, Xu L. (2017). Traumatic Brain Injury–Related Emergency Department Visits, Hospitalizations, and Deaths — United States, 2007 and 2013. *CDC:MMWR*, 66(9):1-16.
- Viewpoint Behavior Technology (2019). Marlau Cage: Environmental Enrichment Cage. <http://www.viewpoint.fr/en/p/equipment/marlau-enrichment-cage>
- Washington PM, Forcelli PA, Wilkins T, Zapple DN, Parsadonian M, Burns MP. (2012). The Effect of Injury Severity on Behavior: A Phenotypic Study of Cognitive and Emotional Deficits after Mild, Moderate, and Severe Controlled Cortical Impact Injury in Mice. *Journal of Neurotrauma*, 29: 2283-2296.
- Weible AP, Rowland DC, Pang R, Kentros C.(2009). Neural Correlates of NOvel Object and Novel Location REcognition Behavior in the MOuse Anterior Cingulate Cortex. *Journal of Neurophysiology*, 102: 2055-2068.
- Zaloshnja E, Miller T, Langlois J A, Selassie AW. (2008). Prevalence of long-term disability from traumatic brain injury in the civilian population of the United States, 2005. *The Journal of Head Trauma Rehabilitation*, 23(6), 394-400.

CURRICULUM VITAE

