ABSTRACT

Abad-Jacobi, Christopher., Persistent Deficits in Cognitive Ability Due to Repetitive Mild Traumatic Brain Injury and Treatment With Sigma-1 Receptor Agonist PRE-084. Masters of Science (Pharmacology and Neuroscience), April 2021, 31 pp, 18 figures.

An estimated 1.6 – 3.8 million sports-related traumatic brain injuries (TBI) occur every year in the U.S. Recent retrospective studies suggest that repetitive mild TBI (rmTBI) is associated with the earlier onset of neurodegenerative diseases. Mild TBI can be hard to detect, and there are currently no widely accepted biomarkers that could aid in the diagnosis of mTBI. Further, there is currently no standard pharmacological treatment for TBI.

Our previous work demonstrated neurological deficits 1 week following 20-25 rmTBI in young male mice. We hypothesized that some of these deficits would persist up to 5-15 weeks following injury and that treatment with an agonist of the Sigma-1 receptor (PRE-084) could reduce these deficits, as has been demonstrated in other neurodegenerative models.

Eight-week-old male C57BK6 mice were divided into sham injury + Vehicle, rmTBI + Vehicle, and rmTBI+PRE084 groups (n=10/gp). Mice were lightly anesthetized with isoflurane and administered either PRE084 (1mg/kg sc or ip) or vehicle immediately prior to experiencing closed head-injury with rotational acceleration via a 65g weight drop 5 days a week for 5 weeks. Five (group 1) and fifteen weeks (group 2) after the final injury mice were assessed for neurological deficits. Injured mice in test 1 demonstrated significant ($P<0.05$) deficits in motor and vestibular-motor. Wake times were significantly increased ($P<0.05$) for Hit mice in both tests one and two. However, cognitive performance in T-maze active avoidance, anxiety-related behavior in the elevated plus maze, and Water Maze on group 1 were not affected. Water maze data on group 2 yielded significant results ($P<0.05$) indicating both groups of Hit mice

performed worse on percent time in annulus 40 centimeters, and on path length in trials one and three. Treatment with PRE-084 did not ameliorate any of these deficits. On group 2, Hit + PRE-084 mice performed significantly worse than their counterparts on the rotarod test. The data suggest that there are some chronic deficits for at least 5 weeks after rmTBI, and that sigma-1 activation does not reverse negative effects of rmTBI. Ongoing studies are examining the persistence of these deficits in mice 15 weeks after the final injury, which are relevant to rmTBI related deficits in military personnel that persist up to a year. Water maze data is beginning to persistent deficits due to rmTBI in the long-term.

THE LONG-TERM NEURODEGENERATIVE EFFECTS OF

REPETETIVE MILD TRAUMATIC BRAIN INJURY

AND TREATMENT WITH SIGMA-1

RECEPTOR AGONIST

PRE-084

Christopher Abad-Jacobi, B.A.

APPROVED:

_____
Derek Schreihofer, Ph.D, Major Professor

_____
Nathalie Sumien, Ph.D., Committee Member

_____
Ann Schreihofer, Ph.D., Committee Member

_____
Caroline Rickards, Program Director

_____
Lisa Hodge, Ph.D., Assistant Dean for Specialized MS Programs

_____
J. Michael Mathis, Ph.D., Ed.D., Dean
Graduate School of Biomedical Sciences

The Long-Term Neurodegenerative Effects of Repetitive Mild Traumatic Brain Injury and Treatment With Sigma-1 Receptor Agonist PRE-084

Practicum Report

Presented to the Graduate Council of the

Graduate School of Biomedical Sciences

University of North Texas

Health Science Center at Fort Worth

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science

By

Christopher Abad-Jacobi

Fort Worth, Texas

April, 2021

ACKNOWLEDGEMENTS

I am grateful to Dr. Schreihofer for accepting me into his lab and taking the time to mentor me throughout this project. Through this mentorship I was able to enhance scientific skills that will surely be of use in the future. I thank all of my committee members, Drs. Nathalie Sumien, Ann Schreihofer, and Derek Schreihofer, for taking the time to support me in this endeavor. I am thankful for Anthony Oppong-gyebi and Daniel Metzger, who went above and beyond in assisting me when help was needed, and were great company during those long days in the lab. A special thanks to Philip Vann, who trained me to perform behavioral tests, showed patience and trust in me, and never hesitated to help me, even if I was asking a question he likely already mentioned. I am grateful to the Department of Pharmacology and Neuroscience for providing me with the tools to succeed during a year full of uncertainty. Finally, I have been blessed with supportive family and friends who never fail to cheer me up.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

CHAPTER 1

BACKGROUND AND LITERATURE

Traumatic brain injury (TBI) is a major cause of death and disability in the United States (5). In 2014, there were approximately 2.87 million TBI emergency department visits, hospitalizations, and deaths (EDHDs) occurred, including over 837,000 occurring among children. (5). Further, those who survive TBI can face the effects that last a few days, or a lifetime. With such a high prevalence, the importance of studying TBI and its effects is well recognized. However, TBI is not a singular condition and can vary greatly in severity from very mild closed head injuries to severe penetrating and open head injuries. Mild traumatic brain injury (mTBI) includes both concussive and subconcussive brain injury. Concussive injuries are defined by those leading to acute behavioral and cognitive dysfunction that persists and then resolves over time. Subconcussive injury includes any head impact that does not lead to acute dysfunction. Although several studies have attempted to define such injuries based on the impact forces involved, there is no consensus on the clinical definition of a subconcussive TBI. Repetitive TBI long has been recognized as an additional type of injury that was initially recognized in boxers demonstrating "dementia pugilistica" or "punch-drunk" syndrome in the early 20th century (4). However, it is now well recognized that other contact sports, including American football, soccer, hockey, and mixed martial arts are also associated with lingering neurological injuries. Recent research shows that repetitive mild TBI (rmTBI) can lead to the development of chronic traumatic encephalopathy and an increased co-morbidity of neurodegenerative disorders like Alzheimer's Disease (AD) and Parkinson's disease (PD) (19). Furthermore, studies have suggested that TBI is associated with earlier onset of neurodegenerative diseases like AD (20).

The prevalence of rmTBI has also demonstrated to be wide. The US Defense and Veterans Brain Injury Center estimates that about 180,000 military personnel were diagnosed with mTBI between 2001 and 2010, and it is estimated that between 1.6 to 3.8 million sports-related TBIs occur each year, with 75% of these estimated to be classified as mTBI (11). Further, many estimates indicate the frequency of mTBI, both in the military and in contact sports, is actually much greater because concussive, and especially subconcussive injuries common in mTBI, are routinely unrecognized (18). While protocols are now in place so that recognized TBIs lead to players being removed from play, it is these unrecognized mTBIs that lead to rmTBI. Finally, diagnosis of mTBI can be difficult not only because mTBIs are often subconcussive, but also because routine imaging approaches do little in helping to evaluate and manage mild concussions (11).

As this review is continued, it is my objective to present a few studies that inspired the questions of my research project.

Indications for Delayed Neurological Deficits

Studies by Lehman et al (2012), analyzed 3439 former NFL players and compared them with the general population. The study found that while the NFL players had a decreased overall mortality rate (standardized mortality ratio: 0.53), they had a nearly threefold rate of neurodegeneration related deaths. Another study on former professional Scottish soccer players found that while mortality from other diseases was lower in the soccer players, their rate of neurodegenerative was higher than that of the general population (14). In a study by Smith et al. (2013), persistent axonal swelling and disconnection has been observed to continue for years after initial trauma. Further, Chronic Trumatic Encephalopathy (CTE) has been demonstrated in athletes, civilians, and military personnel involved in activities with a high risk for repetitive

mild TBI (18). On the other hand, a study by Savica et al (2012) compared 438 former high school football players and compared them with 140 age-matched controls and found no increased risk of dementia, PD or amyotrophic lateral sclerosis. These studies, seemingly at odds, support the need for continued research into the mechanisms underlying the chronic effects of repetitive head injury.

Promising Treatments for Neurological Diseases

Emerging evidence indicates that targeting sigma-1 receptors may be beneficial in attenuating the detrimental effects of a number of neurodegenerative diseases including AD and PD, as well as stroke (21). Sigma 1 receptors are intracellular molecular chaperones that help shuttle proteins from the endoplasmic reticulum (ER) to other membranes, including the plasma membrane. Sigma-1 receptors appear to modulate neurodegeneration and neurological deficits through multiple mechanisms including inhibiting excitotoxicity, production of reactive oxygen species (ROS), stabilizing the mitochondria and ER, and inhibiting ER stress and apoptosis. TBI often results in a sustained released of glutamate, leading to downstream toxic reactions and cell damage/death, oxidative stress, and excessive release of other neurotransmitters like dopamine and serotonin, leading to the generation of ROS and activation of mitochondrial death pathways (21). Additionally, the sustained release of glutamate leads to abnormal increases in intracellular calcium, which then leads to the generation of ROS (27). Figure 2 presents a schematic of how Sigma-1 receptor activation may mitigate excitotoxicity. First, Sigma-1 receptor activation has shown to attenuate the release of glutamate, allowing us to avoid the neurotoxic effects following its sustained release. Sigma-1 receptor activation has also shown to decrease the expression of NMDA receptors, thus attenuating the release of intracellular calcium. Finally, Sigma-1 receptor activation has shown to directly inhibit the generation of ROS and to convey neuroprotection by

preserving anti-apoptotic genes like *bcl-2* (21). Consistent with these hypothesis, Sigma-1 knockout mice show increased ROS production. In order to test these theories, PRE-084 was selected based on research by Allahtavakoli and Jarrot (2012), which showed PRE-084 modulated the inflammatory reaction after stroke in rats, and Mancuso et al. (2012), which showed PRE-084 ameliorated the neurodegenerative effects of amyotrophic lateral sclerosis in rats.

Goal

The rate of neurodegenerative diseases like AD has continued to climb over the past decades, and according to the Center for Disease Control and Prevention (CDC), age-adjusted death rates for AD increased by 39% from 2000 to 2010. As an epidemic of neurodegenerative disease begins to emerge, it is imperative that we diligently investigate its causative factors in order to mitigate its risks.

CHAPTER 2

RESEARCH PROJECT

Specific Aims

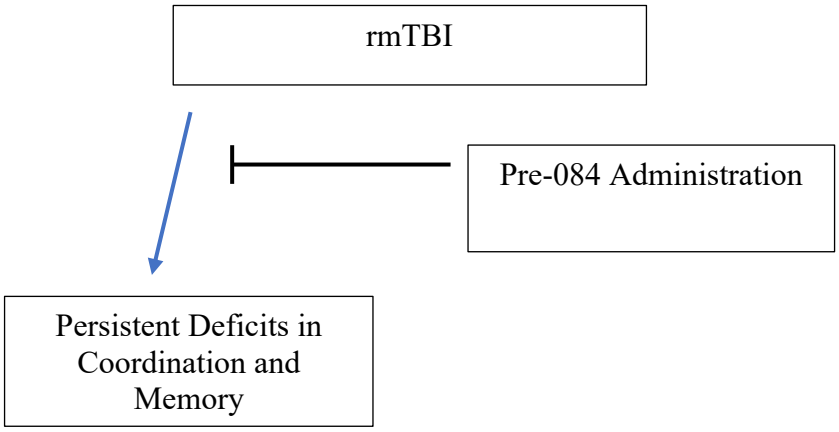

Figure 1. Central Hypothesis. Repeated mild traumatic brain injury will lead to decreased behavioral test performance, indicative of the long-term neurodegenerative effects of rmTBI. Administration of Pre-084 will inhibit the detrimental effects of rmTBI.

The aims of the current study were two-fold. First, to determine if repetitive mild traumatic brain injury (rmTBI) would lead to long-term persistent deficits in coordination and memory. Second. To determine if the detrimental effects of rmTBI could be ameliorated by the prototypical Sigma-1 receptor agonist PRE-084.

Significance

Neurodegenerative diseases occur when nervous system cells (neurons) in the brain and spinal cord begin to deteriorate. Changes in these cells cause them to function abnormally and eventually result in the cells' demise. As neurons deteriorate, an individual may first experience relatively mild symptoms — problems with coordination or remembering names. But as huge

numbers of neurons die, symptoms progressively worsen. In some cases, patients lose the ability to walk independently, think clearly, or generally function in the world. Ultimately, many of these diseases are fatal.

Today, 5 million Americans suffer from Alzheimer's disease (AD) and 1 million from Parkinson's Disease (1). Because neurodegenerative diseases strike primarily in mid- to late-life, the incidence is expected to soar as the population ages. By 2030, as many as 1 in 5 Americans will be over the age of 65. With a greater than 15-fold increase in the rate of AD after the age of 65 (2), finding treatments and cures for neurodegenerative diseases is a matter of increasing urgency. Additionally, with millions of adults and children around the U.S. having some form of TBI each year, it is important that we better understand the long-term effects of rmTBI and improve our ability to assess mTBI.

Innovation

An appropriate animal model of rmTBI must be developed if the scientific community is going to address the aforementioned issues. As Kane et al (2012) state, the availability of a rmTBI animal model would facilitate an understanding of the neurobiological and behavioral outcomes of rmTBI. Biomechanical analyses of head impacts reveal that critical factors in producing mTBIs include high velocity impact and rapid head acceleration (9). Despite analyses showing the significance of these factors, most current animal models do not meet these standards. Instead, they present severe, blunt damage which that yield a high rate of animal death, making it difficult to repeat injuries. Taking these deficiencies into account, the present experiment has adopted, and modified the weight-drop rmTBI method described by Kane et al (2012) to more accurately resemble human injuries (figure 18). The weight was further reduced from the reported studies in an attempt to provide an rmTBI with little to no acute effects on

behavior. Further, a plexiglass door and magnet device, which is further described in the methods section, was implemented. This model allows for reduced force to minimize visible injury and allows for rotational force to be enacted. Such a model is relevant to those engaging in contact sports but not reporting concussive symptoms.

Materials and Methods

Animals. All animal procedures were approved by the University of North Texas Health Science Center Institutional Animal Care and Use Committee. Eight-week old Male C57/Bk6J mice were obtained from Jackson Labs and housed in a centralized animal facility with lights on from 0700 to 1900 h. Mice were fed standard lab chow and had free access to water. They were housed 5 to a cage with suitable bedding material for the entirety of the study. One week after arrival, RFID microchips were placed subcutaneously between the scapulae for identification. The following week, cages were randomized for treatment groups.

Device and rmTBI Induction. A 3 ½ inch two-piece trap door platform was constructed from plexiglass attached to plastic hinges along its length. Two pieces of metal sheet were attached beneath the platform at the head end. Two bar magnets were attached on top of the trap door platform over a piece of metal sheeting that allowed fore and aft movement of the magnets. This configuration allowed for variable magnetic force to be applied to the trap door for mice of different weights. Prior to Hit/Sham, mice were placed in an induction chamber filled with 3-5% isoflurane in 100% oxygen for approximately one minute or until spontaneous movement stopped and then assessed by toe pinch that no longer produced limb movement. Anesthetized mice were placed on the platform in the prone position beneath a guide tube and supported from below. Magnets were then advanced until the platform was able to support the mouse. For Hit groups, a 65 g solid brass cylinder with a 10 x 2 mm flat tip was used and dropped through a 36-

inch plexiglass tube to strike the dorsal surface of the skull between the ears. The weight was arrested with a nylon fishing line so that it only extended 2 cm below the end of the guide tube. Impact with the mouse head resulted in opening of the trap door. After impact, mice underwent a 180-degree rotation to land supine on a foam pad placed 5 inches below the platform. In Sham groups, mice were placed on the platform and slowly dropped through the platform without a weight impact or rotation. Mice were then returned to a holding cage in a supine position, and time to regain an upright position on all four limbs (righting reflex) was recorded.

Treatment and Groups. Mice were divided into control and injury subgroups with and without treatment. Treatment subgroups include those mice who receive rmTBI. Additionally, half of the mice in the treatment subgroups received Pre-084 (rmTBI+Pre-084) and the other half received saline (rmTBI+saline). Control mice were anesthetized with isoflurane, dropped thorugh the plexiglass doors without impact to the head, and received a saline (sham) i.p. injection. 1mg/kg of Pre-084 in saline or saline alone was administered intraperitoneally using a 25mm gauge needle in those mice receiving treatment (13). Two groups of mice were tested. Group one consisted of one batch: n=30. In group one, mice received PRE-084/vehicle immediately post-hit/sham, administered subcutaneously. Group two consisted of two batches, both containing 30 mice. Group two mice received PRE-084/vehicle one-hour post-hit, administered intraperitoneally. Mice in each batch for both groups one and two were split into subgroups as follows: No Hit + Vehicle (control) – n=10, Hit + Vehicle – n=10, Hit + PRE-084 – n=10, totaling to Control: n=10, rmTBI: n=20, Saline: n=20, Pre-084: n=10. Mice received a single hit per day Monday through Friday for 5 weeks, resulting in 25 total impacts and 25 total treatments with saline or PRE-084.

In order to test baseline balance and motor coordination, prior to rmTBI/sham, mice were weighed, and tested by being placed in the middle of either a small square beam (Group 1) or large cylindrical beam (Group 2) with two platforms on either side. Mice were allowed to balance/walk to a platform on either side or fall. Time of fall was recorded. Mice that did not fall were given a time of 60 seconds. This was repeated at the end of each week to assess balance and motor coordination changes.

Behavioral Assessment. Five weeks (Group 1) and 15 weeks (Group 2) post rmTBI/sham, behavioral assessments began. Mice underwent 3 weeks of behavioral assessments. Using the AccuRotor instrument (Accuscan Instruments), the rotarod test was used to evaluate balance and motor coordination. The first day of rotarod was an acclimatization day in which the rotarod rotates at a constant speed and mice continued to be placed on the rotarod until they were able to stay on for three minutes, after which if they fell, time was not restarted but instead they were just placed back on the rotarod until 5 minutes were reached. On the second day, mice were placed on an accelerating rotarod and time of fall was recorded. Four trials were administered with a five-minute break between trials.

The Morris Water Maze (MWM) was used to assess spatial learning and memory deficits after rmTBI. The test consisted of a pre-training stage and an acquisition/probe stage as previously described (7). There was a hidden platform 1.5cm below 24 +/- 1 °C opaque water. Animals were pre-trained to escape the water by locating a hidden platform using a straight alley. The mice had a maximum of 60 seconds to locate the platform, after which the mouse would be directed towards the platform and allowed to stay on for 15 seconds. All mice had two pre-training sessions, each consisting of five trials with two-minute intervals between each. The acquisition and probe stage follow the pre-training stage and consisted of four days. Each day

included 5 trials with two-minute intervals between each. The mouse was placed at a different quadrant from the last in each trial and expected to learn the position of the platform based on external cues in the vicinity of the maze. 90 seconds was the maximum amount of time the mouse was allowed to look for the platform, after which it would be directed towards the platform and allowed to stay on for 15 seconds before removal. Data were expressed as the path length and path-independent swim speed. Probe trials were conducted to underscore the cognitive learning capacity of the mice and involve removal of the platform before the fifth trial on the second and fourth days. The probe trial lasted 30 seconds (32). Percent time spent in the platform quadrant, within a 40 centimeter annulus around the target site was evaluated as a measure for spatial bias. All MWM behavior were recorded and analyzed with AnyMaze software (Stoelting).

Elevated Plus Maze (EPM) was used to test anxiety levels. Mice were placed in the middle of the EPM, and times spent on open and closed wings, and in the middle, was recorded using AnyMaze software (Stoelting). Total time spent on the EPM was five minutes and the test consisted of only one trial per mouse.

T-maze was used to assess function in memory and spatial learning. The T-maze rest on a grid floor wired to deliver a 0.69-mA scrambled shock to the feet (9). The mouse's preferred side was determined by observing which side it went to first, at which point a shock was administered and halted once the mouse went to the other side, the side we then attempted to train the mouse to go into. After every trial the mouse was kept in the appropriate side for ten seconds and then placed back in a clean cage for 1 minute before another trial began. After the preferred side is determined, the mouse had 5 seconds to go into the correct side, after which a shock was administered and halted once the mouse entered the correct side. If the mouse entered the correct

side before 5 seconds, it did not receive a shock. The test was completed once the mouse entered the correct side before being shocked in its last 4 out of 5 times. Additionally, the last 2 times must be correct. This test was performed with each mouse only once.

Statistical Analysis. Data were analyzed by 1-way or 2-way analysis of variance (ANOVA) with Group and time or trial/session as the independent variables. Multiple trials/sessions were assessed with repeated measures ANOVA. Data was analyzed in GraphPad Prism and multiple comparisons were performed using Dunnett's and Sidak's tests. Significance for all experiments was set at $p<0.05$.

Results

Group 1. Subgroups did not significantly differ in weight or bridge time during the injury period (weeks1-5); however, there was a trend for uninjured mice to perform better on the bridge at the conclusion of the study 8 weeks after final injury (figures 3,4). In addition, control mice remained on the top of the balance beam, whereas 19/20 of the mice with injuries were unable to remain on the top of the beam and, instead, clung to the bottom of it, supporting the idea that they had vestibular dysfunction. A difference in main effect was seen in wake time (figure 5), between subgroups ($p=0.0004$), as well as individual differences ($p<0.05$) between control and hit mice (figure 5). No significant differences were seen in EPM, T-maze, WM pathlength or WM percent time in AN 40 (figures 7, 8, 9, 10). When averaged over the four trials, a difference in main effect on the rotarod was observed between subgroups, where ($p=0.0032$), and individual differences between control and hit mice ($p<0.05$) were seen (figure 6).

Group 2. Groups did not significantly differ in weight, bridge time, EPM, or T-maze figure (Figures: 13, 11, 15, 16). A difference in main effect was seen on the group ANOVA ($p<0.05$), and using Dunnett's test, it was found that Hit + PRE-084 mice performed worse controls

($p<0.05$) on trial four (Figure 14). A main effect difference was also observed ($p<0.0001$) in wake time, and Dunnett's test showed significant differences in all control mice vs hit mice on each week ($p<0.05$) except Hit + Vehicle on week 2 (figure 12). Finally, on the Morris Water Maze path length (figure 17), individual differences were seen, where Hit + PRE-084 mice had increased path length as compared to controls on session one ($p<0.05$) and both sets of hit mice had increased path lengths as compared to controls on session three ($p<0.05$). No differences were seen in swim speed between subgroups. Two mice died during the course of the experiment, one in the Hit + Pre-084 group and another in the Hit + Vehicle group, reducing the number each group from n=10 to n=9. Two mice did not meet criterion on the T-maze and were excluded from T-maze results calculations. Further, using Sidak's test, a significant difference on probe trial two was observed, where Hit + Vehicle mice spent significantly less time ($p<0.05$) at a 40cm annulus than control mice. No differences in speed were seen between subgroups (figure

Discussion

The purpose of our study was to observe the long-term effects of rmTBI on brain performance measures like learning and memory, and balance and motor coordination. In addition, we also sought to observe the possible ameliorative effects of Sigma-1 receptor agonist PRE-084. A few important findings are: 1) Control mice in both groups one and two have a significantly lower wake time as compared with Hit groups, 2) Control mice in group one were able to spend more time on the rotarod, 3) Control Mice showed better memory on the Morris Water Maze as compared to Hit mice in group 2, and 4) When compared to Hit + Vehicle and control mice, Hit + PRE-084 mice in group two spent significantly less time on the rotarod.

In group one, the main objective was to determine if our rmTBI device in fact caused a mild head injury, and if behavioral effects of rmTBI were persistent. Accordingly, mice were

subjected to behavioral testing five weeks after induction of rmTBI was complete. A time period of five weeks was chosen based on previous experiments indicating that most people who sustain concussions see their symptoms alleviated by three weeks (17). In order to avoid those possible symptoms affecting behavior, a point in time after the three-week period was chosen. Group one found that mice in both Hit + Vehicle and Hit + PRE-084 groups showed increased wake times. Wake time, or righting reflex, is a measure of neurological restoration. While not a measure of long-term deficit, increased wake time with spontaneous consciousness recovery, gives us confidence mTBI was being delivered (11).

Rotarod data, aimed to determine balance and motor coordination, showed control mice in group one to have increased times. These results are consistent with those seen in athletes who have experienced mild head injuries and showed deficits in balance and postural equilibrium (9). Kane et al., showed that mice who received rmTBI and underwent the accelerating rotarod test one day after 4 hits, performed significantly worse than controls. Tests by Kane et al. further showed no difference in rotarod performance when observed 7 days after rmTBI, indicating a lack of persistent motor deficits. In contrast, our tests displayed motor deficits five weeks after injury, indicating persistent motor deficits due to rmTBI. Results for the balance beam on group one at the end of the experiment, though not significant, also suggest that mice who received injuries continued to have balance issues, as they were unable to remain on top of the beam and instead clung to the bottom. In order to avoid mice clinging to the bottom on group two, a cylindrical beam was chosen. The use of the cylindrical beam made the test more difficult, and this may be a factor contributing to the lack of a trend on the balance beam in group two. The ability of our tests to show persistent motor deficits is likely due to the amount of mTBIs delivered. Kane et al. administered either four or five hits over the course of one day or one

week, as compared to our twenty-five hits administered over the course of five weeks. The present study's number of mTBIs and time period was selected because we believe it more closely resembles the prolonged nature of mTBIs received over the course of a sports season or sports career, where an athlete may receive a number of mTBIs that go undiagnosed (17). Modern sideline concussion assessments further demonstrate the importance of motor tests in determining concussion diagnosis. Currently, sideline trainers use postural stability and tandem gait tests to assess balance and dynamic balance, respectively (32).

Promising results were seen in the Morris Water Maze Data. Diminished cognitive performance was seen on different trials in both Hit groups when compared to control mice and in probe trial two, both Hit mice performed worse than controls. This new finding not observed with behavioral tests performed only five weeks after the last hit (group 1) agree with the hypothesis that long-term cognitive deficits can be observed as a result of rmTBI (12, 14, 25).

A surprising finding was that of decreased performance on the rotarod by Hit + PRE-084 mice on group two. We observed worse rotarod performance by both Hit groups when behavioral tests were performed five weeks after rmTBI, and only by Hit + PRE-084 mice when performed fifteen weeks after rmTBI. A new question now arises from this data; are there long-term detrimental effects to the use of Pre-084 and other sigma drugs? Allahtavakoli et al (2011)., found there to be a neuroprotective effect of PRE-084 48 hours after stroke. Maurice et al. (1994), studied the effects of NMDA receptor antagonism on learning and memory and found that PRE-084 ameliorated some of the effects of NMDA antagonism when performing behavior tests 24 hours after Pre-084 administration. Griesmaier et al. (2012), found PRE-084 administered one hour after glutamate induced excitotoxicity provided neuroprotection. None of the mentioned studies show any detrimental effects due to PRE-084, however, none of these

studies look into the long-term effects of PRE-084. Additionally, none of our results showed any positive impact of PRE-084 on behavioral performance. In the future, it will be important to add a No Hit + PRE-084 group in order to determine if there are long-term detrimental effects to PRE-084 use.

It is important to consider the limitations of the present study, the most important being the assessment of mTBI. As indicated by the Glasgow Coma scale, the diagnosis of TBI in humans is an involved process that includes observing verbal responses to questions, motor abilities in response to commands, and eye movements. It is difficult to determine if a mouse has persistent headaches, and impossible to ask it questions. Due the inability of performing a Glasgow type exam on a mouse, we must resort to something like the righting reflex, which while consistent, lacks the depth of a test where one can ask an experimenter questions and observe cognition. There are also the limitations in administering a mTBI that can fully resemble what the human experience, and equally difficult to make each mTBI the same in terms of rotational speed and acceleration, important aspects of TBI. Furthermore, anesthesia has been hypothesized to ameliorate some of the negative effects of TBI (29). However, these negative effects are seen after prolonged use of anesthesia, in contrast to our experiment where mice were only lightly anesthetized prior to being hit. Additionally, if there were alleviating effects on TBI due to anesthesia, they would only work to decrease the effect of our results. There are also limitations in the fact that we only used male mice thus far. The literature shows that in sports played by both males and females, females have a higher rate of TBI. If this is the case, our data cannot represent the entire population, but only the male population, thus, experiments with female mice are needed. Finally, the groups thus far have been quite small. However, there is yet

another group of 30 mice, n=10 for each group, that are to be examined behaviorally soon, and this will raise the power of group two.

Future Directions. The results of the study demonstrate how difficult it can be to determine cognition in mice. However, many consider it equally difficult to determine TBI in humans. There is still disagreement about which exact tests to use, and when it comes to the most widely used test, the Glasgow Coma Scale (GCS), evidence shows inconsistency and confusion in the use of the GCS in practice. For instance, inter-rater reliability has come into question. In a systematic review by Reith et al (2016), there was found to be a higher reliability for the components of the GCS than for the actual sum score. Unreliable GCS scores can lead to unreliable prognosis for patients. In the future, it is important that we find biomarkers that can more reliably tell us the degree of TBI. An exciting novel approach to biomarkers is the use of exosomal biomarkers. Exosomes are lipid enveloped, thus crossing the blood brain barrier more readily, and are more stable in circulation (30). Finally, we must study the disparities in TBI. Evidence shows that on average, in sports played by both males and females, females are more susceptible to TBI (25). It is imperative that future experiments examine the effects of rmTBI on female mice.

Summary and Conclusion

We assessed persistent cognitive deficits due to rmTBI using a modified model to allow for adjustment of force by body weight and rotation after impact. The rmTBI resulted in increased wake times for hit mice in both groups one and two. Data showing reduced motor performance by Hit + Vehicle vs control on group one, versus none on group two, suggests that motor deficits due to rmTBI persist for at least five weeks post-hit, but do not persist at fifteen weeks post-hit. Additionally, worse performance was seen by individual hit subgroups vs control

in Morris Water Maze path length and AN-40 data in group two, but not in group one, suggesting that memory deficits take an extended period of time to present, similar to humans. Interestingly, no deficits were seen on the T-maze between group one and two mice, suggesting working memory is not affected either in the mid-term or long-term. The major hypotheses of the present study were that rmTBI would lead to persistent cognitive deficits that would be observed in behavioral experiments, and that supplementation with PRE-084 would ameliorate some of the detrimental effects caused by rmTBI. Further studies are needed to fully understand the long-term cognitive effect of rmTBI on mice, and future studies will look at biomarkers to more accurately diagnose mTBI.

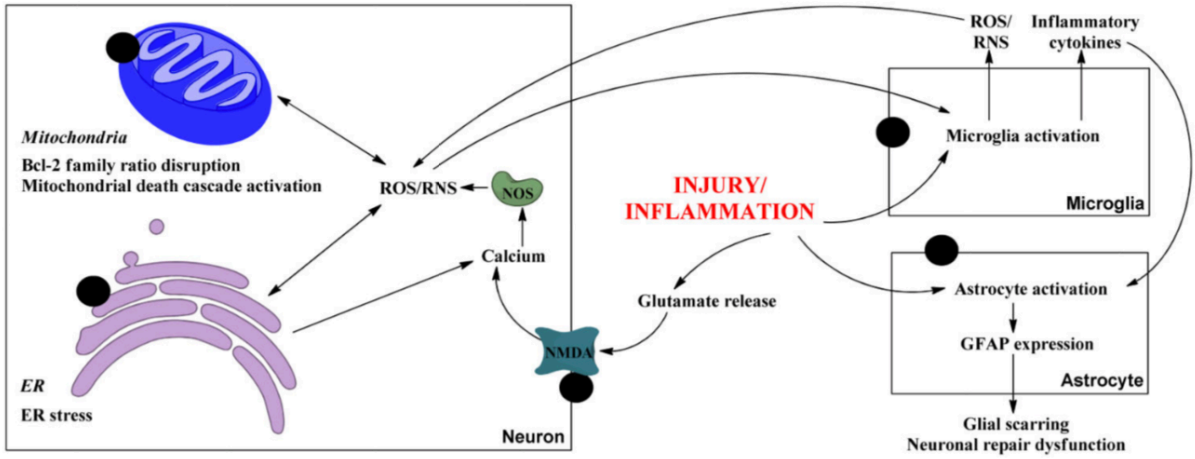

Figure 2. Mechanisms through which Sigma-1 receptor activation is believed to attenuate the detrimental effects of injury and inflammation

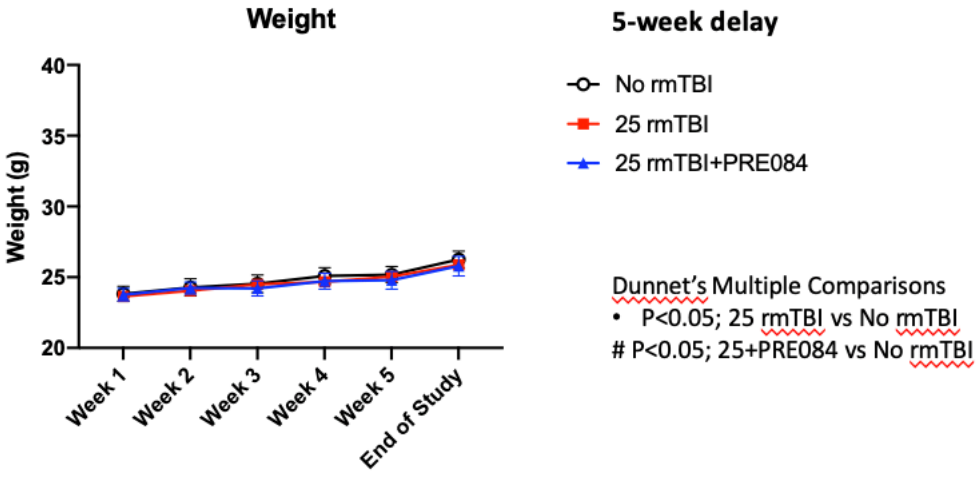

Figure 3. Neither rmTBI or PRE-084 led to a difference in weight for group one mice.

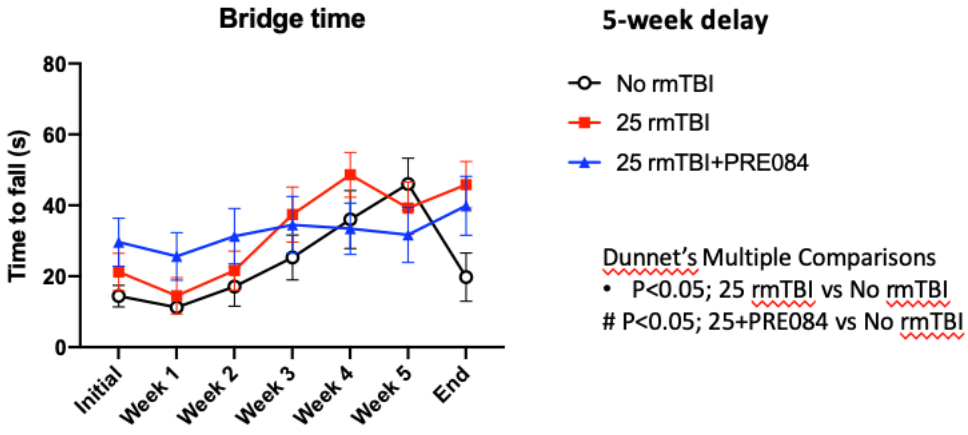

Figure 4. Mice in group one did not differ on balance tests performed at the end of each week.

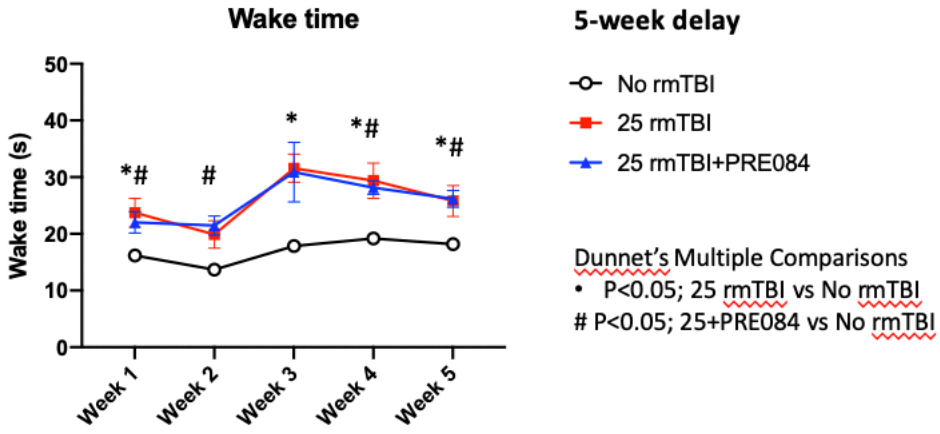

Figure 5. Time to neurological recovery was significantly less in group one control vs hit mice and a main effect difference was seen (ANOVA on Group: P=0.0004).

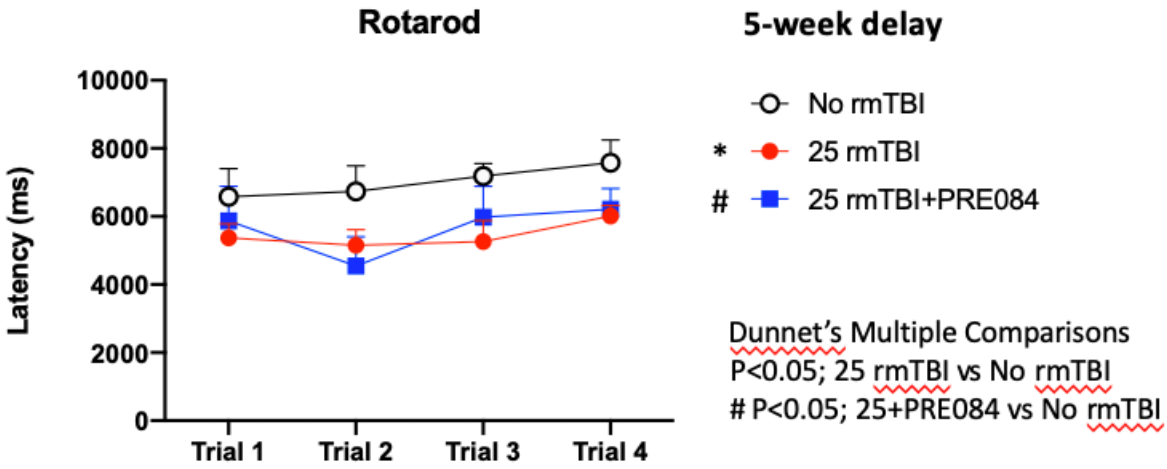

Figure 6. A main effect difference in group one was seen (ANOVA on Groups: $p=0.0032$) as well as individual differences between control and hit mice ($p<0.05$)

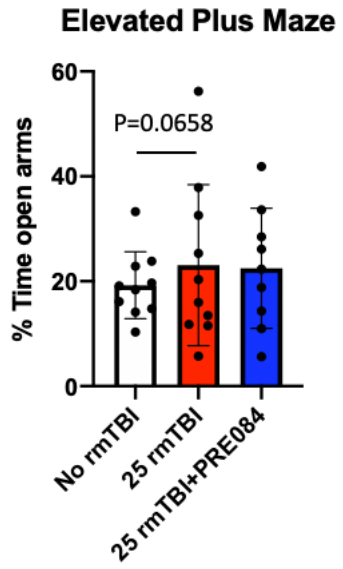

Figure 7. Mice in group one did not significantly differ in anxiety levels.

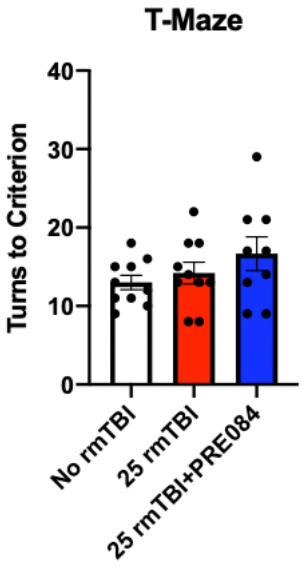

Figure 8. Mice in group one did not significantly differ in tests of working memory.

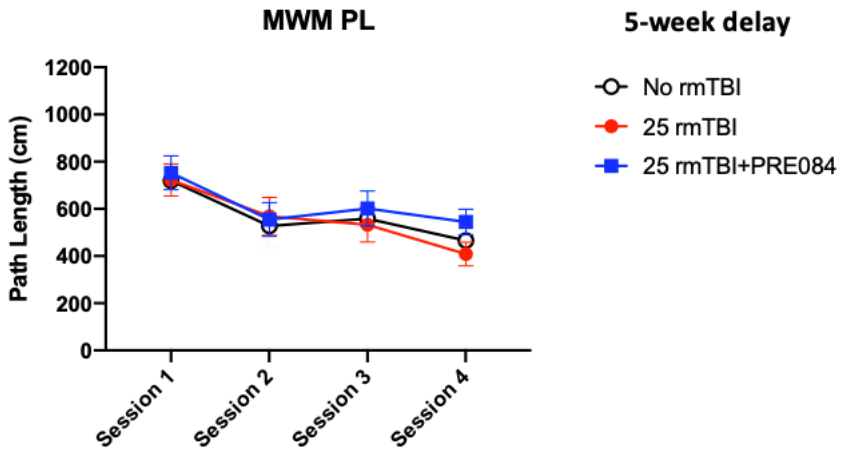

Figure 9. Using the Morris Water Maze to test cognitive performance did not yield significant differences between subgroups in group one.

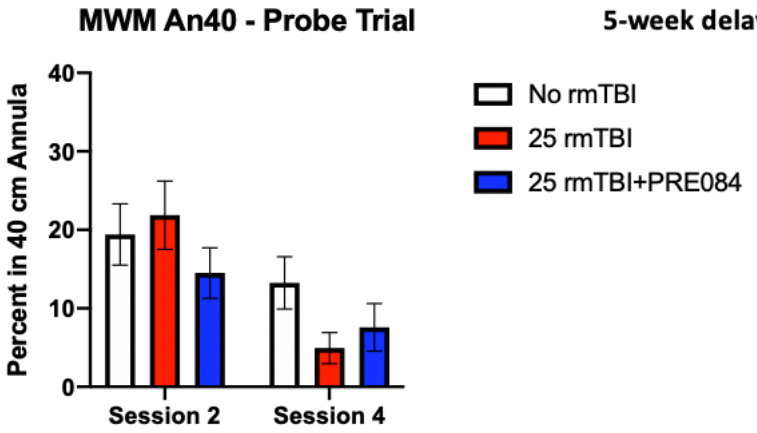

Figure 10. Using the Morris Water Maze to test cognitive performance did not yield significant differences between subgroups in group one.

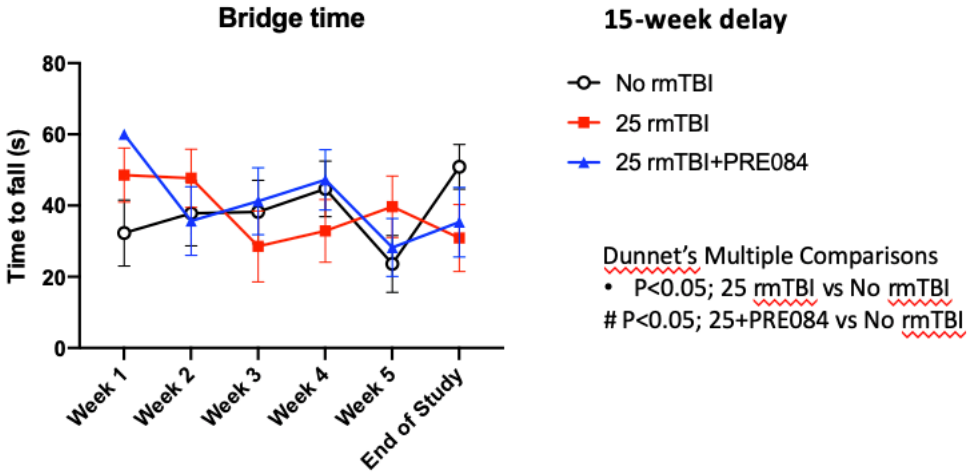

Figure 11. Mice in group two did not differ on balance tests performed at the end of each week.

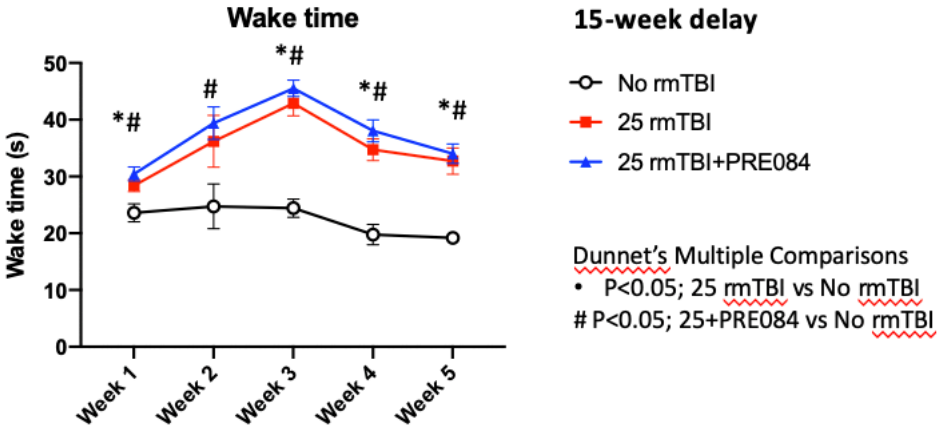

Figure 12. In assessing time to neurological recovery in group two, a main effect ($p<0.05$) was observed between subgroups. A Dunnett's test indicated individual differences in all hit mice vs control except for rmTBI + Vehicle on week 2.

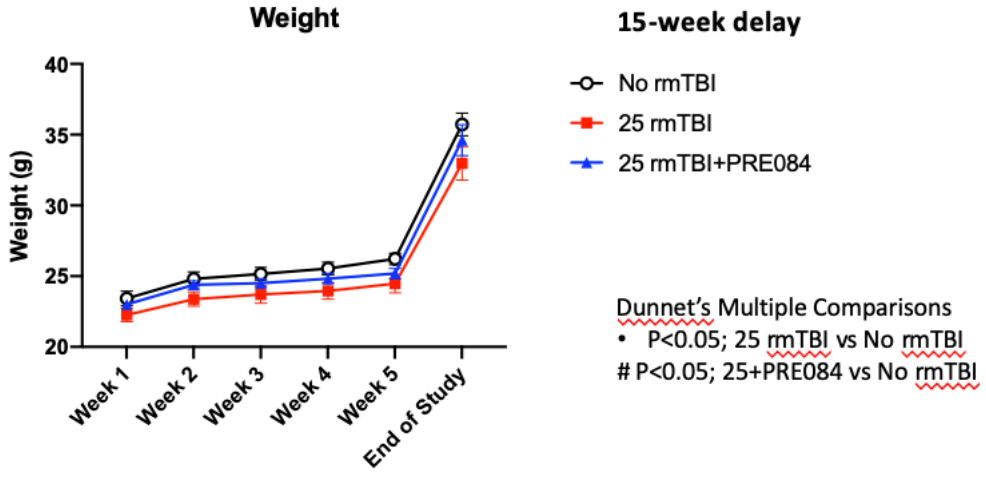

Figure 13. Neither rmTBI or PRE-084 affected the average weight of the mice in group two.

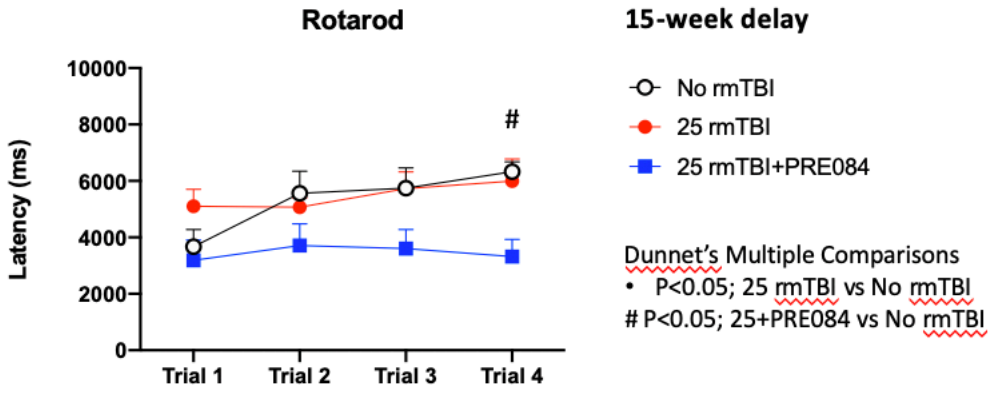

Figure 14. Hit + Pre-084 mice in group two performed significantly worse (P<0.05) than the other groups on tests of balance and motor coordination.

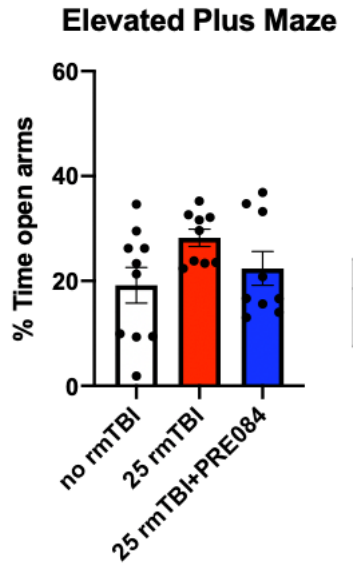

Figure 15. Mice in group two did not significantly differ in anxiety levels.

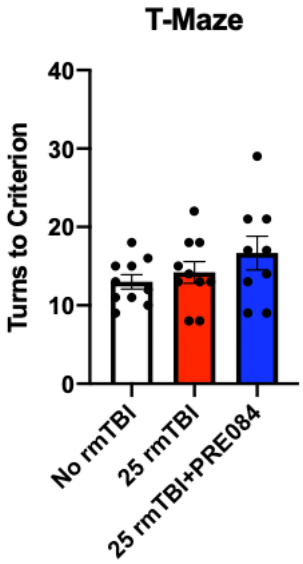

Figure 16. Mice in group two did not significantly differ in tests of working memory.

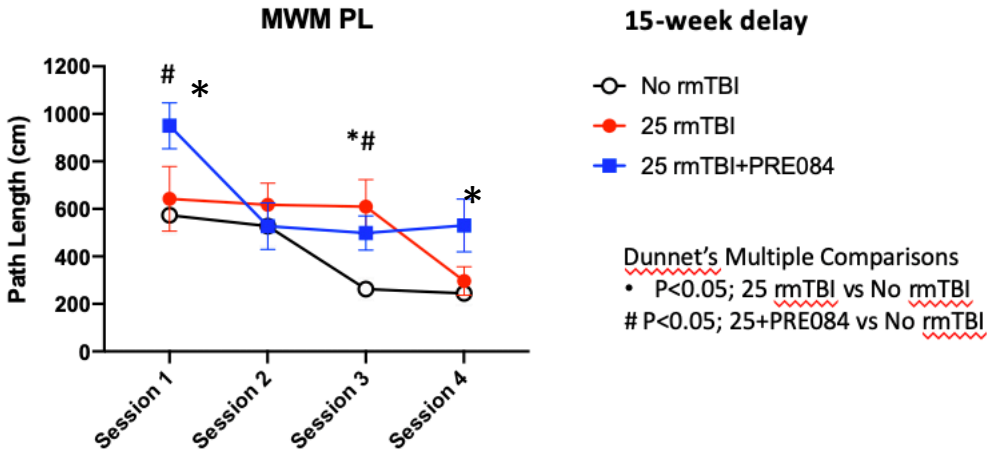

Figure 17. Morris Water Maze Path Length found there to be an overall main effect (Group ANOVA: P<0.0001). Specifically, there was a difference between Hit + PRE-084 vs No Hit mice in trial one and between Hit & Hit + PRE-084 vs No Hit + Vehicle in trial three (p<0.05).

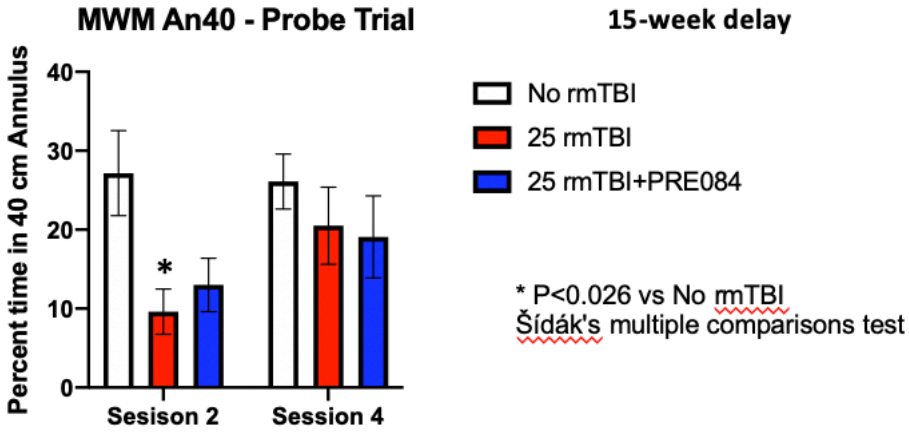

Figure 18. A significant difference was seen in time spent within a 40 centimeter radius of the platform with Hit + Vehicle & Hit + PRE-084 vs Vehicle (No Hit) – Group ANOVA: p<0.05, Dunnets test: p<0.05.

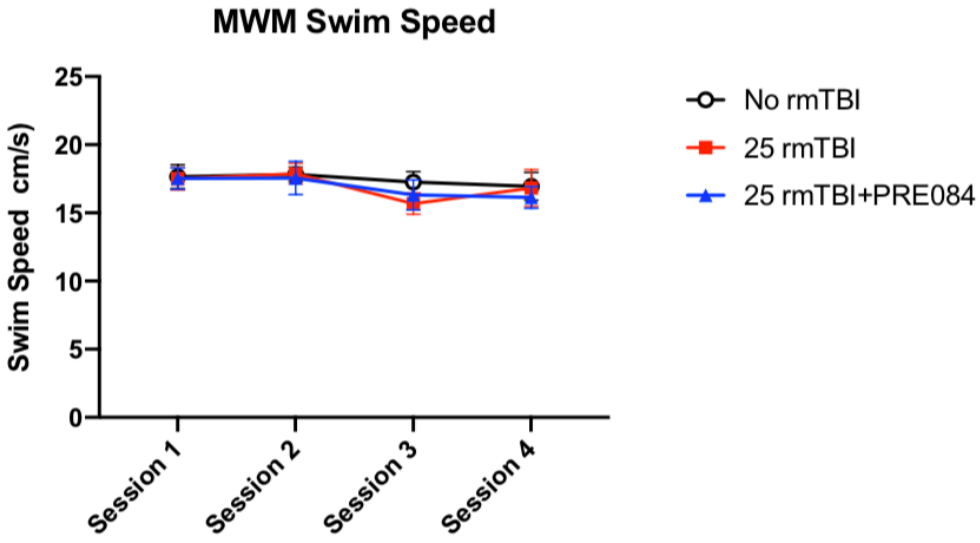

Figure 19. No significant differences were seen in swim speed in group two mice.

Figure 20. Image of the device used to deliver mTBI. At rest, the weight is held taut at the bottom red line. Magnets used to hold the gate closed until hit is delivered can be seen in front of the mouse's head.

REFERENCES

1. (2020), 2020 Alzheimer's disease facts and figures. Alzheimer's Dement., 16: 391-460. https://doi.org/10.1002/alz.12068
2. **Allahtavakoli M, Jarrott B.** Sigma-1 receptor ligand PRE-084 reduced infarct volume, neurological deficits, pro-inflammatory cytokines and enhanced anti-inflammatory cytokines after embolic stroke in rats. *Brain Res Bull* 85:219–224, 2011.
3. **Bishop HI, Guan D, Bocksteins E, Parajuli LK, Murray KD, Cobb MM, Misonou H, Zito K, Foehring RC, Trimmer JS**. Distinct Cell- and Layer-Specific Expression Patterns and Independent Regulation of Kv2 Channel Subtypes in Cortical Pyramidal Neurons. *J Neurosci* 35(44):14922-14942, 2015.
4. **Castellani, R. J., & Perry, G.** Dementia Pugilistica Revisited. *Journal of Alzheimer's disease 60*(4): 1209–1221, 2017.
5. Centers for Disease Control and Prevention. Traumatic Brain Injury in the United States: Fact Sheet [Internet]. CDC.gov. Atlanta (GA): CDC; [updated 2015 Jan 12]. Available from: http://www.cdc.gov/traumaticbraininjury/get_the_facts.html
6. **Erkkinen MG, Kim MO, Geschwind MD**. Clinical Neurology and Epidemiology of the Major Neurodegenerative Diseases. *Cold Spring Harb Perspect Biol*. (2018). doi:10.1101/cshperspect.a033118.
7. **de Fiebre NC, Sumien N, Forster MJ, de Fiebre CM**. Spatial learning and psychomotor performance of C57BL/6 mice: age sensitivity and reliability of individual differences. *Age* 28(3):235-53, 2006.
8. **Griesmaier E, Posod A, Gross M, Neubauer V, Wegleiter K, Hermann M, Urbanek M, Keller M, Kiechl-Kohlendorfer U.** Neuroprotective effects of the sigma-1 receptor

ligand PRE-084 against excitotoxic perinatal brain injury in newborn mice. *Exp Neuro* 237(2):388-95, 2012.

9. **Guskiewicz KM, Mihalik JP**. Biomechanics of sport concussion: quest for the elusive injury threshold. *Exerc Sport Sci Rev* 39:4–11, 2011
10. **Ikonne U. S, Vann P. H, Wong J. M, Forster M. J, Sumien N.** Supplementation with N-acetyl cysteine affects motor and cognitive function in young but not old mice. *Journal of Nutrition* 149(3), 463-470, 2019.
11. **Kane MJ, Angoa-Pérez M, Briggs DI, Viano DC, Kreipke CW, Kuhn DM**. A mouse model of human repetitive mild traumatic brain injury. *J Neurosci Methods* 203(1):41-49, 2012.
12. **Lehman EJ, Hein MJ, Baron SL, Gersic CM.** Neurodegenerative causes of death among retired National Football League players. *Neurology* 79(19):1970-1974, 2012.
13. **Liu DY, Chi TY, Ji XF, Liu P, Qi XX, Zhu L, Wang ZQ, Li L, Chen L, Zou LB**. Sigma-1 receptor activation alleviates blood-brain barrier dysfunction in vascular dementia mice. *Exp Neuro* 308:90-99, 2018.
14. **Mackay DF., Russell E, Stewart K, MacLean J, Pell JP., Stewart W.** Neurodegenerative Disease Mortality Among Former Professional Soccer Players. N Engl J Med 381(19): 1801-1808, 2019.
15. **Mancuso, R., Oliván, S., Rando, A., Casas, C., Osta, R., & Navarro, X.** Sigma-1R agonist improves motor function and motoneuron survival in ALS mice. *Neurotherapeutics 9*(4): 814–826, 2012.

16. **Maurice T, Su TP, Parish DW, Nabeshima T, Privat A.** PRE-084, a σ selective PCP derivative, attenuates MK-801-induced impairment of learning in mice. *Pharmacol Biochem Behav*. 49(4): 859–869, 1994.

17. **McCrory P, Meeuwisse WH, Dvořák J, Echemendia RJ, Engebretsen L, Feddermann-Demont N, McCrea M, Makdissi M, Patricios J, Schneider KJ, Sills AK**. 5th International Conference on Concussion in Sport (Berlin). *Br J Sports Med* 51: 838–847, 2017.

18. **Mckee AC, Daneshvar DH**. The neuropathology of traumatic brain injury. *Handb Clin Neurol* 127:45-66, 2015.

19. **McKee, A. C., Stern, R. A., Nowinski, C. J., Stein, T. D., Alvarez, V. E., Daneshvar, D. H., Lee, H. S., Wojtowicz, S. M., Hall, G., Baugh, C. M., Riley, D. O., Kubilus, C. A., Cormier, K. A., Jacobs, M. A., Martin, B. R., Abraham, C. R., Ikezu, T., Reichard, R. R., Wolozin, B. L., Budson, A. E., … Cantu, R. C.** (2013). The spectrum of disease in chronic traumatic encephalopathy. *Brain 136*: 43–64, 2013.

20. **Nemetz PN, Leibson C, Naessens JM**. Traumatic brain injury and time to onset of Alzheimer's disease: a population based study. *Am J Epidemiol* 149: 32–40, 1999.

21. **Nguyen L, Kaushal N, Robson MJ, Matsumoto RR**. Sigma receptors as potential therapeutic targets for neuroprotection. *Eur J Pharmacol* 743:42-7, 2014.

22. **Parasuraman S, Raveendran R, Kesavan R**. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother* 1(2):87-93, 2010.

23. **Reith FCM, Van den Brandel R, Synnot A, Gruen R & Maas R**. The reliability of the Glasgow Coma Scale: a systematic review. *Intensive Care Medicine* 42: 3–15, 2016.

24. **Saunders RL, Harbaugh RE.** The second impact in catastrophic contact-sports head trauma. *JAMA* 252: 538-539, 1984.

25. **Saigal R, Berger M.** The Long-Term Effects of Repetitive Mild Head Injuries in Sports. *Neurosurgery* 75: S149-S155, 2014.

26. **Savica R, Parisi JE, Wold LE, Josephs KA, Ahlskog JE**. High school football and risk of neurodegeneration: a community-based study. *Mayo Clin Proc* 87: 335-340, 2012.

27. **Sheldon AL, Robinson MB**. The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. *Neurochemistry international* 51:333–355, 2007.

28. **Smith DH, Hicks R, Povlishock JT.** Therapy development for diffuse axonal injury. *J Neurotrauma* 30: 307–323, 2013.

29. **Statler KD, Alexander H, Vagni V, Holubkov R, Dixon CE, Clark RS, Jenkins L, Kochanek PM**. Isoflurane exerts neuroprotective actions at or near the time of severe traumatic brain injury. *Brain Res* 1076:216–24, 2006.

30. **Taylor DD, Gercel-Taylor C**. Exosome platform for diagnosis and monitoring of traumatic brain injury. *Philos Trans R Soc Lond B Biol Sci*. (2014). doi: 10.1098/rstb.2013.0503.

31. **Wang, K. K., Yang, Z., Zhu, T., Shi, Y., Rubenstein, R., Tyndall, J. A., & Manley, G. T.** (2018). An update on diagnostic and prognostic biomarkers for traumatic brain injury. *Expert review of molecular diagnostics* 18: 165–180, 2018.

32. **Yue J, Phelps R, BA, Chandra A, Winkler E, Manley G, Berger M**. Sideline Concussion Assessment: The Current State of the Art, *Neurosurgery* 87: 466-475, 2020

33. **Zhao X, Fan Y, Vann P. H, Wong J. M, Sumien N, He J. J.** Long-term HIV-1 Tat Expression in the Brain Led to Neurobehavioral, Pathological, and Epigenetic Changes Reminiscent of Accelerated Aging. *Aging and Disease 11*(1), 93-107, 2020.

34. **Zongo D, Ribéreau-Gayon R, Masson F, Laborey M, Contrand B, Salmi LR, Montaudon D, Beaudeux JL, Meurin A, Dousset V, Loiseau H, Lagarde E**. S100-B protein as a screening tool for the early assessment of minor head injury. *Ann Emerg Med* 53: 209-18, 2012.