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Spatial and Working Memory in Mice post Traumatic Brain Injury

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Abstract

The objective of this research is to determine how repeated traumatic brain injuries caused by blast waves affect spatial and working memory. Acute traumatic brain injuries (TBI) have been shown to affect spatial and working memory in humans, but less is known about the effect of repeated blast exposures. We hypothesized that three sequential low-level blast waves will impair the spatial and working memory capabilities of these mice when compared to control mice who receive no injury. Mice first trained on a spatial accuracy task, then received three consecutive mild-TBI events. They were tested on the spatial accuracy task at one week and again at 3 months post-TBI. The mice who sustained a TBI spent more time pausing in multiple goal zones than controls following the 3rd rotation of the goal zone location to novel quadrants. This finding suggests that TBI mice are more susceptible to memory interference or could have lower working memory limits than controls. This putative deficit in working memory persisted at 3 months after injury. This data creates a physiological and behavioral framework for relating the underlying causes of TBI-induced cognitive impairment as well as for improving behavioral and cognitive outcomes in individuals who sustain traumatic blast injuries.

Introduction

Traumatic brain injuries lead to death in over 60 thousand individuals in the United States annually (Centers, 2022). A traumatic brain injury is when a sudden external force causes physical damage to the brain. TBIs can cause brain bruising, also known as a concussion, which are not visible on conventional brain scans, as well as more severe brain lesions that can be seen on computed tomography (CT) imaging. In humans, traumatic brain injuries are most commonly caused by falls, motor vehicle collisions, violence, combat injuries, contact sports, and other head traumas. They can cause symptoms including loss of consciousness, coordination, and balance, to nausea and further memory loss. Memory deficits have been well documented in humans after concussion and head injury (McDowell et al, 1997; Azouvi et al, 2017; Chai et al, 2018).

Memory can be divided into a variety of subsections. The first three being short term memory, long term memory and working memory. Short-term memory is memory from a few seconds or minutes before an event while long-term memory can be recalled from days to years in the past. Long term memory can also subsequently be subdivided into explicit and implicit memory and again by subdividing explicit memory into episodic memory and semantic memory. This study will be focusing on working memory and spatial memory which is often used to test the functioning of episodic memory. Episodic memory is information retrieved from the long-term memory that is related to previous experiences with context, such as being able to "recall the what, where and when' of discrete events" (Morris, 2001). Scientists have determined that mice have episodic-like memory, similar to humans, but sometimes question to what extent mice have working memory.

The definition of working memory in humans is debated among researchers but it typically refers to the limited memories that are manipulated and stored in the brain to be used for the

purpose of achieving a goal (Lundqvist et al, 2018; Shipstead et al, 2014). These memories can be anything from memorizing your partner's phone number to the address of your work. Working memory differs from short-term and long-term memory because it is always in use and is where the encoded information in your brain is stored (Chai et al, 2018). Working memory however is limited; it has a capacity and only a certain number of items can be stored there. Primary memory is the part of working memory that allows humans to store anywhere from 3 to 5 pieces of information at the forefront of their memory and protect it from proactive interference (Shipstead et al, 2014). Proactive interference is the interference of previously learned pieces of information while trying to learn and acquire new information (Hoskison et al, 2008). This means that the old information that your brain has already stored prevents more information from being absorbed. Another very important part of working memory is that critical information must be able to be recalled without attention being led somewhere else, leading to loss of information. This is called secondary memory and refers to the ability to recall important information readily from long-term memory or "contextually-relevant information that is not currently maintained by primary memory" (Shipstead et al, 2014). It has been previously discussed that individuals who can recall memories based on relevant cues such as location, have an easier time recalling those memories without interference than those that cannot (Shipstead et al, 2014).

Although it may be difficult to test some aspects of working memory in rodents, spatial memory tasks can be used to study both working and episodic memories. Spatial memory function in rodents provides a robust method for measuring working memory function, and spatial accuracy tasks have also been used as a way to access episodic memory. Spatial memory loss can affect the spatial memory and the integration of location and direction. Spatial memory tasks "required [rodents] to remember a location or set of locations, and either approach or avoid

these locations subsequently" (Dudchenko, 2004). Someone who has spatial memory loss may not be able to remember where a certain event took place, where an object is located, or directions to get to a desired area. This is particularly relevant to humans with post-concussive syndrome or chronic traumatic encephalopathy (CTE), a chronic and debilitating condition which may result from multiple brain injuries (Broussard et al, 2017; Darwish et al, 2012).

Acute traumatic brain injuries (TBI) have been shown to affect spatial and working memory in humans, but less is known about the effect of repeated blast exposures. In this experiment, we study the mechanisms by which sequential, sub-lethal blast wave exposure to the brain affects memory. Traumatic blast injuries can model those suffered by humans, allowing for measurements of putative changes in signaling molecules, behavioral disturbances, memory and learning changes, and differences in brain functioning. Murine blast models like the one that is being used in this experiment, and others such as the fluid percussion model and weight drop model, can be used to help determine the effects of different types of blast injuries on the brains of mice and be subsequently translated to the effects on humans. Other blast models have primarily studied the protein production and the effect on Tau in the brain, but this experiment will specifically study memory and behavioral effects of the blast.

The objective of this research is to determine how repeated traumatic brain injuries caused by blast waves affects spatial and working memory at two time points. We hypothesize that three sequential low-level blast waves will be sufficient to impair the spatial and working memory capabilities of mice relative to mice who receive no injury. To this end we sought to take a rigorous, quantitative approach to testing memory function in mice. The spatial accuracy task used in this experiment was created by Dr. Jeremy Barry from the University of Vermont and is based on

Morris Water Maze and food restricted mouse studies (Getz et al, 2022; Morris, 2001; Rossier et al, 2000).

The Morris water maze, the spatial accuracy model from which this spatial accuracy task is based on, was created by Richard Morris in 1982 and consisted of an opaque pool with a submerged escape platform. The rats were tested on their hippocampal dependent and independent spatial abilities by hiding the platform and determining if the rats could find it using internal and external cues (Morris, 2001). When a task is hippocampal dependent, it means that the hippocampus is required for spatial navigation, memory retrieval and learning. The Morris water maze task was found to be hippocampal dependent based on the ability of the mice to find the goal zone when the platform was visible but unable to find it when it was hidden. When a mouse has a lesion in the hippocampus, the rodent may not be able to find the goal location or know where they are in reference to it. In probe trials, they spend less time swimming in the correct quadrant than sham lesioned animals. Another spatial task that is used to access episodic and spatial memory is the place preference task. Similarly, the place preference task is the use of positive negative reinforcement to create an association with cues in the animal's environment (Buccafusco et al, 2009), where reference memory is used to associate background/static cues with events or locations (Rossier et al, 2000).

This spatial accuracy behavioral task studies goal-directed navigation and the ability to locate the food reward after it has been delivered so it combines ideas from both the Morris water maze and the place preference tasks (Getz et al, 2022). This method of studying goal-directed navigation tests the ability of mice to associate a cue with food reward and to then make spatial decisions relative to the goal's location when the cue is removed. We selected this approach to

measure memory function in mice after serial blast wave exposure because it is reproducible, quantitative, and highly relevant to chronic traumatic encephalopathy.

In relation to this specific cognitive task, the working memory of mice is studied through understanding if the mouse knows where they have been and where they need to go to get to a specific goal zone and receive the positive reinforcement. If the working memory of the mice is intact, they should be able to go to areas that they have been in the past and determine if they are in the correct place or if another place may be better (when the mice try out the earlier target zones and determine that they no longer produce a reward and then move on to the next quadrant). This relates to the spatial accuracy and episodic elements of mouse memory because spatial memory is also used to determine if the mouse is in the correct place. When the mice pause in a certain area, they indicate their spatial decisions and their ability to self-localize relative to static arena and room cues. Pause time and other performance metrics then, provide an operational definition of where the mice think they are in space and time. In the visible goal version, the task is easier and hippocampal dependent. As in the water maze, the hidden goal version is more difficult and hippocampal-dependent.

While reference memory and spatial memory in the mice are operationally defined as the ability to self-localize relative to intra and extra-arena spatial cues and navigate to the goal zone via the hippocampal cognitive map (O'Keefe and Nadel, 1978). Working memory is operationally defined as a short-term memory for an object, stimulus, or location that is used within a testing session, but not typically between sessions. It is distinguishable from reference memory, which is a memory that would typically be acquired with repeated training and would persist from days to months (Olton et al, 1977; Dudchenko, 2004). Spatial and reference memory would therefore be measured when mice remember where the goal zone is when the cue is removed. Working memory

would be the ability to discriminate between all possible goal zones in each quadrant, which would be more taxed when the goal zone has been rotated several times. Multiple aspects of memory are therefore accessible with the spatial accuracy task in this study.

In addition to the cognitive testing, microscope images can be done to examine whether the brains sustained injury or remained un-changed from the blasts. Post-testing, the brains of each mouse were perfused and can later be examined for signs of phosphorylated tau (pTau) and glial fibrillary acidic protein (GFAP). Both of these are markers for a physical brain injury. pTau is a marker of neurodegeneration and a pathognomonic signal of chronic traumatic encephalopathy. pTau is typically only seen in focal (localized) lesions around penetrating blood vessels during the autopsy in subjects with CTE or a blast wave exposure. This protein becomes phosphorylated and accumulates in areas of the brain where certain cell pathways are not working well, often due to damage from a TBI or other neurodegenerative disease (Tai et al, 2012). GFAP is a marker of reactive astrocytes, or an inflammatory marker that is typically seen in a diffuse pattern after any type of brain injury.

This study is one of the first to test the effects of a sequential blast injury model on the effects of spatial navigation and flexible memory associations in mice and data from these experiments will further widen the information known about how different types of brain injuries affect different areas of our bodily functioning. In the future, this research may be used in conjunction with new therapeutic strategies or interventions to understand how to best attenuate memory impairments after a TBI.

Methods

Study Design

The objective of this research is to determine how repeated traumatic brain injuries caused by blast waves affects spatial and working memory. This project consists of a multi-part procedure including training mice on the spatial accuracy test, sustaining the blast injuries, and testing the mice on the apparatus post-TBI (figure 3). These experiments test the hypothesis that three sequential low-level blast waves will impair the spatial and working memory capabilities of mice when compared to mice who receive no injury.

Mouse Model

The ten mice that are tested in the experiment are black male C57BL/6 mice between the ages of 10 and 14 weeks received from Jackson Laboratories in Bar Harbor Maine. The methods used in this thesis follow the protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Vermont. These mice were food restricted to 85% of their baseline body weight and underwent training in the testing apparatus prior to the series of blast injuries in order to familiarize themselves with the apparatus and create a baseline memory of the tests. The mice receive the serial traumatic brain injuries and are then tested again in the spatial accuracy task. The blasting process will be standardized from mouse to mouse so as to limit variability across animals. The data collected in the project will be produced both between the series of blasts and after the blasts from the testing apparatus. The blasting protocol takes place at the UVM Colchester Campus, and the spatial accuracy testing is conducted on the UVM main campus in the Given research facility. The control mice are important for the creation of a behavioral performance baseline relative to all spatial accuracy measures.

Spatial Accuracy Training

Before the traumatic brain injuries can be given, the mice are trained on the spatial accuracy operant task. The spatial accuracy task, that was created by Dr. Jeremy Barry, is based on the

Morris Water Maze task and involves food restricted mice beginning to associate a visible cue and other areas of the arena with food rewards (Getz et al, 2002). The spatial accuracy testing model consists of a large, opaque cylindrical arena with dimensions of 60cm in height and 76 centimeters in diameter. A polarizing cue card (color code gray 9.5; Color-Aid Corp) was taped to the inside wall of the arena on the southeast wall. This arena is monitored by a firewire camera (30Hz sampling rate) and tracking Biosignal software (Tracker, Bio-signal Group Corp) that is elevated above the arena along with an automatic pellet feeder containing Bioserv 20-mg dustless precision pellets. There are 10 training phases in total, comprised of individual 30-minute sessions in phases 1-6. The last four phases are comprised of pairs of 30 minute visible and hidden cue goal sessions. During training on phases 1-6, each mouse is placed in the arena for a with a 2 cm white bottle cap cue attached to the floor of the arena in the NE quadrant. The bottle cap serves as a cue for the goal zone within which the mice would have to remain for a given amount of time to receive an automated food reward. Once the mouse has received 20 rewards in a given phase, they move on to the next phase. In each progressive training phase, the goal zone has a smaller diameter and a longer amount of time is required to trigger release of food pellet reward. After each reward is dispensed, there is a 5 second refractory period before the automated pellet feeder will produce another pellet to encourage the mice to find the previously dropped pellet.

Phases 1-4 require the mouse to be in the goal zone for 500 ms in order to receive the reward with the circular diameter progressing from 51 cm, 28 cm, 19 cm, and 15 cm respectively. Phases 5-7 require a latency period of 750 ms, 1s and 1.2 seconds respectively where the mouse must pause in the goal zone. The diameter of the goal zone remains at 15 cm for these phases and the remaining phases. Phases 7-10 have both visible and hidden cue sessions. Each mouse enters the arena with the bottle cap present, is taken out, the arena cleaned with soap and water, rotated

180º and then the mouse is put back in the arena for another session without the cue present. The goal zone remains in the same area as when the cue was present. In phase 8, the cue is rotated 90º into the next quadrant (NW), then the SW quadrant for phase 9 and finally the SE quadrant for phase 10 (figure 1).

TBI Induction

Once the training is complete, the mice are transferred to the UVM Colchester Research Facility, and neurological scores are recorded on each mouse. These neurological scores consist of grasp, gait, postural reflex, abnormal position (paretic head tilt/contorted body), edge perception, vision, circular motion, posture (pain), apathy (normal exploration), anxiety, aggression, and grooming measurements. Grasp is tested by placing the mouse on the metal grate of the lid of their cage and flipped upside down; if the mouse can stay hanging onto the lid for over 15 seconds, they are considered to have no deficits (Sackheim et al, 2017). Edge perception is measured by holding the mouse by the tail and bringing them bear a table edge to determine if they reach out to it, and vision is tested by slowly moving a cotton swab along the mouse's body in the caudal to rostral direction and determine if the mouse can see it once it comes into their eye line. All other neurological scores are tested visually by watching the mouse in the cage as they move around. The behavioral findings are seen in the posture (pain), apathy, anxiety, aggressive behavior, and grooming measurements while the others are sensory/motor findings.

The mice are then each given 0.15 ml of a ketamine-xylazine cocktail (0.3 ml (200 mg/kg, 100 mg/ml) Ketamine, 0.15 ml (4.5 mg/kg, 20mg/ml) Xylazine, 2.55 ml saline) subcutaneously in the abdomen at a 45º angle, one at a time. This causes them to become unresponsive and unable to feel pain (tested with the pedal reflex) and placed on hot packs. Following that, each mouse is secured into place in a specially designed blasting device

consisting of a driver tube that is filled with pressurized air and a driven chamber. The driven chamber is where the air is released to create a blast wave that propagates down a tube to hit the mouse head on the left side. The driven, or blast chamber is closed around the mouse and a 0.07" aluminum membrane is placed between the driver and driven chambers of the device, closed and clamped. A computer is used to code the blast pressure at 30 psi for each mouse. The blast over pressure wave produced mimics the ideal Friedlander curve with peak of 12.51 PSI with a positive phase of <5 milliseconds for pressure to return to atmospheric (figure 2). Each mouse is taken out of the tube and set on the hot packs to recover. Each of the six TBI and four sham mice are given the same treatment, the only difference is that the sham mice do not receive a blast when in the blast chamber. The mice are given buprenorphine if they exhibit pain within 12-14 hours after the blast. All apparatuses are cleaned with Cavistat. This process occurs two more times with a recovery period of 24 hours between each blast. The mice were transferred back to the Given Research Facility and after 72 hours, rotarod testing occurred. Each mouse was placed on a rotarod device to test motor coordination for 300 seconds (5 minutes) and three trials. The number of seconds that each mouse was able to stay upright and walking on the elevated rod as the speed slowly increased was recorded for each trial.

Cognitive Testing

Twenty-four hours after the final rotarod trial, the mice began testing on the spatial accuracy apparatus. The mice were re-tested only on phases 7-10. The number of rewards given to the mice and the number of entrances in the goal zone during the period is recorded through the tracking system as well as the movement of the mouse around the arena. Each mouse completes one phase per day, consisting of paired visible and hidden cue goal sessions. Each session that the mice complete creates a .dat file that is input into MatLab (MATLAB v R2019A,

MathWorks) to carry out analysis of spatial accuracy performance parameters, create a position map and excel sheet of data that was collected from the position tracker (figure 4). Three months after the initial blast injuries, all mice were again re-tested in phases 7 and 8. General estimating equations (GEE) were used on IBM SPSS statistics software to carry out statistical analyses of the spatial accuracy performance. These GEE are used in statistics to estimate clustered count data and find parameters as well as find correlations in data points. Significance was determined if results provided a p-value of less than 0.05 ($P<0.05$).

Histology

When all mice completed all testing phases, they were anesthetized with 2.5-5% aerosolized isoflurane and 0.8% oxygen inhaled via nose cone, the absence of a pain response was checked with a toe pinch, and perfusions were done on each mouse. After the mice were anesthetized, all four legs were secured. The chest was cut open with scissors until the heart was exposed. A butterfly needle is inserted bevel down into the left ventricle and secured with a clamp. The right atrium is cut with scissors and the perfusion is started through the needle. Attached to the needle is a tube and a large syringe. The syringe is elevated on a test tube stand to control the flow pressure of the liquid in the needle. The flow pressure was set to between 40 and 70 mm of mercury. About 15 mL of PBS buffer is used to initially flush out the blood in the mouse followed by 30 – 40 mL of 0.4% paraformaldehyde (PFA) or until the mouse becomes stiff and fixed. Once the mouse becomes stiff, the head is removed using scissors and the brain carefully extracted from the skull.

Once extracted, each mouse brain was embedded in paraffin, sliced into twenty-micron sections in a cryostat, and placed on glass coverslips. The tissue slices were incubated and stained with antibodies that target and bind to GFAP and pTau as previously described

(Kakinuma et al, 1998; Murakami et al, 2011). The slides can then be scanned under a high-resolution microscope in 16 images with 0.5 micron steps and a computer can combine the images to create a wide field image of the complete brain section in later studies (Larson et al, 2012).

Results

Analysis of tracking data produced many variables for spatial accuracy performance, including number of rewards, number of entrances, RER (rewards versus entrances ratio), mean speed, mean speed to the target, time in each quadrant, time spent in the goal zone, and mean pause angle. Contrary to our hypothesis, none of these performance measures revealed significant group effects for visible or hidden cue goal conditions either in the standard goal location (P7) or during the rotation sessions (P8-P9) ($p > 0.05$). For example, the nonsignificant difference between the entrances and rewards values between treatment groups of TBI and control mice (figure 5).

It was found that there was no significant difference between the time spent in goal zone 2 and goal zone 4 of the hidden session of phase 10 in the TBI mice 4 (mean proportion time in 2=2.6%, mean time in 4=3.5%, $p=0.415$, figure 8). As the TBI mice progressed in rotational phases, the significance between the correct goal zone and goal zone 2 decreased. This trend was evident, but significant, in phase 9. This difference became non-significant in phase 10, or the 4th goal zone location.

The mice, when re-tested 3 months after the initial blast, again showed no significant decrease in performance in rewards, entrances, RER ratio or time spent in the correct goal zone relative to their own past performance. Only the first two phases were tested (7 and 8), but a similar trend in less significant goal times was seen in the TBI mice in the later rotation. Replicating the results of phase 10, the TBI mice in the hidden sessions again demonstrated more confusion

between possible goal zone locations and were unable to differentiate between goal zone 2 and the most recent goal zone in the quadrant 1 (Zone 1/NE quadrant). There was no significant difference ($p=0.728$) between the time spent in goal zone 1 and goal zone 2 in the first phase of the testing (figure 9).

The standard errors for the CTL mice when it came to entrances in visible cue sessions were much higher compared to those of the TBI mice (Phase 7 - 11.103, phase 8 - 12.496, phase 9 - 20.051, phase 10 - 10.334). In addition, there was a significant difference ($p = 0.007$) between the RER (rewards versus entrances ratio) of the hidden sessions in CTL and TBI mice with the TBI mice having a lower mean RER ratio than that of the CTL mice (0.361, and 0.436 respectively; figure 7).

The data collected from the rotarod testing found no significant differences in performance as well as no significant differences between mice in neuroscore testing. Both groups, TBI and control (CTL) mice had marked decreases in rewards received and entrances into the goal zone for the hidden sessions compared to the visible sessions. No significant difference in rewards or entrances was found between the two groups however. Both groups also had an overall trend of decreasing in performance over the course of the four rotations in the amount of time spent in the goal zone (figure 5).

The perfusions done on the mice from this study did not produce images in time for the submission of this thesis and can be used for later research, however images of previously stained brains from mice that underwent the same consecutive blast wave injury protocol are included. These mice (n=12) showed significant pTau accumulation in areas of the brain (figure 10).

Discussion

There are many scientific articles about TBIs and how they affect everything from memory, behavior, learning, reflexes, and mental health (McDowell et al, 1997; Azouvi et al, 2017; Chai et al, 2018; Milman et al, 2005). Only a few of these have studied the convergence of TBI and memory using closed head TBI murine models. Previous studies in this area primarily focused on single mild traumatic brain injuries, mostly utilizing the closed-skull electromagnetically controlled rubber impactor model, and non-invasive closed-head weight-drop models. The previous work in this area concluded that the mice "suffer from long-lasting cognitive deficits, emotional difficulties and behavioral disturbances" (Milman et al, 2005), as well as memory impairments and depressive/anxious behavior (Zohar et al, 2011; Broussard et al, 2017).

Many other well documented TBI models in mice and other animals include incisions of the scalp and skull, and direct cortical impacts to the brains of these animals. The method of injury in the model discussed in this paper is a closed head blast pressure wave model that directly injures the left side of the head without the use of blunt trauma. This creates a reproducible mild head trauma that best recreates the types of injuries seen in blast injuries in the armed forces. This alternative way of inducing mild TBI and studying cognitive deficits has not been studied yet as well as spatial learning and memory which is not well studied in.

The TBI mice in this study exhibited a decrease in the working memory limit compared to the control mice even though there was no visible motor deficit for the mice. The rotarod testing conducted after the TBI induction indicated that there was no significant difference between the motor coordination of the TBI mice compared to the control mice. This indicates that there was no significant damage done to the cerebellum of the mice in the blast since that is the area of the brain that controls balance, motor movement and coordination (Morton and Bastian, 2004). The

neuroscore testing conducted before every blast indicated that neither group had noticeable emotional or behavioral impacts and corroborated that they did not have noticeable motor deficits.

A previous study found that mice that underwent a mild TBI had significant motor deficits for days after the injury (Yang et al, 2013). This study used the weight drop model and two different weights to produce a mild injury and a more moderate injury. These mice were initially trained on the rotarod device prior to receiving the head injury. The moderately injured mice showed significant decreases in rotarod performance while the mild TBI mice performed like the sham mice at first and began to decline over time.

While there were no significant differences in the number of rewards and entrances when comparing the two groups of mice, after a few goal zone rotations these performance variables for both groups of mice declined (figure 5). This indicates that although they had increased difficulty finding the goal zone. While this increase in difficulty was detectable across all animals, group effects were only found with regard to spatial working memory in phase 10 (the 4^{th} zone).

Each group, TBI and control, entered the goal zone and triggered the release of the food reward an equivalent number of times. Although there was no group effect for the number of entrances and rewards, the condition, or whether the cue was visible or hidden, did influence the number of rewards and entrances. All of the mice, regardless of group, had significantly fewer rewards and entrances in hidden sessions compared to visible sessions ($p=<0.001$; figure 6). This is to be expected since the visible cue was a landmark for the mice that indicated goal zone location. In addition to this, the visible task is easier for the mice because the hippocampus is not the only area of the brain that is needed for this, meaning this task is hippocampal independent. In other words, it employs a beacon strategy rather than a hippocampal bases strategy were the

mice have to self-localize relative to stationary room cues to navigate to the hidden goal zone (Morris et al, 1982; O'Keefe and Nadel, 1978).

There was a significant difference ($p=0.007$) between TBI and control mice where TBI mice in the hidden goal session of phase 9 had a lower RER ratio (figure 7). This lower ratio illustrates that these mice had more entrances and received fewer rewards, indicating that the TBI mice could have a spatial accuracy deficit. These mice seemingly entered the goal zone and were not sure if they were in the correct location and moved again. There was an increasing number of entrances which indicated that the mice were less sure of where they were in the arena relative to the goal zone. As mentioned, the spatial accuracy part of this task is when the mouse pauses indicate their memory of the correct goal location. When TBI mice continue to spend time in a previous quadrant, the mice instead indicate they are less able than controls to discriminate between possible goal zone within a session

There was a decrease in performance for the amount of time spent in the goal zone for both groups over the course of the four testing phases. This indicates that after each new rotation, both groups of mice had a more difficult time finding the correct goal zone. Each rotation was pushing the working memory and interference limit of the mice. There was no significant effect of visible cue versus hidden cue on the goal time, unlike for the rewards and entrances. This means that the mice were able to find the goal zone despite not having a visible cue present but had a more difficult time (figure 5).

The major finding of this study was that the TBI mice, by the third rotation (the fourth testing phase (phase 10), are unable to differentiate between the second and fourth goal zone. There is no significant difference between the time spent in goal zone 2 and goal zone 4 ($p=0.415$; figure 8). The TBI mice began the trend of spending time in goal zone 2 during the third and fourth testing

phases and the interference that began in the second rotation became significant by the third rotation. This indicates that there was potential interference between the memory of goal zone 2 and subsequent goal locations. The mice that sustained a traumatic brain injury may have reached their working memory and interference limit and became more confused with each new rotation. The distribution between the correct goal zone and the other goal zones was less different when each new rotation occurred. The difference between the correct zone and other zones becomes less significant as the phases progress.

This finding was replicated when the mice were re-tested 3 months after the initial TBI. The TBI mice were unable to differentiate between the correct goal zone and the other goal zones as early as the first rotation, indicating a further decrease in the working memory limit. In the hidden session of phase 7 of the testing, the injured mice spent a mean of 4.0 % of the time in goal zone 1 and 4.3 % of their time in the correct goal zone 2. These two values are not significantly different ($p=0.728$; figure 9). Since the TBI and control mice did seem to stay in the correct zone more for the second phase however, there was likely some degradation in performance after three months that improved with training and the mice were able to re-learn the task. This could have led to a clearer discrimination between possible goal zones by the second phase. However, even though control animals trend this way, only the TBI animals again showed a lack of discrimination between zone 1 and zone 2 when the zone 1 was the most recently correct goal location. This replicates the tendency of TBI mice to demonstrate an increased susceptibility to interference, as demonstrated by a lack of discrimination between 2/4 possible goal zone locations within the hidden goal session. In addition, because only two phases were completed at the 3-month timepoint, it is unclear as to whether the inability to differentiate between previously learned goal zones and new ones would have become more or less severe as the phases progressed.

The hippocampus is the main area of the brain used for spatial learning, memory, and navigation (O'Keefe and Nadel, 1978; Bast et al, 2009). The working memory deficits and interference present in both timepoints in this model illustrate that these TBI mice are reaching their working memory limit and may be impaired are encoding new information while the control mice are still able to learn new locations and goal zones. The decreased working memory limit appears to be due to the traumatic brain injuries and this is translatable to humans who have been shown to have decreased memory facilities after sustaining a blast injury.

Although the mice from this cohort of TBI and spatial accuracy mice were not able to be perfused and analyzed in time, mice that underwent the same TBI protocol, but did not go through cognitive testing, were imaged. The images (figure 10) show that TBI mice exhibited a higher accumulation of pTau in the brain after receiving the serial blast injuries. This furthers the idea that although the mice did not have motor deficits, there was damage caused by the blast injuries and this could have contributed to the cognitive deficits in the mice.

It is possible that there could be a physical change or network change in the brain due to the TBI, but the brain can compensate for those areas that are damaged. The brain has the ability to take over the roles of damaged neurons by rerouting the information to a different part of the brain and clearing out the damaged or dead neurons (Heather, 2013). A greater change in brain structure would create more visible deficits and could be seen in mice which receive a blast injury that is more severe and has a higher PSI.

We found that sequential blast injury has only a mild effect on spatial accuracy performance without affecting motor responses. We therefore concluded that both hippocampal independent and dependent spatial memory, as determined by the ability of the mice to navigate to the visible goal zone, or to the recent location of the hidden goal zone, is largely intact in the TBI mice and

on part with controls. However, our analyses found that there was a significant group effect for spatial working memory by the 3rd goal rotation. By this phase of training, the TBI mice demonstrated confusion between possible goal locations 2 and 4 (figure 8). This finding suggests that, following several goal rotations, TBI mice were more prone to interference or could have working and spatial memory limitations in comparison to control mice.

The success of these experiments, and the potential establishment of a working memory limit as a result of TBI, creates avenues for combined behavioral and electrophysiological experiments to test the underlying cause of these effects in a hippocampal dependent memory task. It also creates opportunities for pharmacological agents that could attenuate working memory impairments post-TBI. The work conducted with these mice aims to set up a framework for understanding the relationship between brain trauma and learning and memory impairments and will enable researchers in the future to learn how to better treat TBI patients and understand how they function after an injury.

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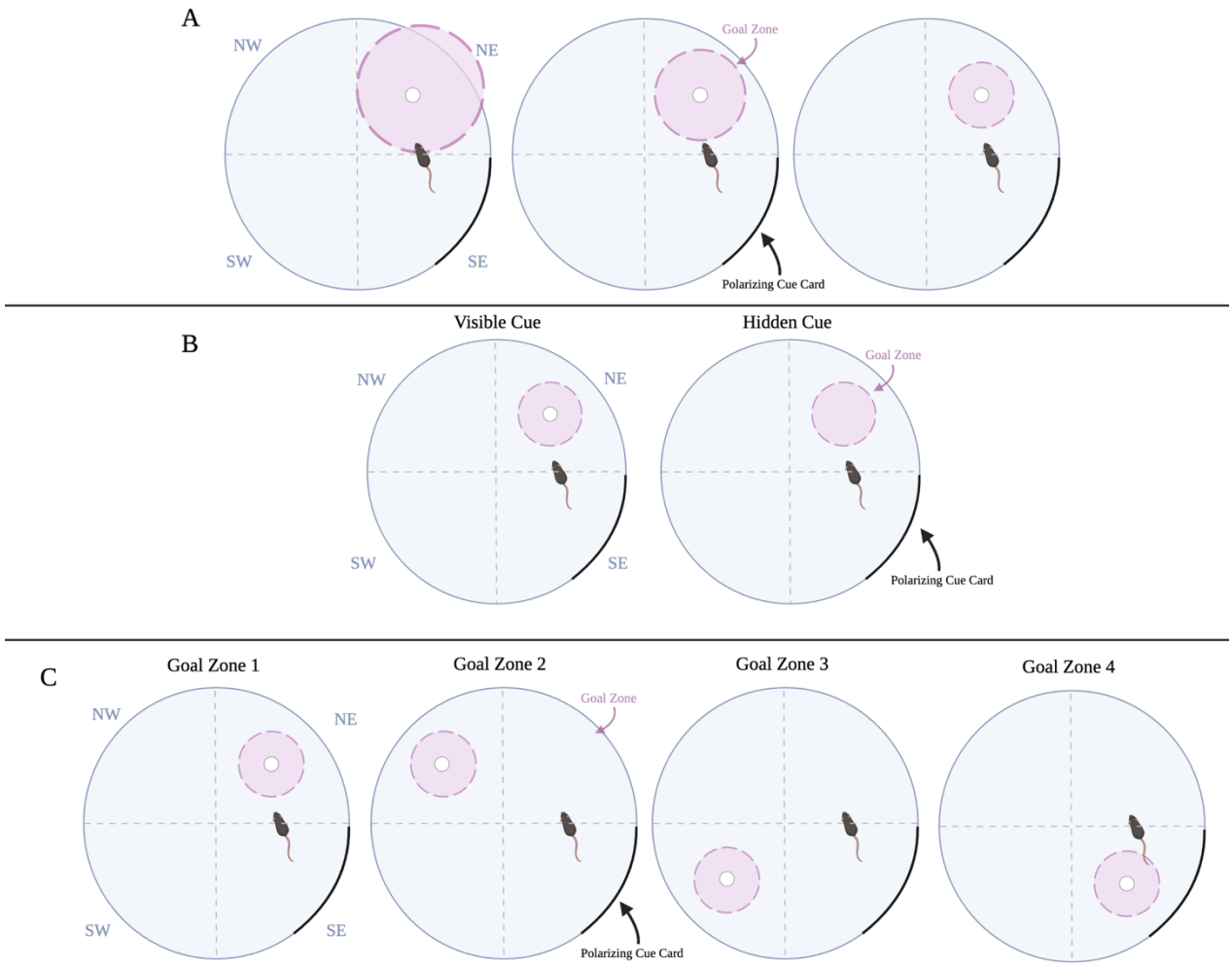

Figure 1. (A) Training phases where the diameter of the goal zone decreases as the training phases progress. The first arena is the approximate size of the goal zone for training phase 1, and it becomes smaller in phases 2-4 until the third arena that represents the goal zone for phases 5-10. (B) Testing phases where there are visible cue sessions and the hidden cue sessions, and how the goal zone stays in the same place but the cue is removed. (C) This is how the arena would look for phase 7 (NE goal 1) followed by rotations of the cue and goal zone to the NW (zone 2), SW (zone 3), and SE (zone 4) quadrants for phases 8, 9, and 10 respectively. This diagram was made using BioRender.com.

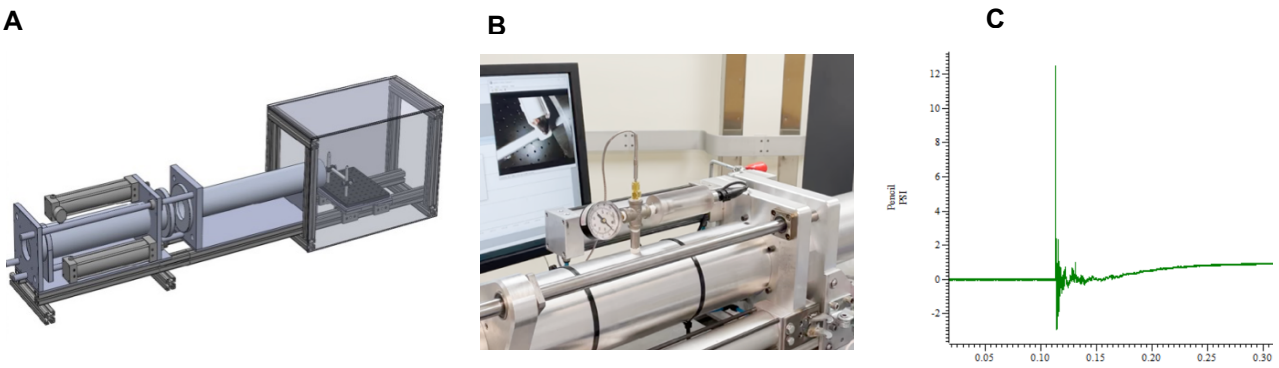

Figure 2. (A) Schematic of custom wave model. A membrane is secured between the driver and driven chambers. The chamber is pressurized, and the membrane pierced, leading to a blast wave propagating down the shock tube to induce injury. (B) Photograph of device with mouse in position viewed on the computer monitor. (C) Blast over pressure wave mimicking ideal Friedlander curve with peak of 12.51 PSI with a positive phase of <5 milliseconds for pressure to return to atmospheric. Source: Freeman Lab

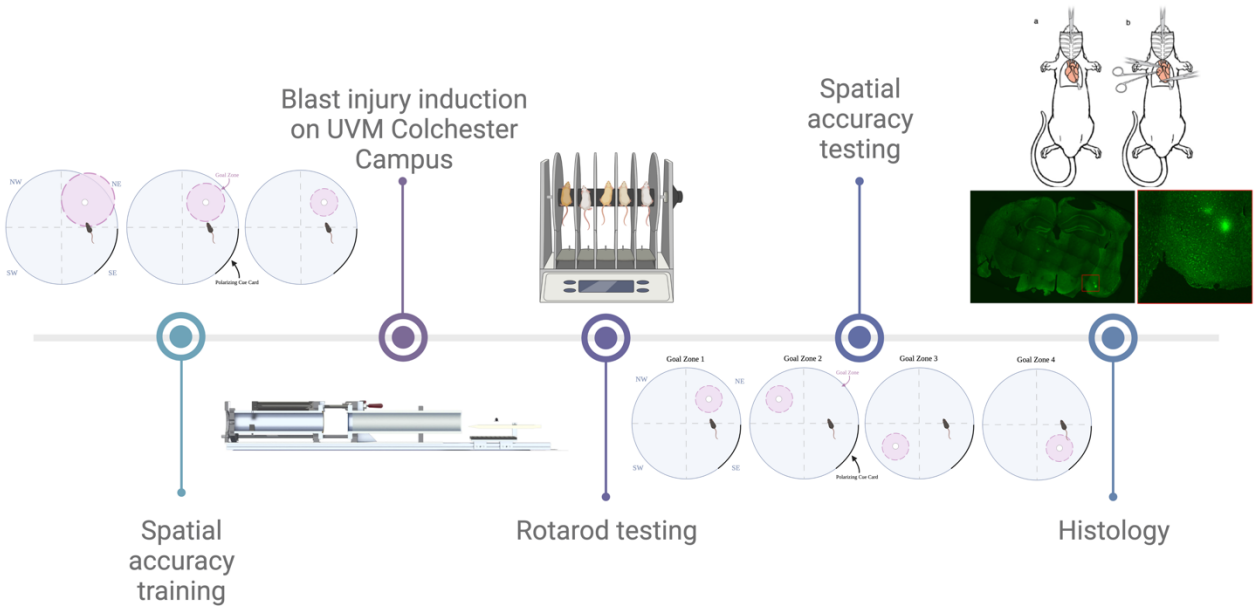

Figure 3. A timeline illustrating the methods used in this experiment. The methods began with training on the spatial accuracy task followed by the blast injuries and followed by testing on the spatial accuracy task again and concluded with perfusion (Gage et al, 2012) and histology. This diagram was made using BioRender.com.

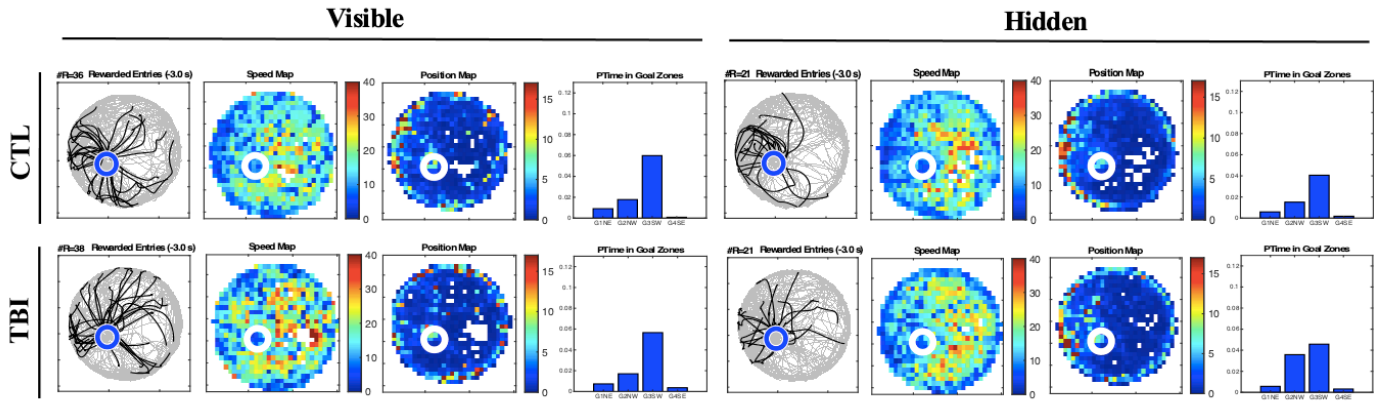

Figure 4. The diagram above illustrates the movement, speed, position, and time spent in each goal quadrant of one control and one TBI mouse in sessions containing a visible cue and without that cue. These diagrams were made from tracking data from the overhead camera and analyzed by custom MatLab code.

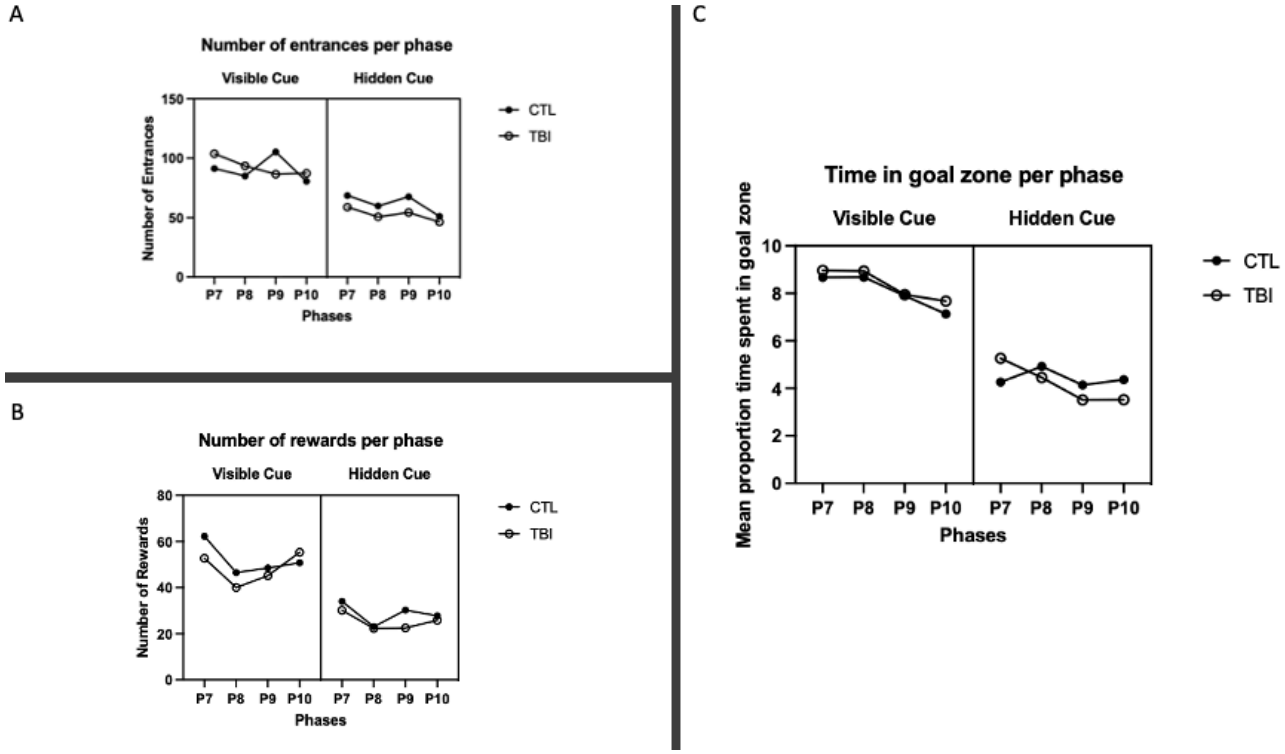

Figure 5. (A) There are no significant differences between the number of entrances or (B) rewards between groups over time, however the visibility of the cue is significant as illustrated more clearly in figure 6. (C) This figure illustrates the trend of decreasing time spent in the goal zones over the course of the four phases. The phases with the visible cue can be found on the top and the hidden cue on the bottom. The mice are pooled, and these values represent their mean time spent in goal zone.

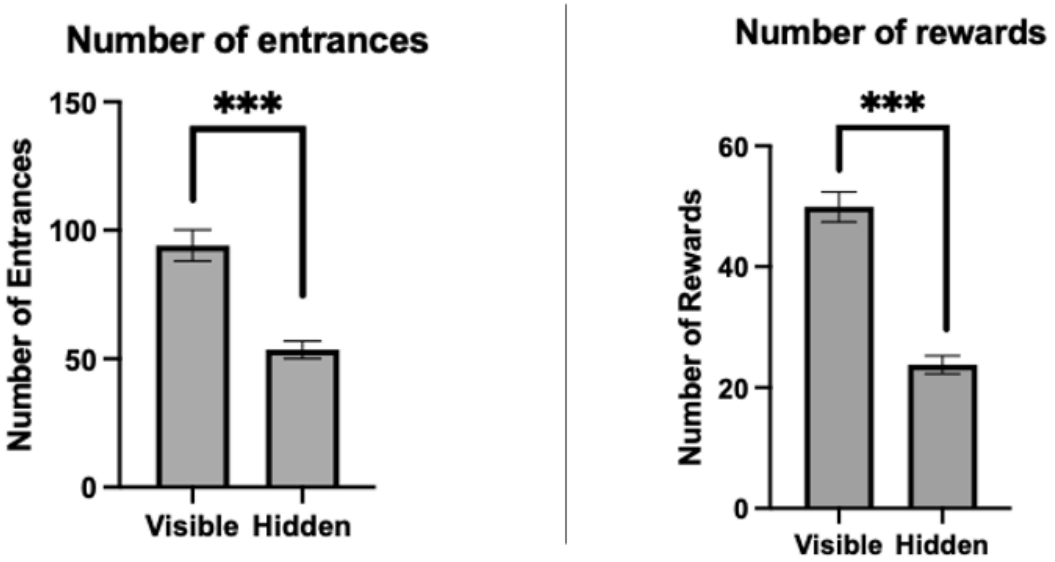

Figure 6. This figure illustrates the significant decrease in the mean number of rewards and entrances for both the CTL (n=4) and TBI mice (n=6) when the cue was hidden (p=<0.001 for both). All animals in each treatment were pooled for these calculations. The mean number of entrances for the visible and hidden sessions were 94.12 and 53.51 respectively. The mean values for the visible and hidden sessions for rewards was 49.91 and 23.76 respectively.

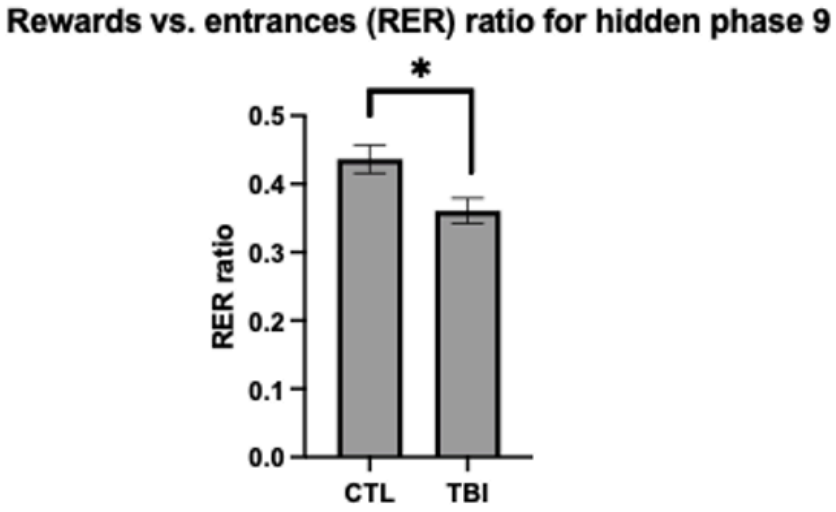

Figure 7. This figure shows the significant difference between the ratio of entrances to rewards of the CTL (n=4) versus the TBI (n=6) mice ($p = 0.007$). The mean value for the control mice was 0.43625 while the mean value for the TBI mice was 0.360833. The RER ratio was calculated for each mouse and then the mean was calculated from those values.

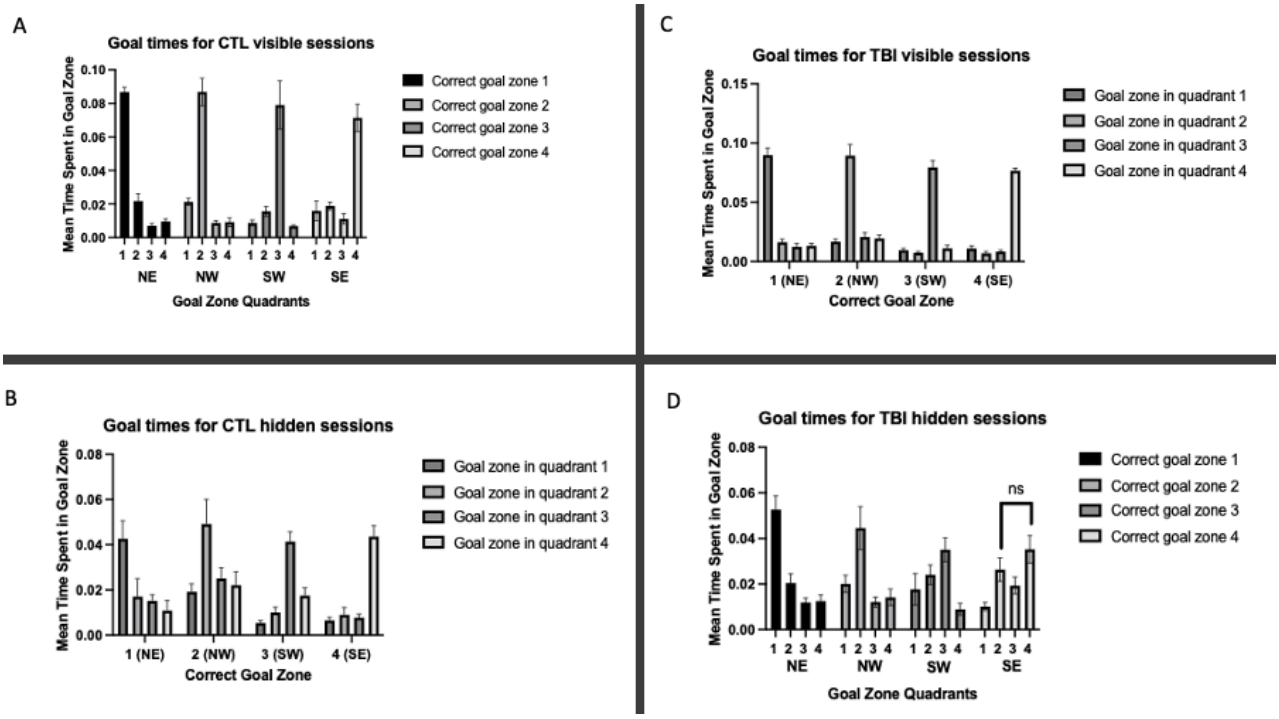

Figure 8. This figure illustrates the mean time that the mice spent in each goal zone in each quadrant compared to the correct goal zone of that phase. (A) The mean goal times for the control (CTL) mice in the visible sessions (n=4). (B) CTL mice in hidden sessions (n=4), (C) TBI mice in visible sessions (n=6) and (D) TBI mice in hidden sessions (n=6). The correct goal zones in each phase of the rotation (1 being the NE quadrant, 2, 3, and 4 are the NW, SW, and SE quadrants respectively) are all significantly different from the other goal zones in incorrect quadrants except for the last rotation phase of the TBI hidden sessions in box D above (p=0.415) (with the mean time in 2 being 0.026 and 4 being 0.035).

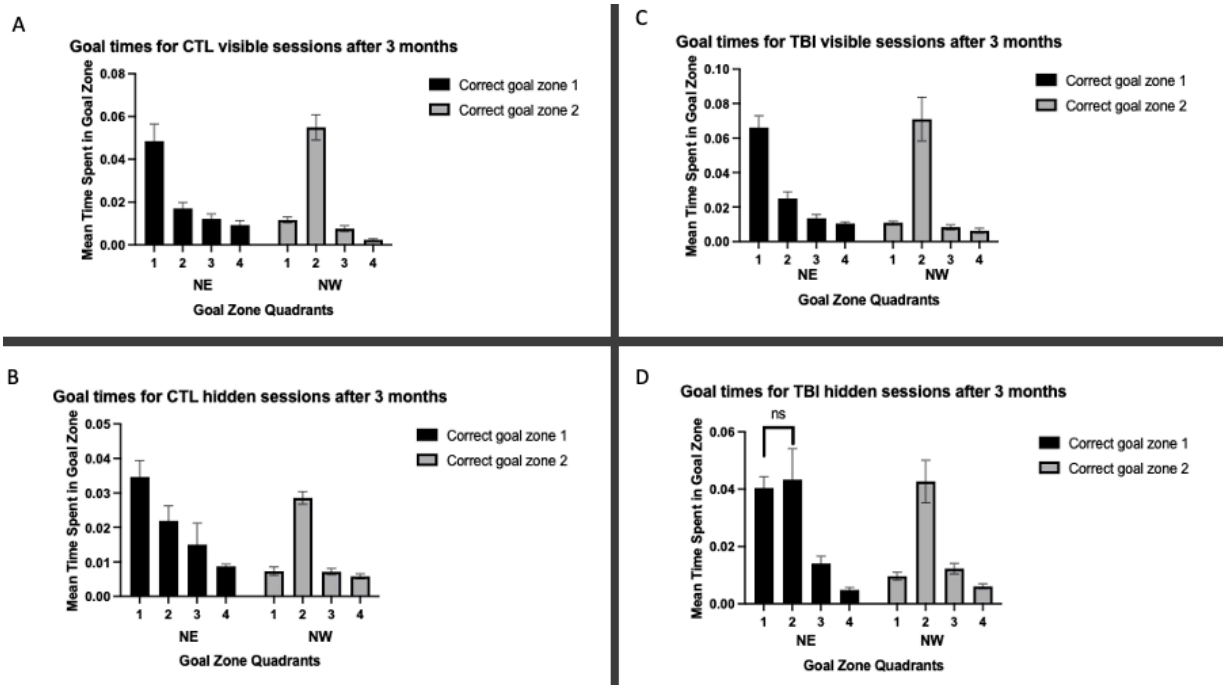

Figure 9. This data was taken 3 months after the initial blast injuries to the mice. This figure illustrates the mean time that the mice spent in each goal zone in each quadrant compared to the correct goal zone of that phase. These mean goal times were only recorded for phases 7 and 8, or goal zones 1, and 2. (A) The mean goal times for the control (CTL) mice in the visible sessions (n=4). (B) CTL mice in hidden sessions (n=4), (C) TBI mice in visible sessions (n=6) and (D) TBI mice in hidden sessions (n=6). The correct goal zones in each phase of the rotation (1 being the NE quadrant and 2 being the NW quadrant) are all significantly different from the other goal zones in incorrect quadrants except for the first phase of the TBI hidden sessions in box D above (p=0.728).

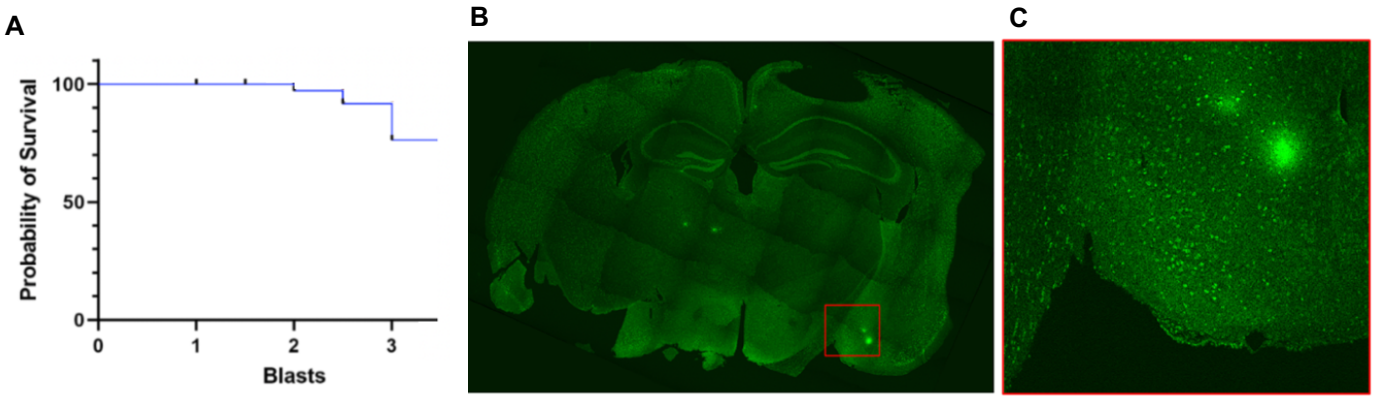

Figure 10. Serial blast injury in mice that did not undergo cognitive testing. (A) Survival of mice after 3 consecutive daily blast injuries (n=12). Surviving mice were allowed to recover for 1 week before euthanasia and perfusion-fixation for immunohistochemistry of p-tau. Representative sections show foci of p-tau accumulation at 10X (B) and 40X (C). Source: Freeman Lab