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Dehydration and Traumatic Brain Injury: A pilot study of a translational rodent model of conditions in professional combat sports

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Abstract

Traumatic brain injuries are a widespread public health issue, with athletes being at an increased risk of traumatic brain injury, especially in contact sports. Dehydration and rehydration prior to use of a weight drop served as a model for conditions experienced by professional boxers in a rodent model of traumatic brain injury. Following dehydration, rehydration, and traumatic brain injury procedures, subjects' symptoms were assessed using the novel object recognition task, sucrose preference test, open field maze, and elevated zero maze. There were significant effects of both dehydration and TBI on time to righting, and there was a significant interaction between manipulations on time to ambulation. During the Elevated Zero Maze, there was a significant main effect of TBI on the number of times subjects entered the open sections of the maze. Post-hoc analyses found that dehydration prior to injury served as a neuroprotective factor on time to ambulation, and that fluid intake resulted in significant difference in fecal boli produced by sham subjects but not TBI subjects. During the Open Field Maze, there was a significant interaction effect on fecal boli. Even with the small sample size of this study, these effects are notable with further research being necessary. Further research would also benefit from the inclusion of food restriction or exercise manipulations.

Keywords

Keywords: Traumatic brain injury, TBI, dehydration, mouse model, weight drop procedure

Dehydration and Traumatic Brain Injury: A pilot study of a translational rodent model of conditions in professional combat sports

Traumatic brain injuries are a widespread public health issue, with 223,135 traumatic brain injury-related hospitalizations reported in 2019 and 64,362 traumatic brain injury-related deaths in 2020 (Centers for Disease Control and Prevention, 2023). With athletes being at an increased risk of traumatic brain injury, especially in contact sports, this population is a useful group for studying mild, moderate, and severe traumatic brain injury.

According to Bernick and colleagues (2021), boxers experienced, on average, a concussion rate of 0.047 concussions per minute of fight time (i.e., total time between both athletes in match), with MMA fighters averaging 0.085 concussions per minute of fight time. With an average between the two estimating to 1 concussion every 12.5 minutes, or 0.061 concussions per minute of fight time, which would roughly translate to one concussion per fight. Additionally, Bernick and colleagues' (2021) work suggests that concussion rates differ based on the outcomes of a fight, with the losing athlete being more likely to receive head trauma. While Bernick and colleagues (2021) have provided estimates of concussion rates in professional fighting athletes, other research suggests that the true rates of traumatic brain injury in these sports are grossly underestimated immediately following in-match injuries (Fares et al., 2019).

A relatively common practice amongst professional boxers and MMA fighters is a "cutting" period prior to a weigh in. During this period, athletes drastically decrease their overall fluid and nutritional intake leading to a roughly 10-15% decrease in body mass (Bernick et al., 2021) in an attempt to be placed in a different weight class. As it stands, professional boxers are already at an increased risk of traumatic brain injury, with typically one mild to moderate head injury occurring with each fight. With past literature suggesting that dehydration affects ventricular volume in the brain (Kempton et al., 2009), which may put individuals at an increased risk of traumatic brain injury (Dickson et al., 2005), there may be a connection between the use of "cutting" periods and rates of traumatic brain injury in professional boxers.

Following sustained periods of reduced fluid-intake, several deficits have been acknowledged, including inefficient cerebral metabolic rates and worsened performance on tasks related to executive functioning (Kempton et al., 2011). Aside from marked cognitive and functional deficits, Kempton and colleagues (2011) also noted an expansion of the ventricular system in participants who underwent a reduced fluid-intake condition, with no overall change in gross cerebral volume.

Kempton and colleagues' (2011) measure of ventricular changes was based on prior research by Dickson and colleagues (2005), who defined intra-cranial volume as "a constant which equals the sum of the volume of the brain, the intra-cranial volume of [cerebral spinal fluid] and the intra-cranial volume of blood" (p. 1), which showed that changes in gross intra-cranial volume may influence susceptibility to traumatic brain injury. Several medical interventions for severe traumatic brain injury focus on the control and maintenance of intracranial pressure and cerebral perfusion pressure—due to these conditions' potential to cause secondary brain injuries. Typically, this is achieved with pharmacologically induced dehydration (Sun et al., 2016).

Following a traumatic brain injury, complications arising from changes in neuronal function can lead to behavioral and cognitive traits that mirror psychiatric disorders in human patients. These can be translated into rodent models of traumatic brain injury by assessing depressed and anxious behaviors in subjects.

Depression following a traumatic brain injury categorized or diagnosed as depression sequelae or depressive disorder due to another medical condition is diagnostically distinct from psychiatric depressive disorders due to the diagnostic criteria of the DSM-5 (American Psychiatric Association, 2013) which states that "symptoms are not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition" (p. 168). Studies have found that between 25% and 50% of patients reported symptoms of depression sequelae within a year following their traumatic brain injury (Jahan & Tenev, 2023; Kreutzer, Seel & Gourley, 2001; Seel et al., 2003). Dysfunction of neurotransmitters, metabolic abnormalities, neuroendocrine dysregulation, and inflammation have been observed in patients following a traumatic brain injury and have been implicated in depression sequelae (Jahan & Tenev, 2023). Within fourteen days following a traumatic brain injury, a significant decrease in immunoreactivity of serotonin reuptake transporters was observed in regions of the brain surrounding the cortical impact site in a subject (Abe et al., 2016). Additionally, after seven days following injury, there is a significant decrease in mRNA and protein expression related to serotonin reuptake transporters (Abe et al., 2016). These findings are consistent with the serotonin hypothesis of depression, which suggests that depression and depression-like symptoms are caused by deficits in serotonin levels in the brain (Albert, Benkelfat & Descarries, 2013). Elevated levels of pro-inflammatory cytokines due to traumatic brain injuries have been suggested to put patients at an increased risk for developing depression-like symptoms (Jahan & Tenev, 2023). While there are notable physiological and psychological benefits to regular exercise and sports participation, these also come with an increased risk for traumatic brain injury (Lee et al., 2019). Depression has been noted as a clinical feature of chronic traumatic encephalopathy (Lee et al.,

2019), a neurodegenerative disease resulting from repetitive traumatic brain injuries predominantly diagnosed in contact sport players (i.e., football players and boxers) (McKee et al. 2014). According to Lee and colleagues (2019), clinical symptoms of depression are associated with cognitive deficits and volumetric changes in professional boxers following repetitive traumatic brain injury.

As with symptoms of depression following a traumatic brain injury, clinical anxiety symptoms as a result of a traumatic brain injury cannot be attributed to a psychiatric disorder and are thus diagnosed as anxiety sequelae. Anxiety disorders have been noted to be more likely to develop in cases of mild traumatic brain injuries rather than moderate or severe traumatic brain injuries (Mallya et al., 2015). Between 1% and 28% of patients exhibited symptoms of anxiety disorders following traumatic brain injuries (Mallya et al., 2015). In terms of specific anxiety or stress-related disorders, several studies have found that between 13% and 24% showed symptoms of post-traumatic stress disorder, between 2% and 15% exhibited obsessive-compulsive disorder-like symptoms, between 0.8% and 10% developed specific phobias, and between 3% and 28% exhibited symptoms of generalized anxiety disorder (Mallya et al., 2015; Hiott & Labbate, 2002; Moore, Terryberry-Spohr & Hope, 2006).

The literature on brain injury seemingly ignores the likelihood of dehydration prior to injury as seen in the populations at higher risk for traumatic brain injury, which raises several questions. Will a dehydration and rehydration period serve as a risk factor in a mouse model of traumatic brain injury? How will dehydration and rehydration prior to the use of a weight drop affect pathology of a mouse model on traumatic brain injury? Dehydration and rehydration prior to the use of a weight drop may serve as a risk factor for symptom expression in a mouse model of traumatic brain injury. Prior research suggests that ventricular expansion is evident following extended periods of dehydration (Kempton et al., 2009) which has been shown to increase susceptibility to brain damage following an injury (Dickson et al., 2005).

Method

Subjects

The subjects in this study consisted of 18 C57BL/6 wild type mice (n = 18) bred in lab. At the start of the experiment, the subjects were approximately 8 weeks old. Please see Table 1 Subject Identification and Assignment for more information on each subject. A mouse model, more specifically the C57BL/6 mouse model, is more practical for use in this study as the physiological differences between mice and rats allow for a shorter period of time necessary to reach the water weight loss

parameters of the translational model. Additionally, the use of the C57BL/6 mouse model can be expanded upon with transgenic subjects in future research. The C57BL/6 mouse model is an ideal model to examine the objectives of this study because of the wealth of scientific knowledge that already exists on stress, anxiety, neuroanatomy, and brain injury of this species, all of which makes the current experiments more feasible.

Table 1

Subject Identification and Assignment

Subject	*Cage*	*Sex*	*Tail Marker*	*Fluid Intake Condition*	*TBI Condition*	*ID (Cage.Sex.Marker)*
1	A	Female	Blue	Dehydration	TBI	A.Female.Blue
2	A	Female	Red	Dehydration	TBI	A.Female.Red
3	A	Female	Null	Dehydration	TBI	A.Female.Null
4	A	Male	Blue	Dehydration	TBI	A.Male.Blue
5	A	Male	Null	Dehydration	TBI	A.Male.Null
6	B	Female	Blue	Dehydration	Sham	B.Female.Blue
7	B	Female	Null	Dehydration	TBI	B.Female.Null
8	B	Male	Blue	Water ad libitum	TBI	B.Male.Blue
9	B	Male	Null	Water ad libitum	TBI	B.Male.Null
10	C	Female	Blue	Water ad libitum	TBI	C.Female.Blue
11	C	Female	Red	Water ad libitum	TBI	C.Female.Red
12	C	Female	Null	Water ad libitum	TBI	C.Female.Null
13	C	Male	Blue	Dehydration	Sham	C.Male.Blue
14	C	Male	Null	Dehydration	Sham	C.Male.Null
15	D	Female	Blue	Water ad libitum	Sham	D.Female.Blue
16	D	Female	Null	Water ad libitum	Sham	D.Female.Null
17	D	Male	Blue	Water ad libitum	Sham	D.Male.Blue
18	D	Male	Null	Water ad libitum	Sham	D.Male.Null

Note. Subject 1 (A.Female.Blue) died as a result of the TBI weight-drop procedure. This subject was not included in behavioral testing, and the subject's brain was unable to be used for biological assays. Subjects 17 (D.Male.Blue) and 18 (D.Male.Null) were from two separate litters, these were the only subjects not housed with one of their own littermates.

Inducing Dehydration in a Mouse Model

In an environment fixed at room temperature, based on research conducted by Bekkevold and colleagues (2013), mice will lose between 10% and 15% of their body weight following a repeated 12-hour period limited access to water (50% ration) for chronic dehydration. However, Bekkevold and colleagues (2013) also found that acute dehydration (no access to water) for 12 hours produced the same decrease in body weight, without the need for additional isolation of subjects. Bekkevold and colleages' (2013) water rationing procedures require that subjects are monitored in isolation to assess average fluid intake, it also requires that testing be done in isolation. By using their acute dehydration method, it will limit the amount of time that subjects need to be isolated, thus not adding additional stress. Subjects can remain in group housing during experimental manipulations with the acute dehydration method.

Following a 12-hour dehydration period the subjects should lose between 10% and 15% of their pre-dehydration body weight. For the purposes of this study, the subjects were randomly assigned to one of two fluid-intake conditions: a 12-hour period with water ad libitum (control condition), and a 12-hour period without water (dehydration condition). Experimental conditions are adaptations of prior research conducted by Bekkevold and colleagues (2013). The amount of water weight loss was recorded in a post-condition "weigh-in," after which the subjects underwent a fluid recovery period with reverse osmosis water and food sources ad libitum for 24 hours before induction of the traumatic brain injury by way of a weight drop procedure. Pre- and post-dehydration, and post-rehydration weigh-ins were conducted to account for water loss and recovery.

Inducing Traumatic Brain Injury

In addition to the fluid intake manipulation, all of the subjects were anaesthetized, and half (n = 9) received a traumatic brain injury by way of a closed-head weight drop device. The following procedures are standardized for use in the Nova Southeastern University animal colony, as validated and used in other studies. The closed-head weight drop device is based on the Kane et al. (2012) apparatus for repetitive mild traumatic brain injury in mice. As a translational model of conditions faced by athletes, a closed-head weight drop method is the most ecologically valid method for inducing traumatic brain injury. Physical impact between an object and the subject's skull mimics how traumatic brain injuries occur during games or matches. While the open-head controlled cortical impact method for inducing traumatic brain injury in rodents allows for the most specific and targeted injury, it is also the least translational to brain injuries experienced by athletes. The apparatus described in Kane et al. (2012) is an effective method for inducing mild traumatic brain injury in a mouse model. The Kane apparatus is a modification of the Marmarou weight drop method (Marmarou et al., 1994) for use

in inducing repetitive mild traumatic brain injuries in lightly anesthetized subjects (Kane et al. 2012). Notably, the Kane et al. (2012) method allows for an unrestrained yet anesthetized subject to experience rapid acceleration with free movement of their head and torso following the injury. This is translational to traumatic brain injury in humans and closely mimics how concussions are experienced by humans. Additionally, this method does not require surgery or procedures, aside for anesthetization, prior to inducing injury, unlike other methods (Kane et al., 2012). Following mild traumatic brain injury induction, subjects recovered spontaneously from anesthesia and the injury with a righting reflex with no signs of behavioral impairments, paralysis, or seizures (Kane et al., 2012). Kane and colleagues (2012) noted minor deficits in motor coordination and locomotive hyperactivity. However, subjects who received 5 mild traumatic brain injuries using the Kane apparatus recovered from these deficits within 30 days (Kane et al., 2012).

The device consists of a plexiglass rectangular box with a sponge/foam pad at the bottom. Across the top of the box, a one-time use aluminum foil stage is attached with tape pulling the foil tight; this foil has a small slit in it cut with scissors near the edge of the foil. Above the foil is a PVC guide tube attached to a ring stand. A small weight, weighing 95g, was dropped through the guide tube to induce injury. Subjects were anesthetized with isoflurane until unresponsive to tail/paw pinch at which point the mouse will be placed directly on top of the aluminum foil slit. The weight was then released through the PVC tube, impacting the head of the mouse and pushing the mouse through the foil and onto the foam base in the process. The foam is wrapped in plastic wrap and then is sprayed down after each TBI and cleaned. Subjects were then monitored for time to righting and time to the first step.

Post-Injury Behavioral Testing

Elevated Zero Maze

The Elevated Zero Maze is a behavioral assessment for anxiety-like behaviors in rodents and is based on approach/avoidance conflict (Tucker & McCabe, 2017). The Elevated Zero Maze is based off of the conflict between approach and avoidance behaviors exhibited in rodents when placed in a novel environment. In the case of the Elevated Zero Maze, rodents exhibiting approach behaviors explore the open segments of the testing area, while those exhibiting avoidance behaviors spend most of the testing period in the enclosed segments (Pawlak et al., 2012). The Elevated Zero Maze consists of a raised circular platform split into four segments. Two of the segments have non-continuous walls allowing for subjects to hide, while the other two segments are open. When placed on the platform, anxious mice are expected to spend the majority of their time in the walled or enclosed segments of the maze rather than exploring the open areas. Subjects were placed in the open

area of the Elevated Zero Maze, equidistant from the two closed sides. Subjects were allowed to explore the environment (i.e., the Elevated Zero Maze) for 10 minutes before being returned to their home cage. While in the Elevated Zero Maze, subjects were video recorded and later analyzed by blinded reviewers.

Sucrose Preference Test

Anhedonia is a symptom of depression, and it refers to the decreased ability to experience hedonic pleasure. The sucrose preference test is a reward-based test used to indicate anhedonia in an animal model. Sweet food or solutions are of natural interest to rodents; however, if they show a lack of interest in food or solution that contains sweetness, that indicates anhedonia (Serchov et al., 2016). The sucrose preference test is designed to test the subjects' capacity for hedonic pleasure by measuring their intake of a sweetened solution. Subjects are given the option between a sweetened solution (typically 0.0013-0.5% saccharin or 0.5-2% sucrose concentrations) and plain water. Other methods for testing depression in animal models, such as the tail suspension test or forced swim test, could be used, however they introduce additional stress as a result of the test methods which can affect pathology following a traumatic brain injury. The use of the sucrose preference test limits added stress experienced by subjects by other depression tests. The following procedures are standardized for use in the Nova Southeastern University animal colony, as validated and used in other studies. The sucrose preference test was executed in the larger rat cages—to ensure accurate assessment, subjects were tested individually—where the rodent can be presented with two sipper tubes. Mice were housed in the rat cages in the same room as their typical housing during the acclimation and testing phases (16 hours). They were exposed to these two bottles in isolation during the four-hour adaptation period. After the adaptation period, typically, subjects would be water-deprived for a predetermined amount of time before the sucrose preference test. However, based on recent findings, performance on the sucrose preference test may be affected by food/water deprivation immediately prior to the test (Fonseca-Rodrigues et al., 2022). Therefore, the sucrose preference testing procedures used in this study omitted the pre-testing water-deprivation. The concentration of the sucrose solution is typically between 0.5-2%. The sucrose solution in this study had a 1% concentration. The solution was made with reverse osmosis water. Water and sucrose solution intake was measured on the day of the behavioral test. As described in Serchov et al. (2016), sucrose preference is calculated as a percentage of the volume intake over the total volume of fluid intake and averaged over the testing period. Sucrose solution will be prepared every day. Isolation took place in the animal holding room, (i.e., not a procedure room) and the subjects were transferred to a clean cage for the duration of the sucrose test. Following the testing period, subjects were returned to social housing.

Novel Object Recognition Task and Open Field Maze

The Novel Object Recognition task is used for the investigation of memory in rodents. For the purposes of this study, data from the Novel Object Recognition task will also be used to assess cognitive flexibility and anxiety in a mouse model. It requires no external motivation, reward, or punishment and can be completed in a short period of time. Without positive or negative reinforcements, the task assesses the preference for the novel object displayed by rodents. The Novel Object Recognition task is evaluated by the differences in the time spent exploring novel and familiar objects, specifically rodents' ability to recognize a novel object in the environment. The initial habituation period of the Novel Object Recognition Task involves the same conditions as an Open Field Maze. The Open Field Maze assesses motor capabilities, exploration of a novel environment, and anxious behavior in rodents (Bailey & Crawley, 2009). The test procedure takes three days and consists of three phases: habituation, familiarization, and the testing phase. During habitation (Day 1), each subject freely explores the open-field arena without objects being present. Data from the habitation period is scored as an Open Field Maze. After the habitation phase is completed, the subject is removed from the arena and placed back in its cage. During familiarization (Day 2), the subject is placed in the open-field arena containing two identical objects. After the retention interval, during the test phase (Day 3), the subject is presented with two objects in the open-field arena. One is identical to the sample presented during the familiarization phase and the second object is novel. If the subject's memory is properly functioning, they spend more time exploring the novel object during the test phase. While completing both the Open Field Maze and the Novel Object Recognition task, subjects were video recorded to be later analyzed using EthoVision XT video tracking software.

Euthanasia

Subjects were placed in a sealed container which was then filled with CO2 to anesthetize the subject and induce hypoxia. Afterwards, scissors were used to perform spinal dislocation to ensure termination of the subject and for easier removal of the brain. Following the removal of the full rodent brain, tissue samples were stored in a deep freezer at -80C.

Statistical Analyses

Percent change in weight loss following the fluid intake manipulation was assessed using a paired t-test to examine group differences. Prior to running a paired t-test, Levene's Test for Homogeneity of Variance was used to assess an assumption for the paired t-test. All other data will be analyzed using a two way between subjects'

analysis of variance (ANOVA). As noted previously, we predicted that the dehydration manipulation may serve as risk factor for traumatic brain injury, as past literature suggests that following extended periods of dehydration, ventricular expansion is evident (Kempton et al., 2009) which has been shown to increase susceptibility to brain damage following an injury (Dickson et al., 2005).

Results

RStudio version 2023.09.1+494 was used for statistical analyses. Additionally, the reshape2 (Wickham, 2007), ez (Lawrence, 2016), psych (Revelle, 2022), ggplot2 (Wickham, 2016), car (Fox & Weisberg, 2019), effectsize (Ben-Shachar, Lüdecke & Makowski, 2020), and Hmisc packages were used for the analyses used. R scripts, outputs, and data frames can be found on Open Science Framework at https://osf.io/pzh6v/?view_only=c7930fcefe264ce7b1364598773bb8d3.

Weight Loss Following Manipulation

See Table 2 for subject weights at each time point, and Table 3 for percent changes in weight between time points. Levene's Test for Homogeneity of Variance was used to verify that the dataset did not violate the assumption of homogeneity of variance. The three Levene's Test for Homogeneity of Variance showed no significant differences between group variance in the percent change in subjects' weights from Time 1 to Time 2 (p = .523), Time 2 to Time 3 (p = .277), and Time 1 to Time 3 (p = .942).

Table 2

Subject Weights Following Fluid Intake Manipulation

ID	**Fluid Intake Condition**	**Pre-manipulation (g)**	**Post-dehydration (g)**	**Post-manipulation (g)**
A.Female.Blue	Dehydration	19.7	18.2	19.4
A.Female.Red	Dehydration	18.8	17.6	18
A.Female.Null	Dehydration	17.7	16.8	17.8
A.Male.Blue	Dehydration	24.5	22.7	24
A.Male.Null	Dehydration	24.7	23	23.5
B.Female.Blue	Dehydration	16.8	15.4	16.9
B.Female.Null	Dehydration	17.5	14.7	16.7
B.Male.Blue	Water ad libitum	23.6	24	23.1
B.Male.Null	Water ad libitum	23.6	23.2	22.9
C.Female.Blue	Water ad libitum	18.1	17.6	17.9

C.Female.Red	Water ad libitum	18.5	17.8	17.2
C.Female.Null	Water ad libitum	17.7	17.7	17.8
C.Male.Blue	Dehydration	25.4	23.6	24.7
C.Male.Null	Dehydration	24.8	19	24.3
D.Female.Blue	Water ad libitum	19.9	20.1	20
D.Female.Null	Water ad libitum	20.4	18.7	20
D.Male.Blue	Water ad libitum	24	23.6	23.6
D.Male.Null	Water ad libitum	23.4	23.2	23.3

Note. For the purpose of assessing percent change in subjects' weight, the pre-manipulation weight will be denoted as time 1, the post-dehydration weight will be denoted as time 2, and the post-manipulation weight will be denoted as time 3.

Table 3

Percent Change in Subjects' Weight

ID ***(Cage.Sex.Marker)***	**Fluid Intake Condition**	**Time 1-2 Change (%)**	**Time 2-3 Change (%)**	**Time 1-3 Change (%)**
A.Female.Blue	Dehydration	-7.614213198	6.593406593	-1.52284264
A.Female.Red	Dehydration	-6.382978723	2.272727273	-4.255319149
A.Female.Null	Dehydration	-5.084745763	5.952380952	0.564971751
A.Male.Blue	Dehydration	-7.346938776	5.726872247	-2.040816327
A.Male.Null	Dehydration	-6.882591093	2.173913043	-4.858299595
B.Female.Blue	Dehydration	-8.333333333	9.74025974	0.595238095
B.Female.Null	Dehydration	-16	13.60544218	-4.571428571
B.Male.Blue	Water ad libitum	1.694915254	-3.75	-2.118644068
B.Male.Null	Water ad libitum	-1.694915254	-1.293103448	-2.966101695
C.Female.Blue	Water ad libitum	-2.762430939	1.704545455	-1.104972376
C.Female.Red	Water ad libitum	-3.783783784	-3.370786517	-7.027027027
C.Female.Null	Water ad libitum	0	0.564971751	0.564971751
C.Male.Blue	Dehydration	-7.086614173	4.661016949	-2.755905512
C.Male.Null	Dehydration	-23.38709677	27.89473684	-2.016129032
D.Female.Blue	Water ad libitum	1.005025126	-0.497512438	0.502512563
D.Female.Null	Water ad libitum	-8.333333333	6.951871658	-1.960784314
D.Male.Blue	Water ad libitum	-1.666666667	0	-1.666666667

D.Male.Null	Water ad libitum	-0.854700855	0.431034483	-0.427350427

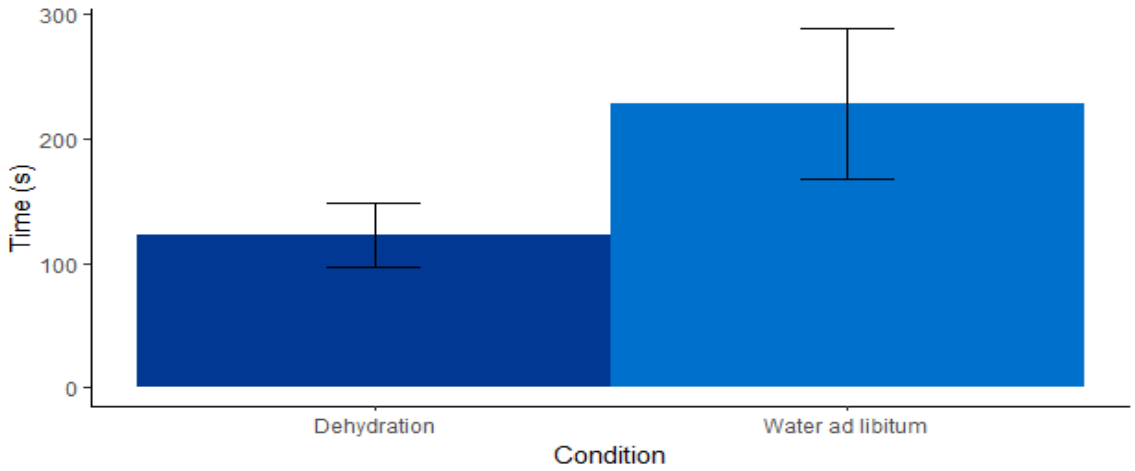
Paired t-tests were conducted to examine the effect of the fluid intake condition and change in the subjects' weight in grams from Time 1 to Time 2, Time 2 to Time 3, and Time 1 to Time 3. There was a significant effect of the fluid intake condition on the change in subjects' weight from Time 1 to Time 2, $t(8) = -4.00$, $p = .004$, $d = -1.33$. There was a significant effect of the fluid intake condition on the change in subjects' weight from time 2 to time 3, $t(8) = 3.37$, $p = .01$, $d = 1.12$. There was not a significant effect of the fluid intake condition on the change in subjects' weight from time 1 to time 3, $t(8) = -0.54$, $p = .607$. There were no significant differences between subjects' weights prior to the dehydration manipulation and following the rehydration period.

Time to Righting and Time to Ambulation

A two-way between subjects' ANOVA was performed to analyze the effects of fluid intake and traumatic brain injury on time to righting. Time to righting was defined as time in seconds following the TBI or sham procedure until the subject stood on their own four feet. A two-way ANOVA revealed that there was not a significant interaction between the effects of fluid intake and traumatic brain injury, $F(1,13) = 3.95$, $p = .068$. Simple main effects analysis showed that fluid intake did have a statistically significant effect on time to righting, $F(1, 13) = 7.205$, $p = .019$, *partial* $\eta^2 = 0.36$), as shown in Figure 1. Simple main effects analysis showed that traumatic brain injury did have a statistically significant effect on time to righting $F(1,13) = 29.138$, $p < .001$, *partial* $\eta^2 = 0.69$), as shown in Figure 2.

Figure 1

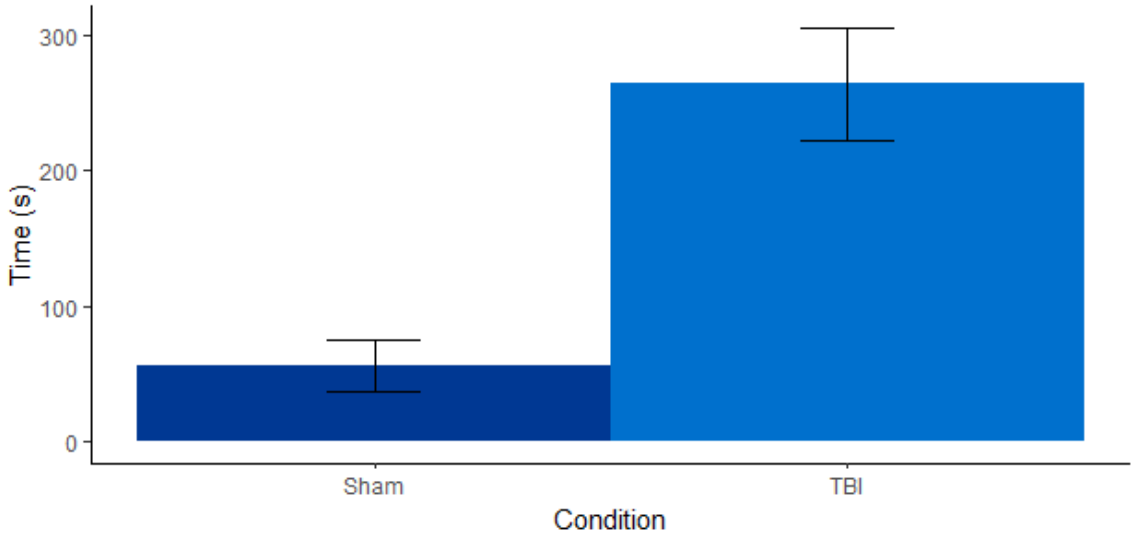
Effect of fluid intake condition on time to righting



Note. Error bars denote standard error.

Figure 2

Effect of TBI condition on time to righting



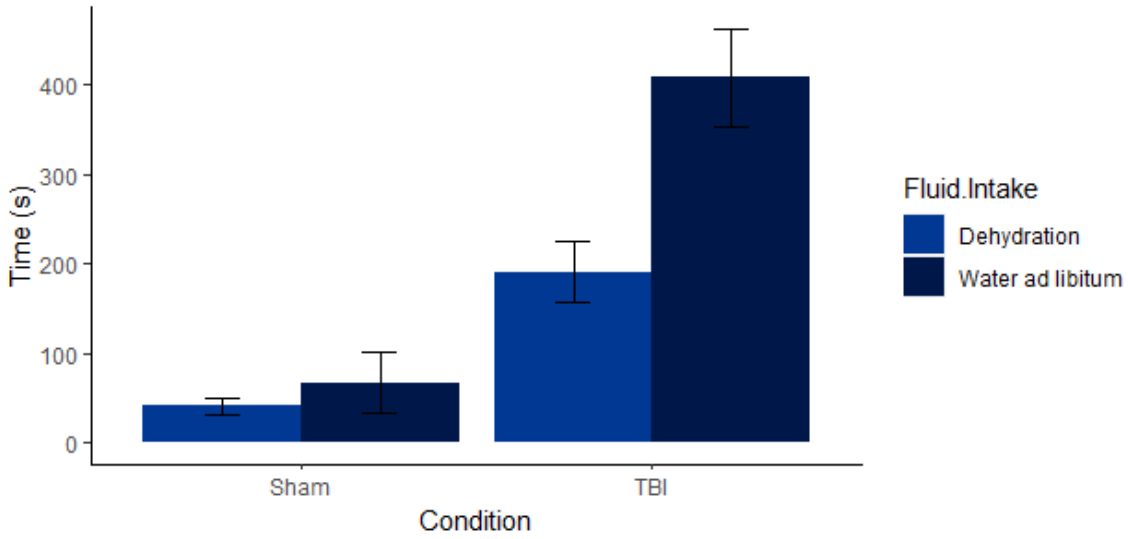
Note. Error bars denote standard error.

A two-way ANOVA was performed to analyze the effect of fluid intake and traumatic brain injury on time to ambulation. See Figures 3, 4, and 5. Time to

ambulation was defined as time in seconds following the TBI or sham procedure until the subject either took their first step or exhibited grasping motions. A two-way between subjects' ANOVA revealed that there was a statistically significant interaction between the effects of fluid intake and traumatic brain injury, $F(1,13) = 5.054$, $p = .043$, *partial* $\eta^2 = 0.28$. Simple main effects analysis showed that fluid intake did have a statistically significant effect on time to ambulation, $F(1, 13) = 8.538$, $p = .012$, *partial* $\eta^2 = 0.73$. Simple main effects analysis showed that traumatic brain injury did have a statistically significant effect on time to ambulation $F(1, 13) = 35.960$, $p < .001$, *partial* $\eta^2 = 0.40$.

Figure 3

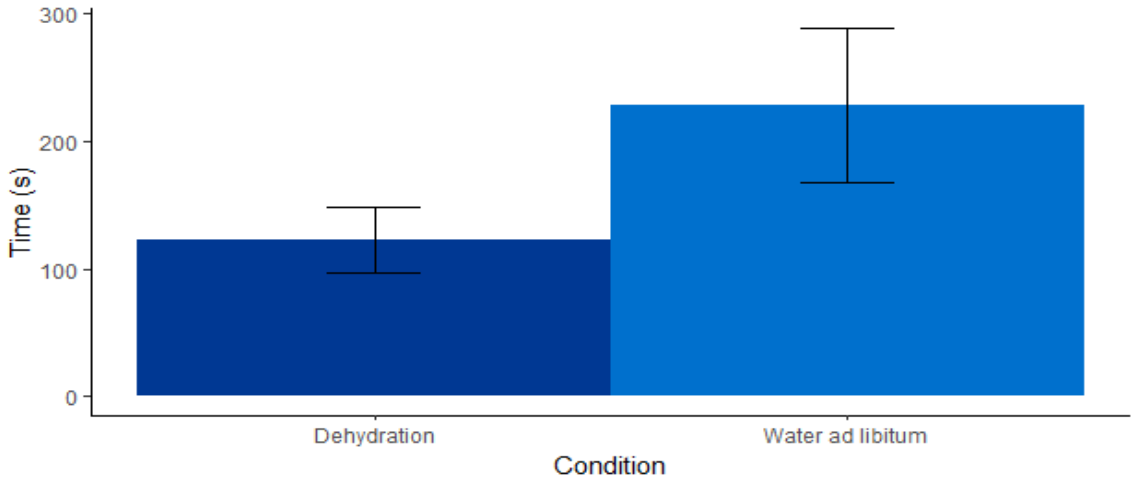
Interaction between fluid intake and TBI conditions on time to ambulation



Note. Error bars denote standard error.

Figure 4

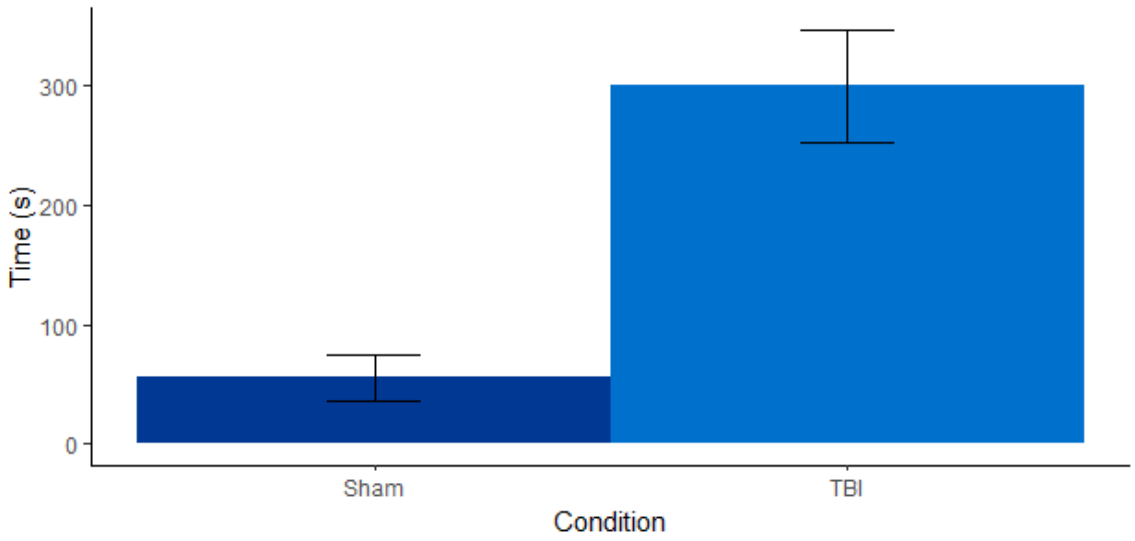
Effect of fluid intake condition on time to ambulation



Note. Error bars denote standard error.

Figure 5

Effect of TBI condition on time to ambulation



Note. Error bars denote standard error.

Behavioral Tests

Elevated Zero Maze

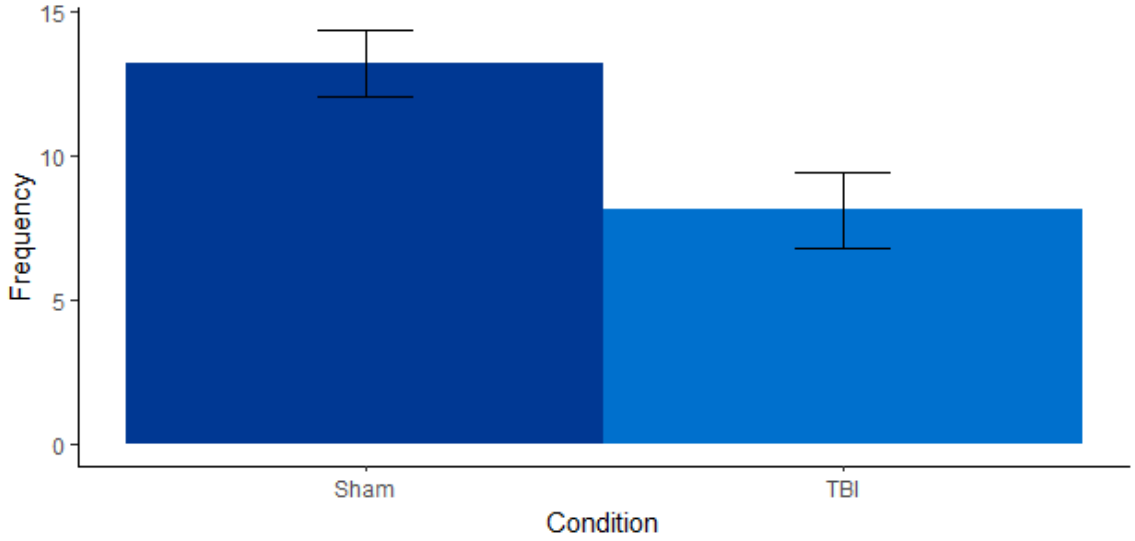
Number of stretch aheads. A stretch ahead refers to when a subject looks over the edge of the platform or looks over the edge of the walls of the closed sections of the Elevated Zero Maze. Two blinded reviewers assessed video recordings of subjects in the Elevated Zero Maze using the Reawr software for video analysis. A Pearson correlation coefficient was computed to assess the linear relationship between stretch aheads reported by each reviewer. There appeared to be a moderate positive correlation between the two variables, $r(15) = 0.47$, $p = .056$, but the linear relationship was nonsignificant. Due to the relatively low correlation between the number of stretches reported by each reviewer, reviewers were changed, and the videos were reassessed. In this secondary correlation, there was a strong positive correlation between the two variables $r(15) = 0.94$, $p < .001$. Following this second correlation test to assess interrater reliability, the number of stretch aheads reported by the reviewers was averaged for each subject. A two-way ANOVA was performed to analyze the effect of fluid intake and traumatic brain injury on the averaged stretch aheads reported. A two-way ANOVA between subjects revealed that there was not a statistically significant interaction between the effects of fluid intake and traumatic brain injury, $F(1, 13) = 0.124$, $p = .730$. Simple main effects analysis showed that fluid intake did not have a statistically significant effect on the averaged stretch aheads reported, $F(1,13) = 0.00$, $p = .987$. Simple main effects analysis showed that traumatic brain injury did not have a statistically significant effect on the averaged stretch aheads reported, $F(1,13) = 0.093$, $p = .765$.

Number of entries into open sections. Two blinded reviewers assessed video recordings of subjects in the Elevated Zero Maze using the Reawr software for video analysis. A Pearson correlation coefficient was computed to assess the linear relation between scores reported by each reviewer on the number of times in open sections. There was a strong positive correlation between reviewers, $r(15) = 0.93$, $p < .001$. Following a correlation test to assess interrater reliability, the number of times in the open section reported by the reviewers was averaged for each subject. A two-way between subjects' ANOVA was performed to analyze the effect of fluid intake and traumatic brain injury on averaged number of entries into open sections of the Elevated Zero Maze. A two-way ANOVA between subjects revealed that there was not a statistically significant interaction between the effects of fluid intake and traumatic brain injury on the averaged number of entries into open sections of the Elevated Zero Maze, $F(1,13) = 1.200$, $p = .293$. Simple main effects analysis showed that fluid intake did not have a significant effect on averaged number of

entries into open sections of the Elevated Zero Maze, $F(1,13) = 1.329$, $p = .270$. Simple main effects analysis showed that traumatic brain injury did have a significant effect on averaged number of entries into open sections of the Elevated Zero Maze, $F(1,13) = 7.132$, $p = .019$, *partial* $\eta^2 = 0.35$, as shown in Figure 6.

Figure 6

Effect of TBI on number of entries into open sections of EZM



Note. Error bars denote standard error.

Duration of time in open sections. Two blinded reviewers assessed video recordings of subjects in the Elevated Zero Maze using the Reawr software for video analysis. A Pearson correlation coefficient was computed to assess the linear relation between scores reported by each reviewer on the duration of time in open sections. There was a strong positive correlation between reviewers, $r(15) = 0.97$, $p < .001$. Following a correlation test to assess interrater reliability, the number of times in the open section reported by the reviewers was averaged for each subject. A two-way ANOVA between subjects was performed to analyze the effect of fluid intake and traumatic brain injury on duration of time in open sections of the Elevated Zero Maze. A two-way between ANOVA between subjects revealed that there was not a statistically significant interaction between the effects of fluid intake and traumatic brain injury on the duration of time in the open sections of the Elevated Zero Maze, $F(1, 13) = 0.083$, $p = .778$. Simple main effects analysis showed that fluid intake did not have a significant effect on duration of time in open

sections of the Elevated Zero Maze, $F(1, 13) = 0.040$, $p = .845$. Simple main effects analysis showed that TBI did not significantly affect the duration of time in open sections of the Elevated Zero Maze, $F(1, 13) = 1.654$, $p = .221$.

Fecal boli. A two-way ANOVA between subjects was performed to analyze the effect of fluid intake and traumatic brain injury on fecal boli produced during the Elevated Zero Maze. A two-way ANOVA between subjects revealed no statistically significant interaction between the effects of fluid intake and traumatic brain injury on fecal boli produced during the Elevated Zero Maze, $F(1, 13) = 1.619$, $p = .226$. Simple main effects analysis showed that fluid intake did not significantly affect fecal boli produced during the Elevated Zero Maze, $F(1, 13) = 0.263$, $p = .226$. Simple main effects analysis showed that traumatic brain injury did not have a significant effect on fecal boli produced during the Elevated Zero Maze, $F(1, 13) = 0.633$, $p = .440$.

Sucrose Preference

A two-way between subjects' ANOVA was performed to analyze the effect of fluid intake and traumatic brain injury on sucrose preference. As described in Serchov et al., (2016), sucrose preference is calculated as a percentage of the volume intake over the total volume of fluid intake and averaged over the testing period. A two-way between subjects; ANOVA revealed that there was no statistically significant interaction between the effects of fluid intake and traumatic brain injury on sucrose preference, $F(1, 13) = 0.052$, $p = 0.823$. Simple main effects analysis showed that fluid intake did not significantly affect sucrose preference, $F(1, 13) = 2.412$, $p = .144$. Simple main effects analysis showed that TBI did not significantly affect sucrose preference, $F(1, 13) = 1.173$, $p = .298$.

Open Field Maze

In center latency to first. A two-way between subjects' ANOVA was performed to analyze the effect of fluid intake and traumatic brain injury on in center latency to first. Center latency to first refers to the time when the subject moves toward the center of the arena for the first time during the testing period. A two-way between subjects' ANOVA revealed that there was not a significant interaction between the effects of fluid intake and traumatic brain injury on in center latency to first, $F(1, 13) = 1.257$, $p = .283$. Simple main effects analysis showed that fluid intake did not have a significant effect on in center latency to first, $F(1, 13) = 0.816$, $p = .383$. Simple main effects analysis showed that traumatic brain injury did not have a significant effect on in center latency to first, $F(1, 13) = 0.541$, $p = .394$.

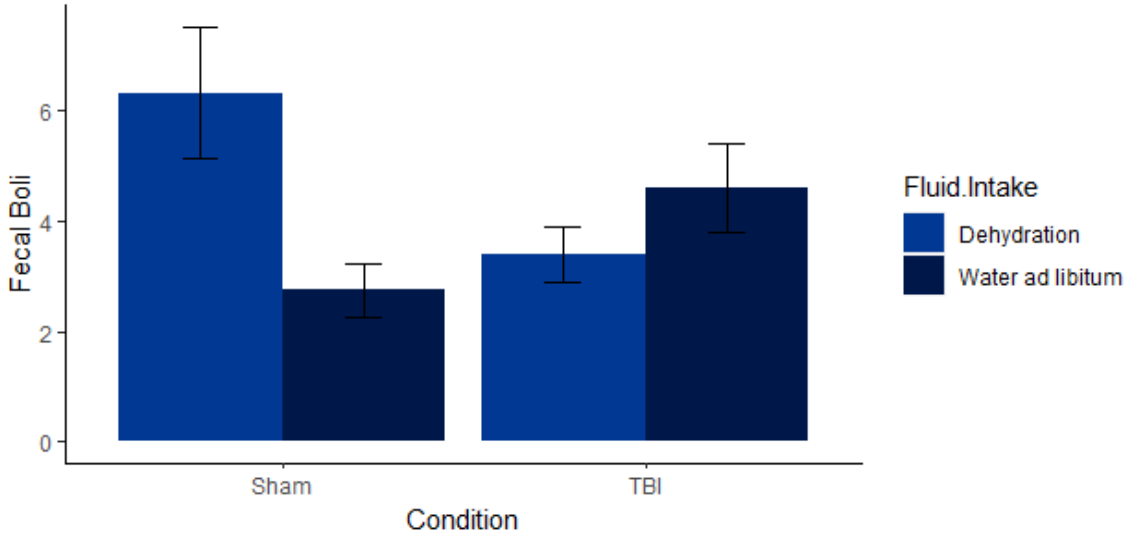
In center cumulative duration. A two-way ANOVA between subjects was performed to analyze the effect of fluid intake and traumatic brain injury on the cumulative duration in the center of the arena. A two-way ANOVA between subjects revealed that there was no statistically significant interaction between the effects of fluid intake and traumatic brain injury on in center cumulative duration, $F(1, 13) = 0.655$, $p = .209$. Simple main effects analysis showed that fluid intake did not have a significant effect on in center cumulative duration, $F(1, 13) = 0.004$, $p = .304$. Simple main effects analysis showed that traumatic brain injury did not significantly affect center cumulative duration, $F(1, 13) = 1.144$, $p = .952$.

Number of entries into center. A two-way between subjects' ANOVA was performed to analyze the effect of fluid intake and traumatic brain injury on number of entries into center. A two-way between subjects' ANOVA revealed that there was not a statistically significant interaction between the effects of fluid intake and traumatic brain injury on number of entries into center, $F(1, 13) = 0.253$, $p = .623$. Simple main effects analysis showed that fluid intake did not have a significant effect on number of entries into center, $F(1, 13) = 0.000$, $p = .623$. Simple main effects analysis showed that traumatic brain injury did not significantly affect number of entries into center, $F(1, 13) = 1.641$, $p = .623$.

*Fecal boli***.** A two-way between subjects' ANOVA was performed to analyze the effect of fluid intake and traumatic brain injury on fecal boli produced during the Open Field Maze. A two-way between subjects ANOVA revealed that there was a significant interaction between the effects of fluid intake and traumatic brain injury, $F(1, 13) = 10.145$, $p = .007$, *partial* $\eta^2 = 0.44$, as shown in Figure 7. Simple main effects analysis showed that fluid intake did not significantly affect fecal boli produced during the Open Field Maze, $F(1, 13) = 0.963$, $p = .344$. Simple main effects analysis showed that TBI did not have a significant effect on fecal boli produced during the Open Field Maze, $F(1, 13) = 0.205$, $p = .658$.

Figure 7

Interaction between fluid intake and TBI on fecal boli produced during OFM



Note. Error bars denote standard error.

Novel Object Recognition Task (5 minutes)

Novel object nose point latency to first. A two-way between subjects' ANOVA was performed to analyze the effect of fluid intake and traumatic brain injury on novel object nose point latency to first. Novel object nose point latency to first refers to when the subject moves towards the novel object for the first time during the testing period. A two-way between subjects' ANOVA revealed that there was not a statistically significant interaction between the effects fluid intake and traumatic brain injury on novel object nose point latency to first, $F(1, 13) = 2.447$, $p = .142$. Simple main effects analysis showed that fluid intake did not have a significant effect on novel object nose point latency to first, $F(1, 13) = 1.013$, $p = .333$. Simple main effects analysis showed the traumatic brain injury did not have a significant effect on novel object nose point latency to first, $F(1, 13) = 0.081$, $p = .780$.

*DI index***.** A two-way between subjects' ANOVA was performed to analyze the effect of fluid intake and traumatic brain injury on DI index in the first 5 minutes of the Novel Object Recognition Task. DI index is calculated by subtracting time spent looking at the familiar object from the time spent looking at the novel object and then dividing that new value from the total time spent near either object. A two-way between subjects' ANOVA revealed that there was not a significant interaction between the effects of fluid intake and traumatic brain injury on DI index in the first 5 minutes of the Novel Object Recognition Task, $F(1, 13) = 0.006$, $p = .941$. Simple main effects analysis showed that fluid intake did not have a significant

effect on DI index during the first 5 minutes of the Novel Object Recognition Task, $F(1, 13) = 1.332$, $p = .269$. Simple main effects analysis showed that traumatic brain injury did not have a significant effect on DI index during the first 5 minutes of the Novel Object Recognition Task, $F(1, 13) = 0.939$, $p = .350$.

Novel Object Recognition Task (10 minutes)

DI index. A two-way between subjects' ANOVA was performed to analyze the effect of fluid intake and traumatic brain injury on DI index in the first 5 minutes of the Novel Object Recognition Task. A two-way between subjects' ANOVA revealed that there was not a significant interaction between the effects of fluid intake and traumatic brain injury on DI index, $F(1, 13) = 0.119$, $p = .736$. Simple main effects analysis showed that fluid intake did not have a significant effect on DI, $F(1, 13) = 0.854$, $p = .372$. Simple main effects analysis showed that traumatic brain injury did not have a significant effect on DI index, $F(1, 13) = 1.039$, $p = .327$.

Fecal boli. A two-way between subjects' ANOVA was performed to analyze the effect of fluid intake and traumatic brain injury on fecal boli produced during the Novel Object Recognition Task. A two-way between subjects' ANOVA revealed that there was no statistically significant interaction between fluid intake and traumatic brain injury on fecal boli produced during the Novel Object Recognition Task, $F(1, 13) = 1.841$, $p = .198$. Simple main effects analysis showed that fluid intake did not have a significant effect on fecal boli produced during the Novel Object Recognition Task, $F(1, 13) = 0.001$, $p = .977$. Simple main effects analysis showed that traumatic brain injury did not significantly affect fecal boli produced during the Novel Object Recognition Task, $F(1, 13) = 0.702$, $p = .417$.

Post-hoc analyses

`Significant interaction effects were further assessed using Tukey's multiple comparison of means. There appears to be a significant neuroprotective effect of dehydration in the TBI condition on time to ambulation ($p\text{-}adj = .007$). There was no significant difference between sham subjects regardless of fluid intake ($p\text{-}adj = .978$). During the Open Field Maze, there was a significant difference in fecal boli produced by sham subjects by fluid intake condition ($p\text{-}adj = .037$). However, there was no significant difference between fluid intake conditions for TBI subjects ($p\text{-}adj = .606$).

Discussion

While the results suggest some significant differences between the condition groups, the size of the sample does not allow for an accurate judgment of statistical

significance. Further studies with larger sample sizes are necessary to confirm the results of this pilot study. Subjects' brains collected immediately following euthanasia will be used in protein assays to examine any biological differences between conditions.

There were significant changes in subjects' weight from Time 1 to Time 2, this suggests that the dehydration manipulation was successful in decreasing subjects' water weight. Additionally, there were significant changes in subjects' weight from Time 2 to Time 3 along with there being no significant changes in subjects' weight from Time 1 to Time 3 suggests that the rehydration returned subjects to their approximate pre-manipulation weight.

There were significant differences between both dehydration and TBI manipulations on time to righting, and there was a significant interaction between manipulations on time to ambulation. These findings suggest that dehydration does have some effect on recovery immediately following the injury. Post-hoc analysis suggests that dehydration prior to injury may serve as a neuroprotective factor allowing for decreased time to ambulation. In the translational human population, this could suggest that "cutting" periods prior to injury may prevent or lessen traumatic brain injury pathology.

During the Elevated Zero Maze, the TBI manipulation had a significant effect on the number of times subjects entered the open sections of the maze. This suggests that subjects were less anxious than the sham group as they were more likely to explore the open areas. This is consistent with past research and testing using the Elevated Zero Maze. The Elevated Zero Maze was also conducted under bright light, on subjects housed in a reverse light cycle. This could potentially affect exploratory behaviors in nocturnal animals, such as rodents.

During the Open Field Maze, there was a significant interaction between manipulations of fecal boli. This increase in fecal boli present suggests that these subjects were more anxious than subjects in the other conditions. Post-hoc analysis revealed that there was a significant difference between fluid-intake conditions on the sham subjects, but not the TBI subjects.

When initially assessing the interrater reliability of the blinded reviewers for Elevated Zero Maze data, the correlation between the numbers reported by reviewer 1 and reviewer 2 was not high enough (i.e., $r > 0.9$). As a result, another reviewer was brought in to compare, and when correlated with reviewer 2, met the set criteria. The discrepancy exhibited by the first reviewer was most likely due to the fact that times peaked was meant to be recorded separately from the other two variables that were recorded using Reawr. The strong interrater reliability scores

for both the number of times and cumulative duration in the open sections of the Elevated Zero Maze suggests no evidence that retesting is necessary.

This translational model of boxers does not account for training as done by athletes. This could be addressed by adding some form of exercise enrichment to subject housing, such as an exercise wheel, or by giving each subject a 2-hour voluntary exercise period daily outside of subject housing. However, this could potentially introduce additional stress, which prior research out of Nova Southeastern University's Animal Colony suggests can further worsen symptoms of traumatic brain injury. The "cutting" period also typically includes food and fluid restriction. This may add additional stress that, compounded with fluid restriction, could worsen traumatic brain injury pathology. Additionally, the small sample size used (n=18, n=17 for behavioral tests) does not allow for an accurate determination of significance. Further research is necessary to confirm these results.

Conclusions

As noted previously, it was initially hypothesized that dehydration and rehydration prior to a traumatic brain injury manipulation may serve as a risk factor for traumatic brain injury, as past literature suggests that following extended periods of dehydration, ventricular expansion is evident (Kempton et al., 2009), which has been shown to increase susceptibility to brain damage following an injury (Dickson et al., 2005). Findings regarding time to righting and time to ambulation immediately following induction of a traumatic brain injury by way of a closed head weight drop method shows that dehydration and rehydration prior to injury do not pose a significant risk to immediate recovery and serve as a neuroprotective factor, based on post-hoc analysis. However, the death of one subject immediately following injury induction may suggest some other variables interacting with dehydration could worsen the effects of traumatic brain injury.

While the sample size in this study is small and further research with a larger sample is necessary to confirm the results, the significant effects of the dehydration condition on time to righting and time to ambulation immediately following induction of traumatic brain injury are noteworthy. These significant differences in immediate post-traumatic brain injury recovery suggest that dehydration alters the initial effects of traumatic brain injury but has little to no lasting effects. Dehydration, either short-term or extended periods of dehydration, can impact how individuals recover from an initial traumatic brain injury. With the two populations most likely to receive a traumatic brain injury (i.e., athletes and military personnel) also being more likely to experience dehydration prior to receiving an injury, these findings could have large implications for these populations.

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