

Characterization of Differing Time-Course of Cognitive Deficits and Emotional Changes Following Rotational Traumatic Brain Injury in the Rat

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Abstract Quantifying injury tolerance for concussion is complicated by variability in the type and severity of post-injury physiological and behavioral changes, as well as differences in the time course of these deficits. The current study outlined acute and chronic changes in behavioral metrics following rotational acceleration-induced concussion in rats. A unique injury model independently controlled magnitude and duration of the rotational acceleration pulse. Increasing rotational acceleration magnitude produced longer unconsciousness times, which were used as an assessment of acute injury severity. However, longer duration rotational accelerations produced changes in emotionality measured using the Elevated Plus Maze. Cognitive deficits were not apparent in the Morris Water Maze assessment, possibly due to the lower severity of rotational acceleration pulses incorporated in this study. Changes in emotionality evolved between acute and chronic assessments, in some cases increasing in severity and in others reversing polarity. These findings highlight the complexity of quantifying injury tolerance for concussion and demonstrate a need to incorporate rotational acceleration magnitude and duration in proposed metrics. Rotational velocity on its own was not a strong predictor of the magnitude or type of behavioral changes following concussion.

Keywords traumatic brain injury, rotational acceleration, injury metrics, emotionality, behavioral assessment, rodent model.

I. INTRODUCTION

Traumatic brain injury occurs through a variety of mechanisms including penetration, blunt impact, rotational acceleration, and blast overpressure [1]. Whereas penetration and impact produce primarily focal injuries, rotational acceleration and blast produce pathologies spread more diffusely throughout the brain [2, 3]. Diffuse brain injury resulting from head rotational acceleration can occur in a variety of environments, but is commonly associated with motor vehicle collisions and sporting events such as boxing and American football [4, 5]. Understanding biomechanical injury tolerance and kinematic metrics associated with different injury outcomes is important for development of safety enhancements for vehicle and sporting environments (i.e., airbags and helmets). Given advancing technology for head impact measurement in sports [6, 7], understanding biomechanics of concussion can also influence player participation and return to play guidelines [8]. Unfortunately, biomechanical tolerance for lower levels of traumatic brain injury (i.e., concussion) is complicated by a varied clinical course that includes physical symptoms such as headache and vestibular disturbances, cognitive difficulties such as working memory impairments, and changes in emotionality including depression, aggressive behavior, and anxiety. Complicating the issue even further is that the time course and duration of these issues varies between individuals, injury severity, and injury characteristics (i.e., head acceleration in different directions). Therefore, understanding injury tolerance for traumatic brain injury and development of tolerance metrics may require a different approach than for other body regions [9, 10] by possibly accounting for the temporal characteristics of these injuries. The first step is to better understand the influence and sensitivity of mechanical metrics on the acute and chronic outcomes following concussion.

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Following impact, the head experiences acceleration or deceleration that occurs over a relatively short time period. Development of injury tolerance requires an understanding of sensitive characteristics of the head rotational acceleration versus time pulse (i.e., peak magnitude and positive duration) and quantification of values associated with different levels of injury severity and time-course of outcomes. Accordingly, in 2003 Gennarelli and colleagues reported human injury tolerance metrics that were based on scaled data from primate experimentation and outlined peak rotational acceleration magnitudes associated with six levels of diffuse brain injury between mild concussion and severe diffuse axonal injury (DAI) [11]. Other metrics have incorporated rotational velocity in addition to rotational acceleration. In the case of the Head Impact Power (HIP), Newman and colleagues developed an injury metric that incorporated both linear and rotational acceleration and velocity [12]. Similarly, the more recently proposed Brain Injury Criterion (BrIC), advanced by the US NHTSA incorporates rotational velocity in the three primary planes versus computationally-derived critical values [13]. Studies incorporating data collected from humans during sporting events have highlighted the influence of rotational kinematics for the onset of concussion [14]. It is clear from these prior studies, and others, that head rotational kinematics are important in the onset of concussion. However, those studies focused on concussion onset and did not differentiate between different injury outcomes (i.e., cognitive deficits versus changes in emotionality) or changes in clinical course over time. Outlining these factors will be key in the development of a more robust understanding of concussion tolerance.

Animal models are ideal for the study of concussion tolerance, given the ability to accurately control input head kinematics and quantify injury outcomes over time. While the primate model is closest to the human in terms of anatomical characteristics and inertial tolerance, ethical considerations have essentially eliminated the possibility of using this model for injury tolerance research. However, given different anatomical characteristics, the rodent model is ideal for this type of study due to the ability to incorporate larger numbers and a variety of well-characterized behavioral assessments to quantify time course of specific changes following injury. Unfortunately, a majority of rodent concussion research has incorporated injury models that are not biomechanically relevant for the study of injury tolerance including controlled cortical impact [15] and fluid percussion [16] models. Even impact acceleration models are limited in their application to injury tolerance by the focus on primarily linear acceleration and the lack of quantification of head kinematics in a majority of studies [17]. Other groups, including ours, have begun to incorporate rotational acceleration in rodent injury models [18-21] and models incorporating other animals [22]. These models are ideal for the study of concussion injury tolerance for rotational acceleration due to their repeatability, independent control over rotational characteristics [23], and ability to quantify behavioral deficits at acute and chronic time points following injury. Therefore, the purpose of this study was to quantify acute and chronic behavioral changes in rats following rotational acceleration-induced concussion with different magnitude and duration characteristics.

II. METHODS

The current protocol consisted of exposing rats to rotational injury or sham procedure followed by behavioral assessments during the first week or four weeks following the injury or sham procedure to characterize acute and chronic cognitive and emotional changes in the rats associated with different head rotational acceleration pulses. All injury and behavioral testing were conducted with approval from the Institutional Animal Care and Use Committee at our Institution.

Experimental Model

Rats were exposed to high rate head rotational acceleration using the MCW Rotational Injury Device (Figure 1) [23]. The model consists of a rat helmet with laterally extended moment arm, impactor mass, and pneumatic launcher [18]. The helmet fits securely to the head of the rat without surgical intervention. Our prior work confirmed that greater than 90% of helmet rotational acceleration is transferred to the head [18]. The impactor was accelerated by the launcher down a drop tower to impact the moment arm and generate sufficient force to rotate the device in the coronal plane. Characteristics of the rotational acceleration versus time pulse were modulated by the mass of the impactor, initial drop height, and characteristics of the elastomer interface

material between the impactor and the moment arm [24]. An accelerometer attached to the distal end of the moment arm measured tangential linear acceleration, which was converted to rotational acceleration of the helmet versus time. Our previous studies and extensive testing ensured that magnitude and duration of the rotational acceleration versus time pulse were independently modulated and could be accurately measured for each injury [23].

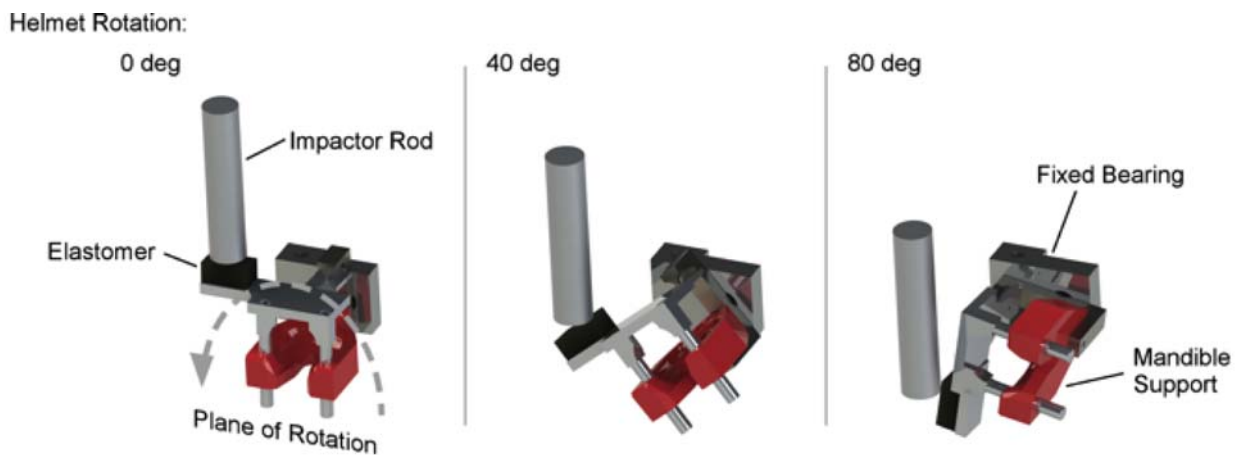


Fig. 1. MCW Rotational Injury Device that induces concussion in anesthetized rats through pure coronal plane head rotational acceleration without surgical intervention.

Acceleration data were collected at 1.0 MHz (National Instruments Corporation, Austin, TX) and low pass filtered at 10 kHz for analysis. Kinematic measurements of the helmet were used to classify the exposure severity. Peak helmet rotational acceleration was measured as the maximum positive acceleration and rotational acceleration duration was measured as the positive duration of the acceleration pulse. Rotational velocity was computed as the integral of the positive portion of the rotational acceleration versus time pulse.

Sprague Dawley rats were anesthetized using Isoflurane anesthesia, fitted in the helmet, and subjected to head rotational acceleration. Control rats were subjected to a sham procedure that consisted of anesthesia and placement in the helmet without exposure to head rotational acceleration. A total of five groups were included in this study, which consisted of a sham control group and four injury groups defined by different rotational acceleration magnitude and duration characteristics (Table 1). Three levels of rotational acceleration magnitude and two levels of rotational acceleration duration were included across the four injury groups to assess the independent effects of magnitude and duration on behavioral outcomes following mild traumatic brain injury (Figure 2). Following exposure to head rotational acceleration, rats were placed on a warming blanket and allowed to recover. Rats were placed back in their cages and monitored for at least 15 min after regaining the ability to independently ambulate, then returned to the veterinary medical unit and monitored for a minimum of an additional six hours.

TABLE I
ROTATIONAL ACCELERATION CHARACTERISTICS FOR THE EXPERIMENTAL GROUPS

Group	Number	Magnitude (krad/s ²)	Duration (ms)	Rotational Velocity (rad/s)
Sham	16	0	0	0
M1D2	39	207 ± 33	3.6 ± 0.5	265 ± 72
M2D1	34	358 ± 46	1.5 ± 0.4	234 ± 78
M2D2	25	368 ± 36	3.4 ± 0.5	383 ± 97
M3D2	19	503 ± 26	3.2 ± 0.2	613 ± 13

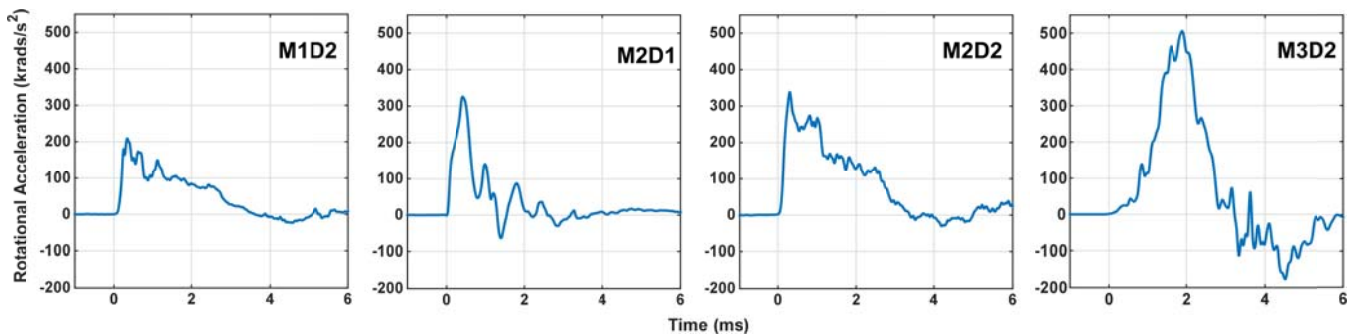


Fig. 2. Representative rotational acceleration versus time traces for the four experimental pulses.

Behavioral Assessments

Duration of unconsciousness was assessed immediately following exposure to head rotational acceleration to provide an acute assessment of injury severity. This time period was quantified as the time from removal of anesthesia, just prior to head rotational acceleration exposure, until return of the righting reflex. These times were delineated following the injury procedure by blinded observers using real-time video of the recovery area with technicians verbally noting specific steps during the injury protocol. Longer unconsciousness times were associated with acutely more severe injuries.

Rats were exposed to a series of behavioral assessments either during the first week (acute) or 30 days (chronic) post injury. Separate groups of animals were subjected to the behavioral protocol at each time point. The Morris Water Maze Visuo-Spatial Learning Paradigm (VSLP) was performed on days 1, 2, and 3 or 29, 30, and 31 post injury [25]. The VSLP was used to assess cognitive deficits associated with spatial learning. The protocol consisted of three consecutive days of testing using the Morris Water Maze, a 1.83-m diameter circular pool filled with water to 25 cm deep and located in a laboratory room with numerous visual cues external to the maze. The walls and interior sides of the maze were painted black such that the rats could not see the bottom of the maze. A hidden circular platform (10-cm diameter), also painted black, was placed inside the maze, such that the surface of the platform was 2 cm below the surface of the water. Rats were exposed to a total of 12 trials in the Morris Water Maze, divided evenly across three days. The four trials per day consisted of placing the rat into the water at one of the four cardinal locations (N, E, S, W) and allowing it to swim until finding and mounting the hidden platform, which was located at 45 deg to the cardinal locations (i.e., NE) mid-way between the outer wall and the center of the maze. The location of the platform was changed for each of the four trials per day and sequence of platform rotation was changed between days. Rats were allowed to remain on the platform for 30 s after mounting it. If rats did not find the hidden platform within 60 s, the rat was guided to the platform by the technician and allowed to remain on the platform for 30 s. Each trial was recorded using a digital camera located above the maze and with the entire maze within the field of view. The path of each rat was analyzed for specific metrics using Ethovision software (Noldus Information Technology, Leesburg, Virginia, USA). Latency (s) to find the hidden platform was measured from the time that the rat was placed in the maze until mounting the platform. The number of unsuccessful trials, assessed as a trial wherein the rat did not find the platform during the 60 s duration, was counted across the 12 trials. The amount of time spent in the target quadrant (i.e., the quadrant of the maze that contained the platform) was also measured for each trial.

Rats were exposed to the Elevated Plus Maze protocol on the second (acute) or 30th (chronic) day post injury, which was used to quantify activity and emotional-type behaviors following TBI in rats. The maze consisted of four perpendicular 10x90 cm arms suspended 50 cm above the floor. A 10x10 cm central platform connected the arms. One pair of opposing arms was enclosed by 40-cm-high walls, while the other two arms and the center platform were uncovered. Rats were initially placed on the central platform facing one of the two open arms. The animals were allowed to explore the maze for five minutes and tracked using a digital video camera mounted above the maze. Metrics quantified during the test included: total distance traveled, total number of arm changes, and the number of entries into and time spent in open areas (center platform and uncovered arms). These metrics were automatically quantified using Ethovision video tracking system. Behaviors associated with post-injury activity included total number of arm changes and total distance traveled.

Behaviors associated with changes in emotionality included the number of entries and amount of time spent in open arms.

Statistical Analysis

In general, group-wise statistics were performed to determine statistically significant differences ($p < 0.05$) in behavioral metrics at each assessment time. However, because unconsciousness was only measured immediately following the injury protocol, rats from acute and chronic assessments were grouped and a single factor analysis of variance (ANOVA) was performed to determine significant differences ($p < 0.05$) between the five experimental groups highlighted in Table 1. Mean latency for the second through fourth trials of each MWM set was computed for the first, second, and third sets and statistically compared between groups on a set-by-set basis. Two-factor repeated measures ANOVA analysis was used to identify statistically significant differences between experimental groups and testing days in latency to find the hidden platform, with testing day as the repeated factor. The number of unsuccessful trials in the second through fourth trials of each MWM set were counted and compared between groups for the acute and chronic assessment periods using ANOVA analysis. Likewise, group-wise statistical comparison of all EPM metrics was accomplished for the acute and chronic assessments using ANOVA analysis. Bonferroni post-hoc analysis was used to determine statistically significant differences between specific groups (i.e., sham compared to M3D2).

III. RESULTS

A total of 132 Sprague Dawley rats were included in this study, 78 were exposed to behavioral assessments in the acute phase (i.e., within one week post injury) and 54 rats were exposed to behavioral assessments one month following injury. All rats survived the experimental procedure without skull or cervical spine fracture and regained consciousness within seven minutes following the injury procedure. Head rotational acceleration magnitude was significantly different ($p < 0.0001$) between controls, M1, M2, and M3 magnitude levels, as outlined in Table 1. Similarly, head rotational acceleration duration was significantly different ($p < 0.0001$) between controls, D1 and D2 duration levels. Rotational velocities were also significantly different ($p < 0.0001$) between injury groups, with each group different from all others except M1D2 and M2D1, which were not significantly different from each other.

Duration of unconsciousness was significantly different ($p = 0.0121$) based on experimental group (Figure 3). Acute unconsciousness was generally longer for higher magnitude rotational accelerations (M2, M3) than shams and lower magnitude accelerations, indicating higher acute severity of concussion for the higher magnitude accelerations. Independent comparisons between each of the groups revealed that unconsciousness times for each of the three M2 or M3 groups were significantly longer ($p < 0.05$) than the shams and M1 groups. However, unconsciousness time was not different between M2 and M3 groups.

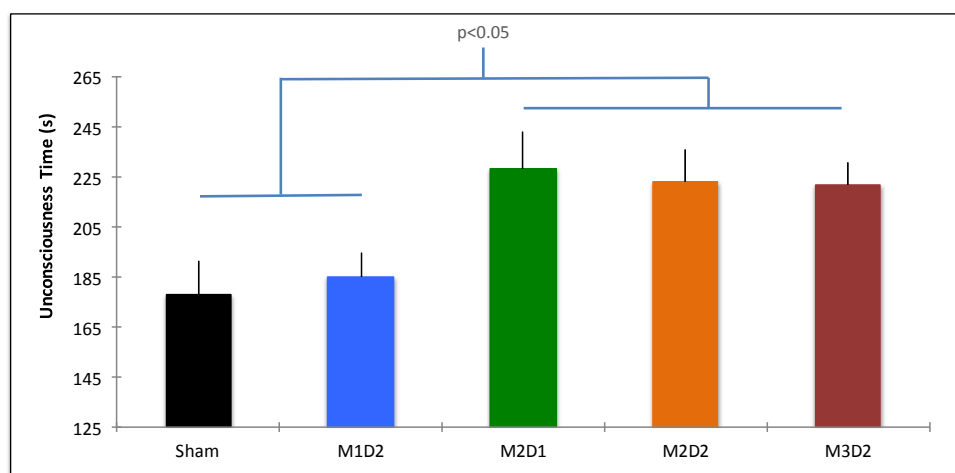


Fig. 3. Duration of unconsciousness (s) for each of the experimental groups following the experimental procedure. Data represented as mean $\pm$ standard error.

The Morris Water Maze assessment was used to identify cognitive deficits in experimental rats at acute and chronic time points following concussion. Repeated measures ANOVA analysis revealed latency to find the hidden platform during the acute and chronic time points were not significantly different between groups (Figure 4), although set-to-set decreases in latency across the four experimental groups were statistically significant ($p < 0.05$), indicating a process of spatial learning for assessments at both the acute and chronic time points. Similar to latency, the number of unsuccessful trials during the second through fourth trials of Sets 1-3 were not significantly different between experimental groups. The percentage of time spent in the target quadrant was also not significantly different between experimental groups during the acute or chronic assessment. These findings indicate a lack of cognitive deficits in rats experiencing TBI as a part of this study.

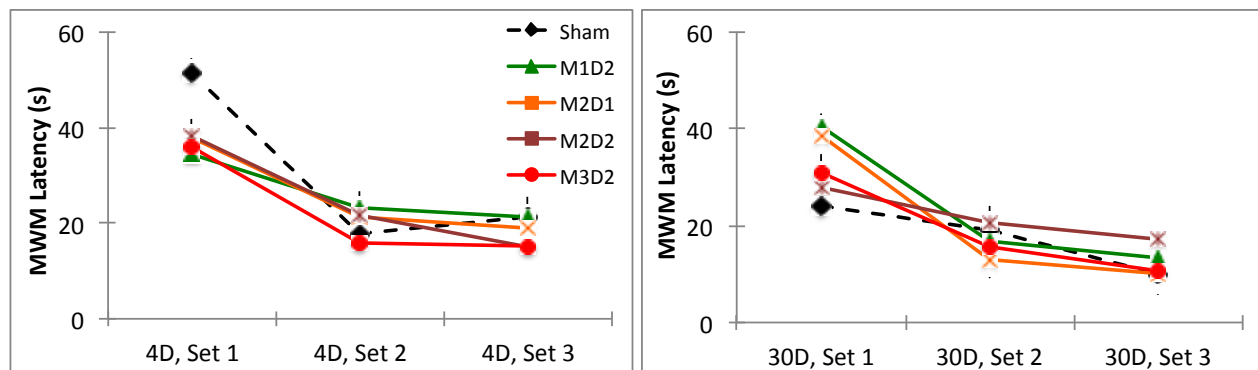


Fig. 4. Mean latency to find the hidden platform across the second through fourth trials for Sets 1-3 in the MWM assessment at acute (left) and chronic (right) time points. Data represented as mean $\pm$ standard error.

The Elevated Plus Maze was used to assess changes in post-injury activity and emotionality in experimental rats at acute and chronic time points following concussion. Analysis of post-injury activity demonstrated differing trends with regard to experimental groups between acute and chronic assessments. During the acute assessment, injured rats demonstrated significantly greater activity than controls as evident in the analysis of total EPM arm changes ($p = 0.0009$) and shown in Figure 5, left. The number of post-injury arm changes during the acute assessment demonstrated a dose-dependent response, with greater activity in rats with higher severity head rotational accelerations. All D2 groups had a greater number of arm changes than controls and D1 groups and arm changes increased from M1D2 to M2D2 to M3D2. Post-hoc analysis revealed significantly increased arm changes ($p < 0.05$) for all three D2 groups compared to controls. Total distance traveled in the EPM was also significantly dependent upon injury group ($p < 0.005$) and trends mirrored those of total arm changes. The number of entries into the open areas of the maze (i.e., center and open arms) during the acute phase demonstrated similar statistically significant trends (Figure 5, right). Specifically, the number of open entries in the acute phase was significantly different based on experimental group ($p = 0.0014$), with injured animals demonstrating a greater number of open entries.

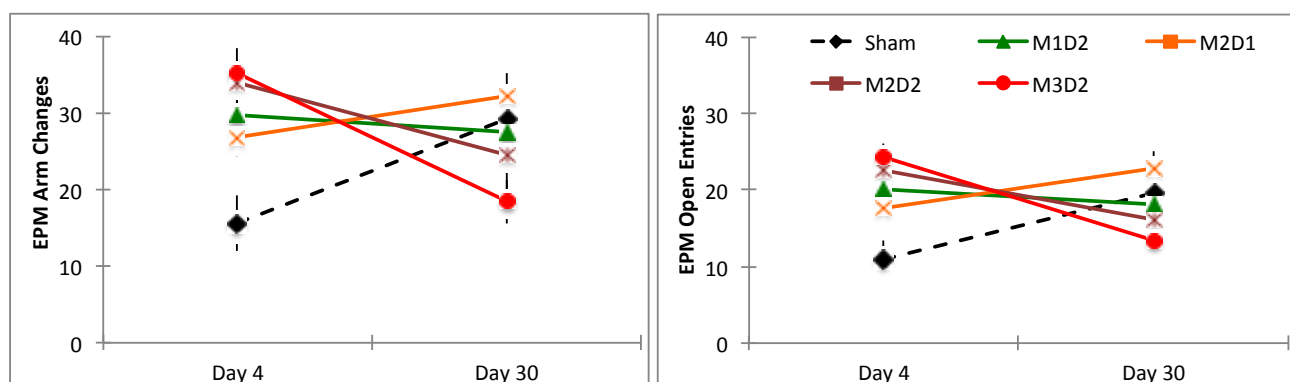


Fig. 5. Assessment of post-injury activity at acute and chronic time points using the Elevated Plus Maze and analyzing the total number of arm changes (left) and open arm entries (right). Data represented as mean $\pm$ standard error.

Whereas injured animals demonstrated increased and dose-dependent activity in the acute phase during EPM assessments, with greater activity for longer duration pulses, chronic changes in the Elevated Plus Maze demonstrated different trends. Injured rats demonstrated similar or decreased activity in terms of the total number of arm changes or open area entries at the chronic assessment time point (Figure 5). However, those differences were not statistically significant, although a group-to-group comparison demonstrated significantly decreased activity in the M3D2 group compared to controls ($p < 0.05$). Once again, identical trends were evident for total distance traveled, with group-based differences not statistically significant but the direct comparison of the M3D2 to controls revealed significantly less activity ($p = 0.05$).

Analysis of the amount of time spent in the open areas of the Elevated Plus Maze revealed somewhat similar trends to the activity metrics highlighted in Figure 5. Injured rats spent significantly more time in the open areas of the EPM ($p = 0.0005$) during the acute assessment (Figure 6), with the long duration groups demonstrating dose-dependent response (M1-M2-M3). Post-hoc analysis revealed the M3D2 group spent significantly more time in the open areas ($p < 0.05$) than shams and the M2D1 group. Although group differences were not statistically significant at the chronic assessment, the M3D2 spent less time in the open areas than controls and the other injured groups. However, the M2D2 and M2D1 groups spent more time in the open areas, which was more consistent with the acute assessment.

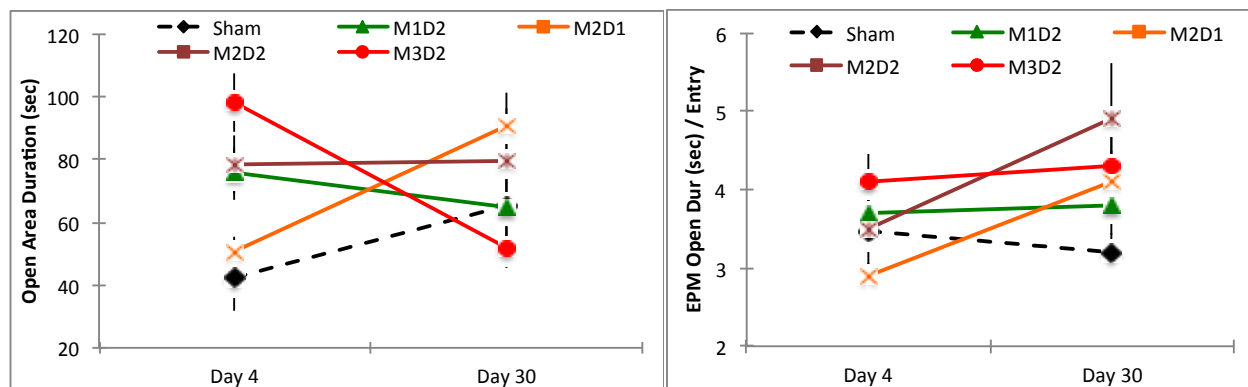


Fig. 6. Assessment of post-injury emotionality at acute and chronic time points using the Elevated Plus Maze and analyzing total amount of time spent in the open areas (left) and the amount of time spent in the open areas per entry right). Data represented as mean $\pm$ standard error.

Normalizing the amount of time spent in the open areas to the number of open area entries revealed a different trend (Figure 6, right). Although not significantly different across the five groups, trends with regard to timing were reversed from the other metrics, wherein injured rats behaved similarly to shams during the acute assessment and spent more time in the open arms of the EPM than shams at the chronic time point. Group-to-group comparisons demonstrated significantly increased time in the open areas per entry for M2D2 compared to shams ($p < 0.05$) or trends that approached statistically significant differences for the M3D2 and M1D2 groups.

IV. DISCUSSION

The current study demonstrated a complex relationship between head rotational acceleration biomechanics and injury outcomes at acute and chronic time points. Specifically, higher magnitude rotational accelerations produced injuries with greater acute severity, assessed as the duration of unconsciousness time. This trend, however, was only true when comparing higher magnitude groups (i.e., M2 and M3) to lower magnitude groups and shams. Step increases in unconsciousness time were not evident for each increasing rotational acceleration magnitude. In general, this finding is in line with prior work that has generally associated greater head accelerations with more severe injuries in the acute phase [11] or predicted the onset of concussion using peak rotational acceleration [14]. However, the finding that rotational velocity did not influence acute severity, with equivalent velocities (M1D2 and M2D1) producing significantly different unconsciousness times is somewhat contradictory to other studies that outlined the influence of rotational velocity on acute outcomes [13]. The prior association of rotational velocity with acute injury severity may be due, in part, to the inherent correlation

between rotational acceleration magnitude and rotational velocity, given similar acceleration durations. Prior work highlighting the importance of rotational velocity for injury risk made no apparent attempt to outline the role of different pulse shapes at a given rotational velocity magnitude. Therefore, increasing cumulative strain in the finite element brain with higher rotational velocity-related metrics [13] is likely a function of a more severe rotational acceleration pulse, with greater rotational acceleration magnitude and duration or greater magnitude at a constant duration. However, results of the current study have demonstrated a more complex relationship, with head accelerations of equivalent rotational velocity producing different acute and chronic outcomes depending on the rotational acceleration pulse shape (i.e., magnitude and duration). Our earlier work also demonstrated different distributions of diffusion tensor imaging-identified structural damage within the brain [23] and different strain distributions in a finite element brain model [26] for rotational acceleration pulses of equivalent velocities, depending on the magnitude and duration characteristics. These findings indicate that rotational velocity on its own was not a strong predictor of the onset of or outcomes following traumatic brain injury and that injury tolerance should incorporate an assessment of pulse shape.

Rotational acceleration pulses incorporated in the current study did not produce evidence of significant cognitive deficits for any of the Morris Water Maze Metrics. This finding is consistent with our previous outcomes from this model [23]. This finding is different from other studies incorporating the rotational acceleration mechanism in rodents, wherein significant and transient impairments were identified in working and reference memory [19]. That study also identified no significant differences in Elevated Plus Maze behaviors following concussion, whereas the current study produced a number of significantly different metrics based on acceleration characteristics including total number of arm changes and time duration in the open areas. This finding may highlight outcome differences based on the direction of rotational acceleration, sagittal in the prior study and coronal in the current study. Tolerance differences between the different planes of head rotational acceleration were previously identified by Gennarelli and colleagues [27] and the present findings may highlight differences in either the severity of tissue damage based on the direction of rotation or, more likely, the distribution of tissue injury. Changes in the distribution of tissue injury involve different affected brain regions and may produce differing injury outcomes and, presumably, a different clinical course in humans.

However, differences in acceleration pulse characteristics between the two studies also cannot be overlooked. Rostami and colleagues incorporated higher magnitude and shorter duration accelerations (1.5 Mrad/s^2 , 0.4 ms) [19] compared to the lower magnitude and much longer durations incorporated in the current study. Interestingly, given the different rotational acceleration pulse shapes, rotational velocities were actually similar between the two studies. Scaling of peak magnitudes from the rat to the human can be accomplished using a brain mass scaling ratio outlined by Holbourn [28]. Accordingly, human-equivalent peak accelerations incorporated in the current study were approximately $4,500 \text{ rad/s}^2$. That magnitude would produce classical concussion according to the previously cited scale [11]. In the absence of prior concussion, that level of injury may be below the threshold to produce significant cognitive impairments in the acute or chronic phase. It should also be acknowledged that differences in cognitive assessments between the two studies, Morris Water Maze in the current study and Radial Arm Maze in the prior, may also explain different outcomes.

A primary outcome from the present study was the finding of significant changes in emotionality following different rotational acceleration pulses. In the acute phase, injured rats had both greater activity and more time in the open areas of the Elevated Plus Maze. Activity metrics were predicted by a combination of magnitude and duration with all longer duration groups (D2) having more activity and more time in the open areas than shorter duration groups and shams. Within the long duration groups, a dose dependence was evident for increasing arm changes and open area time, with increasing magnitude associated with more activity and more open area time. Computational modeling has demonstrated changing injury distributions with longer duration rotational acceleration pulses [29]. Longer duration pulses were associated with higher strains in deeper brain regions. Those findings were supported by our prior medical imaging outcomes that showed structural damage spread more diffusely and in deeper regions of the brain for longer duration rotational accelerations [23]. From a mechanistic standpoint, longer duration head accelerations provide more time for strains to be transferred from superficial to deeper brain regions. A number of structures associated

with the limbic system are located deeper within the rat brain, including the hippocampus, amygdala, and the thalamus. The limbic system plays a primary role in the regulation of emotion and behavior. Therefore, longer duration acceleration pulses produce strains deeper in the brain and higher magnitude accelerations produce higher strain magnitudes for greater tissue damage.

Another novel outcome from this study is the demonstration of different trends in behavioral deficits between the acute and chronic phases. Significant differences in activity metrics (i.e., total arm changes and total distance traveled) between injured groups and controls tended to resolve by the chronic phase. Resolution of changes over time is expected for the relatively low severity injuries produced in this study. However, trends for metrics more associated with emotionality tended to reverse between the acute and chronic phases. The most striking trends were with regard to the amount of time spent in the open areas of the maze. Total open area time for the M3D2 group was 22% lower than controls for the chronic assessment after being 133% greater ($p < 0.05$) at the acute assessment. Trends with regard to open area time were not as dramatic for the other injury groups. However, normalized open area time (open area time/# open entries) was greater for injured groups at the chronic assessment after being similar to controls at the acute assessment. This demonstrates a progressive deficit that may be attributable to secondary damage in the brain tissues following the initial insult. This type of phenomenon was previously shown using diffusion tensor imaging for a different rodent injury model [30], wherein structural damage patterns within the brain tissues were far more extensive at the chronic compared to acute time points.

V. CONCLUSIONS

Results of this study demonstrated a complex relationship between head rotational acceleration characteristics, including rotational acceleration magnitude and duration, and rotational velocity, and the type, severity, and time course of behavioral changes following concussion. In general, higher rotational acceleration magnitudes (M2 and M3) resulted in greater acute severity of concussion than the lower magnitude group and shams, and longer duration rotational accelerations produced greater differences in emotionality in the acute phase. Rotational velocity was not a strong independent predictor of acute severity or acute changes in emotionality. However, in the case of higher severity acceleration pulses, trends with regard to emotionality developed or reversed between the acute and chronic phase. This may indicate progressive damage due to secondary processes following the initial injury and complicates the quantification of injury tolerance for concussion, where a majority of injury tolerance research is focused on the onset of injury and not the time-based progression of damage.

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VII. REFERENCES

1. Stemper, B.D. and F.A. Pintar, *Biomechanics of concussion*. Prog Neurol Surg, 2014. **28**: p. 14-27.
2. Adams, J.H., D.I. Graham, and T.A. Gennarelli, *Head injury in man and experimental animals: neuropathology*. Acta Neurochir Suppl (Wien), 1983. **32**: p. 15-30.
3. Magnuson, J., F. Leonessa, and G.S. Ling, *Neuropathology of explosive blast traumatic brain injury*. Curr Neurol Neurosci Rep, 2012. **12**(5): p. 570-9.
4. Bazarian, J.J., S.G. Fisher, et al., *Lateral automobile impacts and the risk of traumatic brain injury*. Ann Emerg Med, 2004. **44**(2): p. 142-52.
5. McCrea, M., K. Guskiewicz, et al., *Incidence, clinical course, and predictors of prolonged recovery time following sport-related concussion in high school and college athletes*. J Int Neuropsychol Soc, 2013. **19**(1): p. 22-33.

6. Duma, S.M., S.J. Manoogian, et al., *Analysis of real-time head accelerations in collegiate football players*. Clin J Sport Med, 2005. **15**(1): p. 3-8.
7. Camarillo, D.B., P.B. Shull, J. Mattson, R. Shultz, and D. Garza, *An instrumented mouthguard for measuring linear and angular head impact kinematics in American football*. Ann Biomed Eng, 2013. **41**(9): p. 1939-49.
8. Guskiewicz, K.M., J.P. Mihalik, et al., *Measurement of head impacts in collegiate football players: relationship between head impact biomechanics and acute clinical outcome after concussion*. Neurosurgery, 2007. **61**(6): p. 1244-52; discussion 1252-3.
9. Pintar, F.A., N. Yoganandan, and L. Voo, *Effect of age and loading rate on human cervical spine injury threshold*. Spine (Phila Pa 1976), 1998. **23**(18): p. 1957-62.
10. Ivarsson, B.J., D. Genovese, et al., *The tolerance of the femoral shaft in combined axial compression and bending loading*. Stapp Car Crash J, 2009. **53**: p. 251-90.
11. Gennarelli, T.A., F.A. Pintar, and N. Yoganandan, *Biomechanical tolerances for diffuse brain injury and a hypothesis for genotypic variability in response to trauma*. in *47th Proceedings of the Association for the Advancement of Automotive Medicine*. 2003.
12. Newman, J.A., N. Shewchenko, and E. Welbourne, *A proposed new biomechanical head injury assessment function - the maximum power index*. Stapp Car Crash J, 2000. **44**: p. 215-47.
13. Takhounts, E.G., M.J. Craig, K. Moorhouse, J. McFadden, and V. Hasija, *Development of brain injury criteria (BrIC)*. Stapp Car Crash J, 2013. **57**: p. 243-66.
14. Rowson, S., S.M. Duma, et al., *Rotational head kinematics in football impacts: an injury risk function for concussion*. Ann Biomed Eng, 2012. **40**(1): p. 1-13.
15. Lighthall, J.W., *Controlled cortical impact: a new experimental brain injury model*. J Neurotrauma, 1988. **5**(1): p. 1-15.
16. Shultz, S.R., D.F. MacFabe, K.A. Foley, R. Taylor, and D.P. Cain, *A single mild fluid percussion injury induces short-term behavioral and neuropathological changes in the Long-Evans rat: support for an animal model of concussion*. Behav Brain Res, 2011. **224**(2): p. 326-35.
17. Marmarou, A., M.A. Foda, et al., *A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics*. J Neurosurg, 1994. **80**(2): p. 291-300.
18. Fijalkowski, R.J., B.D. Stemper, et al., *New rat model for diffuse brain injury using coronal plane angular acceleration*. J Neurotrauma, 2007. **24**(8): p. 1387-98.
19. Rostami, E., J. Davidsson, et al., *A Model for Mild Traumatic Brain Injury that Induces Limited Transient Memory Impairment and Increased Levels of Axon Related Serum Biomarkers*. Front Neurol, 2012. **3**: p. 115.
20. Xiao-Sheng, H., Y. Sheng-Yu, Z. Xiang, F. Zhou, and Z. Jian-ning, *Diffuse axonal injury due to lateral head rotation in a rat model*. J Neurosurg, 2000. **93**(4): p. 626-33.
21. Gutierrez, E., Y. Huang, et al., *A new model for diffuse brain injury by rotational acceleration: I model, gross appearance, and astrogliosis*. J Neurotrauma, 2001. **18**(3): p. 247-57.
22. Smith, D.H., M. Nonaka, et al., *Immediate coma following inertial brain injury dependent on axonal damage in the brainstem*. J Neurosurg, 2000. **93**(2): p. 315-22.
23. Stemper, B.D., A.S. Shah, et al., *Head rotational acceleration characteristics influence behavioral and diffusion tensor imaging outcomes following concussion*. Ann Biomed Eng, 2015. **43**(5): p. 1071-88.
24. Fijalkowski, R.J., B.M. Ellingson, et al., *Interface parameters of impact-induced mild traumatic brain injury*. Biomed Sci Instrum, 2006. **42**: p. 108-13.
25. Cheney, J.A., A.L. Brown, et al., *The novel compound LOE 908 attenuates acute neuromotor dysfunction but not cognitive impairment or cortical tissue loss following traumatic brain injury in rats*. J Neurotrauma, 2000. **17**(1): p. 83-91.
26. Fijalkowski, R.J., N. Yoganandan, J. Zhang, and F.A. Pintar, *A finite element model of region-specific response for mild diffuse brain injury*. Stapp Car Crash J, 2009. **53**: p. 193-213.
27. Gennarelli, T.A., L.E. Thibault, et al., *Directional dependence of axonal brain injury due to centroidal and non-centroidal acceleration*, in *31st Stapp Car Crash Conf*. 1987: New Orleans, LA. p. 49-53.
28. Holbourn, A.H.S., *Mechanics of head injuries*. Lancet, 1943. **2**: p. 438-441.
29. Lamy, M., D. Baumgartner, N. Yoganandan, B.D. Stemper, and R. Willinger, *Experimentally validated three-dimensional finite element model of the rat for mild traumatic brain injury*. Med Biol Eng Comput, 2013. **51**(3): p. 353-65.
30. Budde, M.D., A. Shah, et al., *Primary blast traumatic brain injury in the rat: relating diffusion tensor imaging and behavior*. Front Neurol, 2013. **4**: p. 154.