

# INDUCING MILD TRAUMATIC BRAIN INJURY IN THE RODENT THROUGH CORONAL PLANE ANGULAR ACCELERATION

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## ABSTRACT

This investigation used a new experimental model to apply pure coronal plane angular acceleration to a rodent, inducing concussion (mild traumatic brain injury). A mass was propelled down a drop tube toward the laterally extended moment arm of a rodent helmet fixture. Upon impact, the rodent helmet-head complex experienced mean angular acceleration of  $368 \text{ krad/s}^2$  (standard deviation = 30). All 26 specimens demonstrated a period of transient unconsciousness, mild ventriculomegaly, and absence of severe pathology. Injuries were classified as concussion based on scaled biomechanics, transient unconsciousness, macroscopic damage and minimal histological abnormalities.

**Keywords:** angular acceleration, closed head injury, concussion, diffuse brain injury, mild traumatic brain injury

**DIFFUSE BRAIN INJURY (DBI)** continues to be a major public health problem. In the United States these injuries number upwards of one million each year, with an estimated 90,000 patients suffering long-term disability (Centers for Disease Control and Prevention 2006). DBI most commonly occur during motor vehicle collisions, falls, assaults and sports-related activities (Centers for Disease Control and Prevention 2006; Williamson and Goodman 2006). A demographic injury profile indicated young adults and elderly are most susceptible (Lee *et al.* 2006; Steudel *et al.* 2005). In particular, 17% of injuries occurred in the 15- to 30-year age group, and 39% of injuries occur in the 65 and older age group (Steudel *et al.* 2005). Although considerable advances have been made in prevention of head injury, such as bicycle helmets, seatbelts, and airbags, head injuries continue to affect a significant portion of society.

Neurological deficit associated with DBI ranges from temporary impairment of brain function (confusion, amnesia) to transient or prolonged unconsciousness with death, or vegetative outcome (Alexander 1995; Gennarelli *et al.* 1982; Ommaya and Gennarelli 1974; Vos *et al.* 2002). As a result, DBI is considered a spectrum of injuries spanning from mild concussion to severe diffuse axonal injury (DAI) (Abel *et al.* 1978; Adams *et al.* 1983; Blumbergs *et al.* 1989; Margulies and Thibault 1992; Ommaya and Gennarelli 1974; Viano 1985). It is well documented that whether the initial injury is mild or severe, a high potential exists to initiate a dynamic sequence resulting in neuronal loss (Buki *et al.* 2000; Hovda *et al.* 1991; Kelley *et al.* in press; Maxwell *et al.* 1997; Oppenheimer 1968; Povlishock 1992; Povlishock *et al.* 1983). Pathologies associated with DAI are well defined, while the extent of injury resulting from mild traumatic brain injury (MTBI) is less clear. Previous experimentation demonstrated that biomechanical measures modulate injury severity, with higher magnitude insults leading to more severe injuries (Douglass *et al.* 1968; Gennarelli *et al.* 1987; Higgins and Schmall 1967; Hodgson *et al.* 1983; Margulies *et al.* 1990; Ommaya and Hirsch 1971; Ono *et al.* 1980; Shatsky *et al.* 1974; Stalnaker *et al.* 1973). However, a direct correlation between specific injury biomechanics such as insult magnitude and duration, and resulting pathologies, particularly for low-severity injuries, has not been well defined.

DBI generally occurs via impact often without skull fracture and is referred to as closed head injury (Gennarelli and Thibault 1982; Gennarelli and Thibault 1995; Goldsmith and Plunkett 2004). The impact causes rotation of the head and inertial forces are distributed to the underlying brain tissue.

Clinical studies, physical modeling, and finite element analysis have hypothesized that resulting strains are the primary cause of neurological deficiencies following DBI (Adams *et al.* 1991; Bandak and Eppinger 1994; Bradshaw *et al.* 2001; Gennarelli and Thibault 1995; Goldsmith and Plunkett 2004; Holbourn 1943; Ivarsson *et al.* 2000; Kelley *et al.* in press; Margulies and Thibault 1992; Margulies *et al.* 1990; Miller *et al.* 1998; Povlishock 1992; Strich 1961; Zhang *et al.* 2004; Zhou *et al.* 1994). The severity of injury is directly attributed to, among others, four biomechanical factors: magnitude and duration of angular acceleration, brain mass, and plane of rotation. It was previously demonstrated that an inverse relationship between brain mass and peak angular acceleration exists, wherein decreasing brain mass requires increasing levels of angular acceleration for similar injury levels (Douglass *et al.* 1968; Margulies and Thibault 1992; Ommaya and Hirsch 1971; Ommaya *et al.* 1967). A separate study demonstrated that coronal plane rotations are most injurious due to decreased inertial properties and geometric constraints (Gennarelli *et al.* 1987; Hodgson *et al.* 1983; Margulies and Thibault 1992; Margulies *et al.* 1990; Thibault and Gennarelli 1990). Other experimentation has shown a positive correlation between magnitude of angular acceleration and severity of injury (Abel *et al.* 1978; Higgins and Schmall 1967; Hodgson *et al.* 1983; Margulies and Thibault 1992; Ono *et al.* 1980). Furthermore, duration of angular acceleration is a determinant of injury type, wherein short duration impacts result in focal injury while long duration impacts result in DBI (Margulies and Thibault 1992; Ono *et al.* 1980; Shatsky *et al.* 1974; Stalnaker *et al.* 1973). Development of a relationship between impact parameters and resulting pathology requires a methodology to strictly control input biomechanics and identify resulting pathology at all levels of the DBI spectrum.

Previous experimental rodent models have focused on high severity DBI, wherein injury biomechanics were not quantifiable. The present study developed an experimental model to consistently produce MTBI in rodents using pure coronal plane angular acceleration without translation and skull fracture. The present methodology permitted direct comparison between measured injury biomechanics and outcomes, behaviors, pathology and histology.

## METHODS

The experimental protocol was approved by our institutional review board. The experimental model consisted of a spring-loaded ejection system (Figure 1b) and rodent helmet fixture (Figure 1c). The spring-loaded ejection system propelled an impactor down a drop tube to contact a laterally extended moment arm on the rodent helmet fixture (Figure 1a). Energy was transferred from the impactor to the moment arm. The impactor force was directed inferiorly. A pin-joint was used to constrain helmet motion to pure coronal plane angular rotation without translation. Therefore, linear force from the impactor was converted to angular helmet motion. Because the helmet and rodent head were assumed to be rigidly connected, purely rotational motion of the helmet resulted in purely rotational motion of the head.

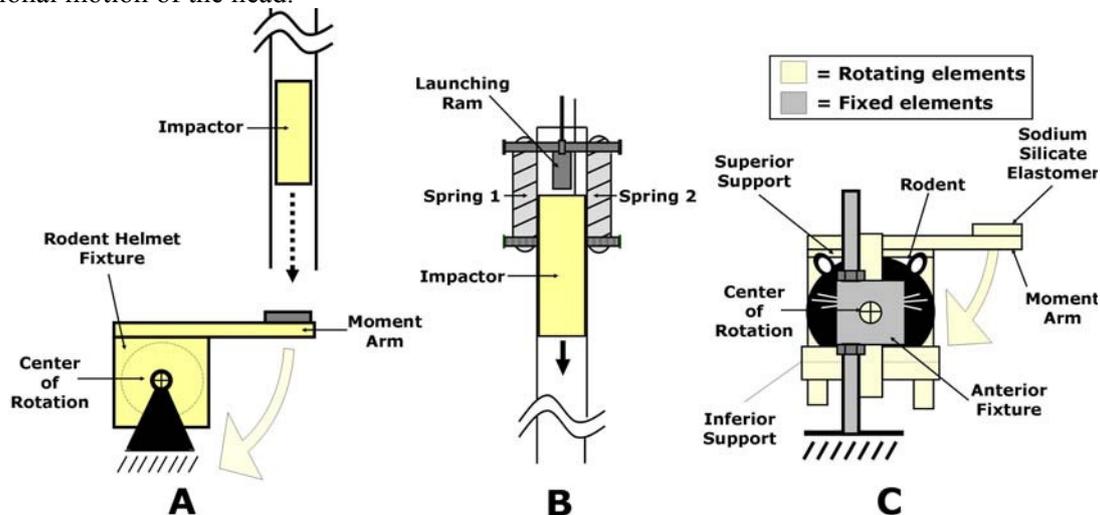


Fig. 1 – a. general illustration of the induction system, b. spring loaded ejection system, c. rodent helmet fixture

**Spring-Loaded Ejection System.** A 488-g impactor was positioned inside a 2.3-m drop tube in contact with the launching ram (Figure 1b). Two vertically oriented springs (4,435.5 N/m) were vertically displaced 15 cm. Upon spring release, the impactor was accelerated down the drop tube to a closing velocity of 10 m/s (standard deviation 1.5), and impacted the moment arm of the rodent helmet fixture causing pure coronal plane angular acceleration.

**Rodent Helmet Fixture.** The rodent helmet fixture consisted of four parts: moment arm, superior and inferior supports, and anterior fixture (Figure 1c). The moment arm extended laterally from the superior support, transferring energy from the impactor to the rodent head without skull fracture. A sodium silicate elastomer was placed on the contacting surface of the moment arm to control contact time and modify pulse width. Superior and inferior supports secured the head and maximized energy transfer from the impactor. The inferior support was molded to match the bone structure of the rodent mandible, minimizing sliding of the head inside the helmet during impact. The anterior fixture was a pin joint that constrained helmet motion to pure coronal plane rotation with the center of rotation 2-mm inferior to the brain base in the mid-sagittal plane. Helmet rotation was limited to 90° by a damping mechanism. The anterior fixture was constrained by two locking bolts and a support post to prevent translation of the head with respect to the body.

Rotational helmet displacement was measured by tracking reflective targets attached to the moment arm and anterior fixture at 13.5 kfps using a high-resolution digital imaging system (Redlake Inc., San Diego, CA). Image resolution was 0.875 mm/pixel. Rotational displacement data were low-pass filtered at 1,000 Hz, and angular acceleration was computed as the second derivative of rotational displacement. Differentiation error was limited to 5.1% of the peak angular acceleration.

Vital signs were monitored for five minutes prior to experimentation to obtain basal levels. Specimens were detached from the monitoring equipment during impact. Immediately following impact, vital signs were monitored continuously for 10 minutes and intermittently for the remaining survival period. Temperature was monitored by a tympanic membrane thermometer, heart rate was monitored via ECG, and respiratory status was monitored using pulse oximetry via the tail (Datex-Ohmeda, Madison, WI). Systolic blood pressure was measured with tail cuff sphygmomanometer and pulse transducer (IITC, Woodland Hills, CA).

Twenty-six adult male Sprague-Dawley rats, each weighing 255 to 325 g, were administered general anesthesia prior to impact using a mixture of ketamine (75 mg/kg) and medetomidine (0.5 mg/kg). A reversal agent consisting of atipamizole (1 mg/kg) was administered subcutaneously immediately following impact. This anesthetic regimen was previously shown to be safe and effective on rodents, with minimal effect on neurologic function (Hahn *et al.* 2005; Sleeman and Gaynor 2000; Sun *et al.* 2003; Yamashita *et al.* 1996). Fifteen controls were subjected to an identical protocol without impact.

The criterion for onset of concussion was defined as the loss of coordinated response to external stimuli and the absence of severe pathology. A reversal agent was administered immediately after injury to assess the presence or absence of six reflexes (corneal, escape, pinch, pinna, startle and whisker). Apparent reflexes were scored a value of one and results of all six tests were summed to evaluate the level of unconsciousness. Unconscious time was assessed as the time to first reflex. This type of reflex assessment was shown to be an effective measure of unconscious time (Abel *et al.* 1978; Adelson *et al.* 1996; Dixon *et al.* 1987; Smith *et al.* 2000). A T-test was used to assess differences in unconscious time between controls and experimental specimens. The time point associated with presence of all six reflexes was measured in both control and experimental specimens for a quantitative measure of recovery.

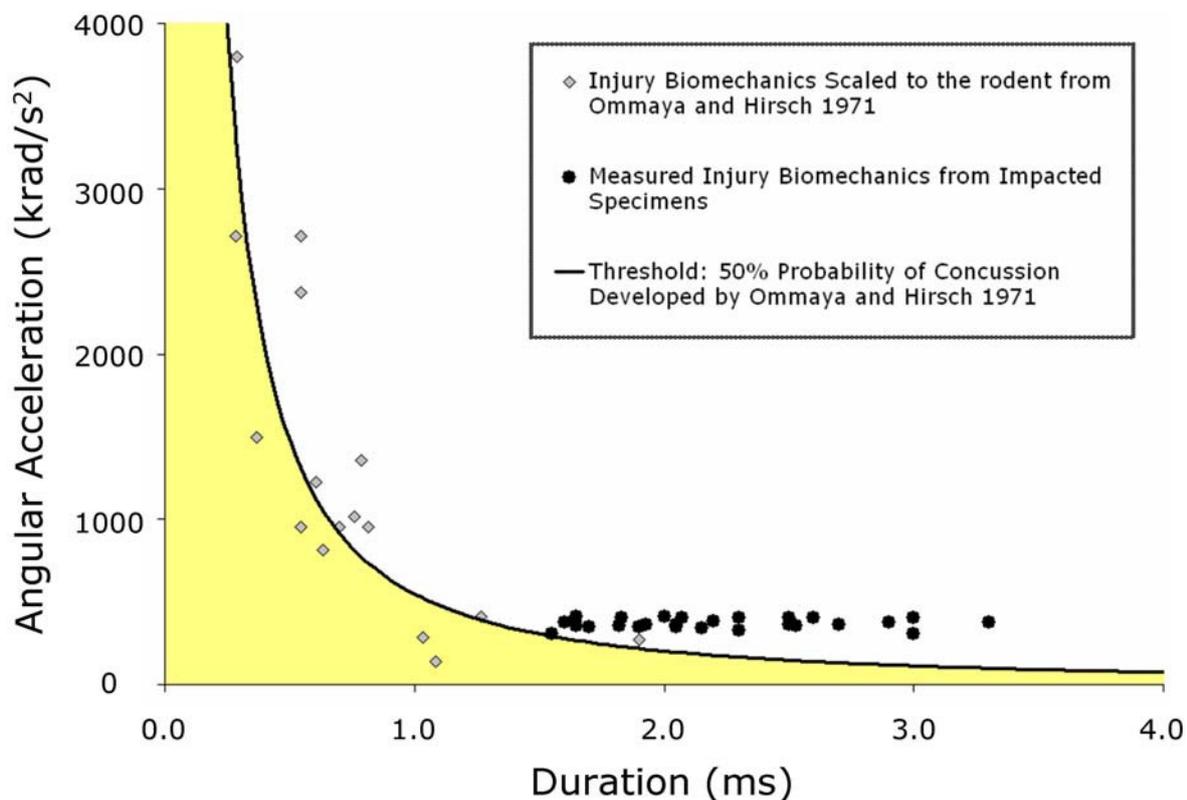
Specimens were euthanized 24, 48, 72 or 96 hours post impact with an overdose of sodium pentobarbital (100 mg/kg IP). The thoracic cavity was opened, and a perfusion catheter was inserted into to the ascending aorta. The right atrium was incised, and 300 ml of warm buffered saline was perfused. This was followed by fixation using 4% paraformaldehyde, pH 7.4, at 4 deg C. Upon

extraction, the brain was macroscopically assessed and then put into a post fix consisting of 4% paraformaldehyde and 20 g of sucrose/100 ml for 24 hours. The tissue was frozen and sectioned (10 - 20  $\mu\text{m}$ ). Sections were assessed histologically using hematoxylin and eosin (H&E) and beta amyloid precursor protein ( $\beta$ -APP) staining protocols. Mean ventricle size was approximated by digitally measuring cross-sectional area of the coronal histology sections in the area of fornix decussation.

Magnitude and duration of angular acceleration obtained in the present study were compared to a previously defined concussion threshold criterion (Ommaya and Hirsch 1971). Therefore, peak angular acceleration values indicated by Ommaya and Hirsch were scaled to the rodent based on a brain-mass scaling ratio applied in previous studies (Margulies and Gennarelli 1985; Ommaya *et al.* 1967). The concussion criterion compared magnitude and duration of angular acceleration to predicted values required to induce concussion.

## RESULTS

Impacts resulted in mean peak angular accelerations of 368  $\text{krad/s}^2$  (standard deviation = 30), with mean durations of 2.1 ms (standard deviation = 0.5). Figure 2 compares peak angular acceleration and duration parameters from all 26 tests to a previously developed concussion threshold (Ommaya and Hirsch 1971). This comparison suggests that all 26 experimental tests resulted in input biomechanics sufficient to induce concussion in the rodent.

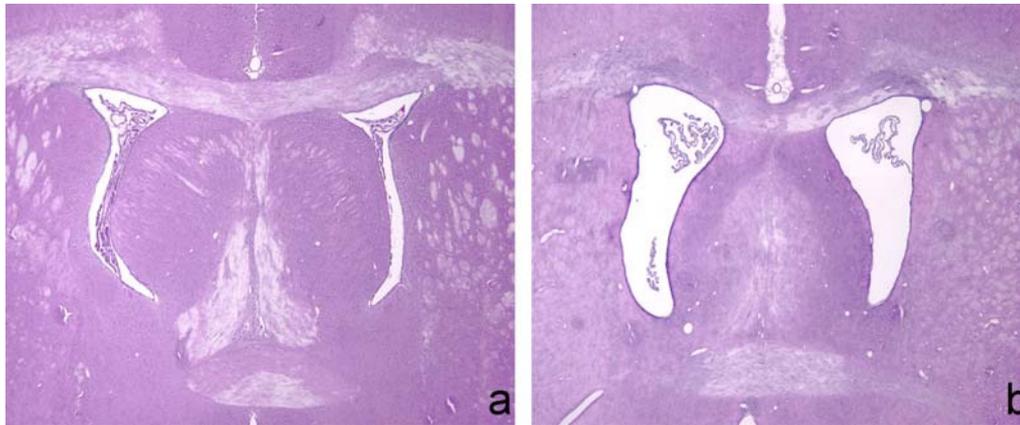


**Fig. 2 – Comparison of experimental results to concussion threshold.**

Consistency of physiologic function was demonstrated during recovery as vital signs maintained basal levels. Immediately after impact, specimens were considered unconscious as all reflexes were absent. Unconscious time, assessed as the time to regain the first reflex, was significantly longer ( $p < 0.05$ ) in experimental specimens ( $8.8 \pm 3.7$  minutes) than controls ( $2.0 \pm 0.3$  minutes). The order in which the six reflexes re-appeared was not uniformly distributed across the 26 experimental and 15 control specimens. Control specimens attained complete consciousness (regained all reflexes)  $2.5 \pm 0.2$  minutes post impact, whereas experimental specimens did not attain complete consciousness until  $39.7 \pm 18.9$  minutes post impact. In addition, experimental specimens exhibited minimal ambulation

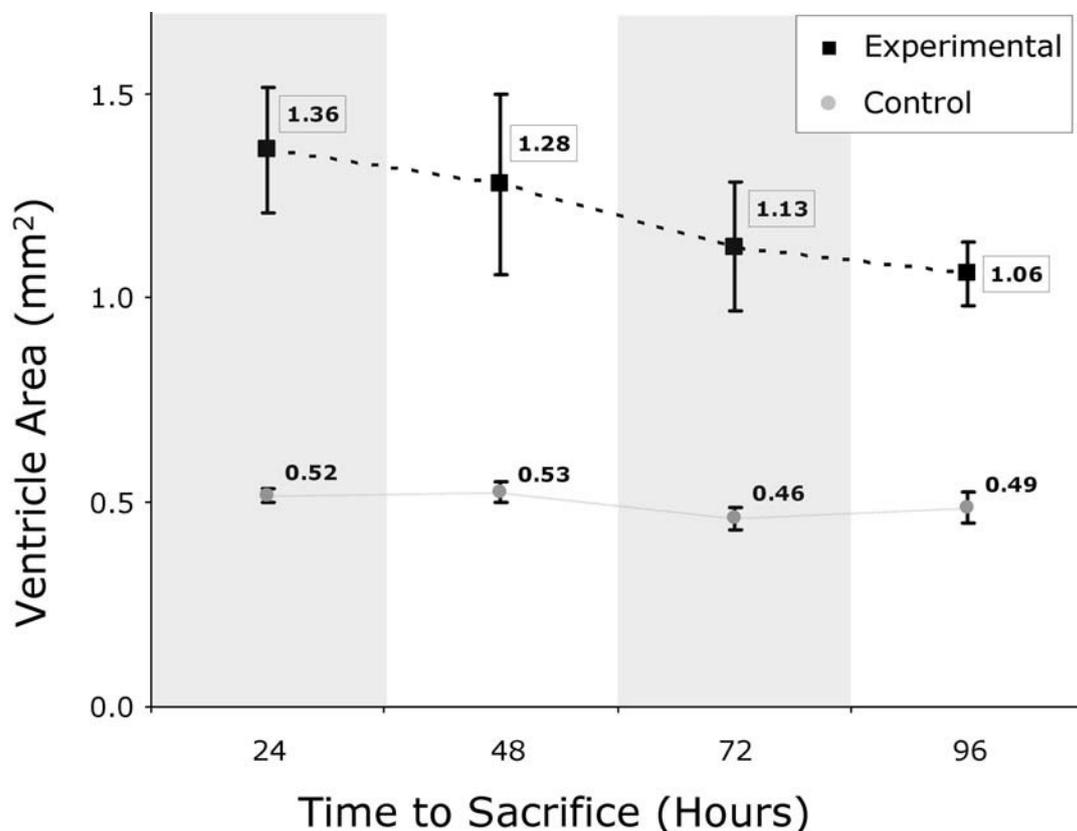
for up to 180 minutes post impact, a phenomenon that did not occur in controls. All specimens demonstrated normal ambulation prior to sacrifice.

Skull fracture was absent in all experimental specimens. Macroscopic analysis revealed ten specimens experienced subarachnoid hemorrhage (38%), characterized by significant blood formation spread thinly but diffusely over the cerebral hemispheres. Four separate specimens (15%) experienced intraparenchymal lesion in the subfrontal lobe. H&E staining demonstrated all impacted specimens suffered mild ventriculomegaly (Figure 3).



**Fig. 3 – Histological findings of ventriculomegaly (a. control, b. experimental).**

Mean ventricle area decreased in experimental specimens at increasing time points (Figure 4). However, mean ventricle size was significantly larger than controls at all time points ( $p < 0.05$ ).  $\beta$ -APP staining demonstrated a lack of retraction bulbs in coronal sections at all time points.



**Fig. 4 – Ventricle size at 24, 48, 72, and 96 hours post-injury**

## DISCUSSION

In 1943, Holbourn hypothesized that sudden head rotation (i.e. angular acceleration) was a possible concussion injury mechanism. Ommaya (1964) later confirmed this hypothesis in a study subjecting primates to linear or angular head accelerations. In that study, specimens were concussed more frequently under head angular acceleration. Clinical studies report that diffuse brain injuries occur most commonly due to occupant impact with deformable or padded surfaces (Gennarelli 1983). This loading typically results in high rates of head angular acceleration. However, patients often suffer injuries that are focal and diffuse in nature, suggesting multiple factors contributing to resulting pathology. The current head injury criterion (HIC) focuses entirely on linear acceleration over a finite duration. As such, this may not fully characterize the mechanism and rotational injuries may be completely excluded (Margulies and Thibault 1992; Viano 1988; Viano *et al.* 1986). The present investigation focused on development of a MTBI model incorporating pure coronal plane angular acceleration, with the capability of expanding to characterize biomechanics and resulting pathologies across the entire spectrum of DBI. A clear understanding of the effects of linear and angular acceleration on brain injury, associated pathologies, and clinical outcomes may lead toward a comprehensive injury metric incorporating both injury mechanisms. In addition, due to correlation between biomechanics and pathology the present model may be used to investigate other aspects of DBI such as gradations of macroscopic damage, axonal injury and other pathological and behavioral sequelae shown to affect prognosis.

The current model was intentionally focused on producing MTBI in the rodent. As such, an angular acceleration greater than  $350 \text{ krad/s}^2$  was required. Previous experimentation suggested this level of angular acceleration is sufficient to induce concussion in the rodent (Fijalkowski *et al.* 2006). Experimental findings demonstrated that this model accurately and repeatedly produced angular accelerations of  $368 \text{ krad/s}^2$  (standard deviation = 30). Future work will investigate different injury severity levels along the DBI spectrum by experimentally increasing magnitudes of angular acceleration magnitudes and modulating pulse widths.

The present definition of concussion was a transient unconscious period with an absence of severe pathology. All experimental rodents demonstrated a transient period of unconsciousness, defined as the time to initial reflex, lasting between five and fifteen minutes. This unconscious time was significantly greater than controls. In addition, all experimental rodents exhibited a lack of substantial pathology at 24, 48, 72, and 96 hours. More severe DBI is associated with advanced pathological outcomes. Therefore, all experimental rodents sustained concussion as a result of pure coronal plane angular acceleration. Further validation of this injury level is discussed below. Heart rate, respiration rate and blood pressure did not deviate from basal levels. Such deviation is associated with more severe trauma levels (Adelson *et al.* 1996; Dixon *et al.* 1988; Dixon *et al.* 1987). In addition, subarachnoid hemorrhage noted in only 38% of specimens was limited to the cerebral hemispheres, as previously noted in concussion literature (Abel *et al.* 1978; Alexander 1995; Bazarian *et al.* 2005; Gennarelli *et al.* 1982; Jane *et al.* 1985; Smith *et al.* 2000). Subarachnoid hemorrhage resulting from more severe trauma involves the brain stem or spinal cord, and hematoma that infiltrates the hypothalamus, ventral pons and cortical gray and white matter (Dixon *et al.* 1988; Gutierrez *et al.* 2001). Furthermore, microscopic analysis was unremarkable with no petechial hemorrhage or significant neuronal damage, indicative of more severe DBI if present (Dixon *et al.* 1988; Foda and Marmarou 1994; Kelley *et al.* in press; Lammie *et al.* 1999). This definition of concussion is consistent with previous concussion literature and general descriptions of concussion in experimental animals (Abel *et al.* 1978; Adelson *et al.* 1996; Dixon *et al.* 1987; Gennarelli 1982; Ommaya and Hirsch 1971; Smith *et al.* 2000). Therefore, according to these indications, it remains likely that experimental rodents consistently sustained low-level DBI (concussion) under the present protocol. To our knowledge, this is the first study to subject rats to extremely high magnitudes of head angular acceleration required to induce MTBI.

Injury biomechanics were compared to a previously defined concussion threshold criterion based on magnitude and duration of head angular acceleration (Ommaya and Hirsch 1971). The injury biomechanics applied in the present study were sufficient to induce concussion according to scaled

biomechanics from the previously defined injury criterion (Figure 2). Although not used as a direct indicator for concussion in this study, this comparison is further validation of the level of injury sustained by experimental rodents.

Gennarelli et al. (2003) hypothesized the magnitude of angular acceleration required to induce increasing levels of DBI in the human correlated with the abbreviated injury scale (AIS). To further define the present injury level, peak angular accelerations obtained in this study were scaled to the human according to brain mass (Ommaya and Hirsch 1971), and the corresponding injury level was determined (Table 1). Biomechanical parameters measured in this study suggest injury severity consistent with AIS level 2. Pathological results in some specimens suggest an AIS level 3 injury due to intraparenchymal lesion and subarachnoid hemorrhage. However, due to the low incidence rate (15% and 38%, respectively) the overall grouping may still be considered an AIS level 2 injury based on consistency of biomechanical insult and post-injury behavior. Findings of transient unconsciousness, absence of retraction bulbs and acute ventriculomegaly support this assumption.

**Table 1 - Categories of DBI based on biomechanical insult (Gennarelli et al. 2003).**

AIS level	Clinical category	Angular Acceleration	Angular Acceleration
		Human (1350 grams) (krad/s <sup>2</sup> )	Rodent (3 grams) (krad/s <sup>2</sup> )
1	Mild concussion	3	176
2	Classical concussion	5.8	341
3	Severe concussion	8.6	505
4	Mild DAI	11.5	675
5	Moderate DAI	14.4	845
6	Severe DAI	16.5	968

Present experimental rodents sustained a minimum of 325 krad/s<sup>2</sup> and maximum of 409 krad/s<sup>2</sup> (mean 368, standard deviation = 30) coronal plane angular acceleration. Using the mass scaling ratio previously described (Margulies and Gennarelli 1985; Ommaya et al. 1967), equivalent angular acceleration in humans is a mean of 6.2 krad/s<sup>2</sup> (standard deviation= 0.51). According to Gennarelli et al. (2003), this value was sufficient to induce concussion. These numbers agree with recent sports concussion literature. Newman et al. (2000) reported a minimum of 4.2 krad/s<sup>2</sup> and maximum of 9.68 krad/s<sup>2</sup> (mean 6.8, standard deviation = 2) and Pellman et al. (2003) reported a minimum of 3.4 krad/s<sup>2</sup> and maximum of 9.6 krad/s<sup>2</sup> (mean 6.4, standard deviation 1.8). Although the present mean angular accelerations are slightly lower than previously reported mean values, they are within the standard deviation. Another study demonstrated that boxers sustained up to 16 krad/s<sup>2</sup> without concussion. In addition, the previous author admitted that pulse width in these situations was likely too small to result in concussion. Study of the complex interplay between magnitude and pulse width of angular acceleration remains a future direction of MTBI research using this model.

Angular acceleration of the helmet fixture was used to quantify the biomechanical insult applied to experimental rodents. Due to a fixed center of rotation (Figure 1) the helmet was constrained to pure rotation without translation. To test the assumption of a rigid interface between the head and the helmet, a secondary experimental protocol was used to quantify the magnitude of loss during energy transfer from the helmet to the head. Twenty five tests were performed using postmortem rodents with photo reflective targets rigidly attached to the cranium, along with targets attached to the helmet as per the experimental protocol. These experiments determined that peak head angular accelerations attained an average of 94.5% (standard deviation = 3.25%) of peak helmet accelerations. In all cases, head angular accelerations exceeded 90% of the helmet. Therefore, helmet angular accelerations, as reported in this manuscript, may slightly overestimate head accelerations in experimental specimens. Due to the invasive nature of rigidly attaching targets to the cranium, it was not possible to implement head targets during experimentation. However, due to minimal loss in the transfer of energy from the helmet to the head, it is the opinion of these authors that helmet accelerations are suitable approximations for head accelerations used to induce MTBI in experimental specimens.

Histology demonstrated the presence of acute mild ventriculomegaly in all experimental specimens between 24 and 96 hours post impact (Figure 3). While cross-sectional ventricle area of controls remained constant over this time period, ventricle area of experimental specimens decreased at each increasing time point (Figure 4). However, ventricle area was significantly greater in experimental specimens than controls at all time points. Although more commonly demonstrated in severe injury levels such as DAI (Adelson *et al.* 1996; Kishore *et al.* 1978; Marmarou *et al.* 1996), mild ventriculomegaly has also been shown to occur secondary to less severe injury levels (Meyers *et al.* 1983). Decreasing ventricle size over time indicates an acute phenomenon that may recede over time. This acute phenomenon may be another indicator of MTBI.

The rodent model, as implemented in this investigation, has the capability of enabling genotypic manipulation through targeted deletion (knockout) of specific genes. A previous investigation hypothesized a role of specific genotypes in modulating DBI severity and outcome (Gennarelli *et al.* 2003). In particular, apolipoprotein (APOE) was shown to have considerable influence on brain injury severity in clinical studies (Friedman *et al.* 1999; Liaquat *et al.* 2002; Nathoo *et al.* 2003; Samatovicz 2000). A possible explanation of this clinical correlation is that APOE was shown to maintain structural integrity of the neuron microtubule. Disruption of microtubule function was shown to be a result of DBI that may lead to long-term symptomatology (Huh *et al.* 2003; Li *et al.* 2000). This association may affect susceptibility to brain injury. Future research implementing genetically engineered rodents may provide insight into adverse consequences of certain genetic factors.

The current model has reliably and consistently produced concussion-type DBI in the rodent such that a direct relationship to resulting injury can be quantified. In addition, the model has the ability to expand to other levels of the DBI spectrum by altering biomechanical input. Continued research will focus on issues such as biological implications of transient ventriculomegaly, parametric analysis of biomechanics and development of a relationship between injury biomechanics and resulting pathology throughout the entire spectrum of DBI. Information obtained will aid in the development of protective measures and treatment options for DBI by identifying tolerance criteria at each severity level.

## CONCLUSIONS

Twenty-six rodents were experimentally exposed to coronal plane angular acceleration levels sufficient to induce MTBI. The injury level can be classified as concussion according to recoverable unconsciousness, macroscopic damage, minor histological results and scaled biomechanical data. This experimental model is the first to induce MTBI in the rodent using pure coronal plane angular acceleration, a clinically accurate injury mechanism. The model remains adaptable through magnitude and pulse width modification to explore the entire spectrum of DBI, correlated with resulting gradations of pathology.

## ACKNOWLEDGMENTS

This study was supported in part by the Department of Neurosurgery at the Medical College of Wisconsin and VA Medical Research.

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