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Mechanically Induced Diffuse Axonal Injury: Brain Injury Due to Periodic and Indicial Traumatic Events

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ABSTRACT

Diffuse axonal injury (DAI) is the predominant mechanism of traumatic brain injury (TBI) after sudden periodic and impulsive events. It is common in all TBI, regardless of severity, and is characterized by a shear action induced on axons by surrounding tissue. DAI leads to progressive changes that may ultimately lead to the loss of connections between nerve cells. The slow progression of events in DAI (that continues long after injury) further complicates the effects of transportation-related injuries, but also creates a window of opportunity for therapeutic intervention. A common denominator between these two contradicting factors is the need for a small animal model that will not only allow multiple repetitions of deceleration induced injuries cost effectively, but also enable the drug efficacy trials combat DAI.

Use of small animals is customary in most fields of biomechanics, but in the case of mechanically induced injury, reduction in brain mass implies scaling of kinetic parameters for similarity in inertia forces and strain levels to be maintained. Parameters that have to be "scaled-up or down" for a successful comparison are then ones affecting the order of magnitude of the rotational motion causing brain tissue to sustain shear strains, namely, acceleration and impulse duration. This scaling was implemented in the design of testing platforms used to test more than 250 rodents. DAI was observed on several animals tested.

A test platform was designed and built for periodic and indicial (sudden, single-stroke) events. Constraining adaptors were fabricated to constrain and isolate motion around various axis of motion. Furthermore, motion of the head was isolated from motion of the rest of the body.

INTRODUCTION

Mechanically induced TBI results in a combination injury that includes DAI as well as a contusion, subarachnoid bleeding, penetrating injury, and/or ischemic events. For practical

purposes, the most useful experiments would attempt to isolate purely occurring forms of injury in order to allow their individual study. Mimicking a rare but sometimes occurring pure form of DAI was the focus of this work.

Presently, no small animal model exists that is based purely on DAI, and this may be one of the reasons for the failure of many TBI clinical trials. An injury model that closely mimics the predominant type of traumatic human head injury is essential in developing and evaluating treatments that are specifically directed at DAI. Most importantly, such a model would facilitate the development of an understanding of the level and types of dynamic events and energy transfer required to cause DAI. Its implications on transportation related injury are certain to precede the development of any new near and long-term therapeutic practices.

Background Information

The predominant mechanism of injury in approximately 40%-50% of the TBI admissions in the United States is DAI. A component of DAI is present in all motor vehicle crashes where the patient has lost consciousness. The predominant anatomical sites of injury to the brain are most often the midbrain, corpus callosum, and the pontine-mesencephalic junction adjacent to the superior cerebellar peduncles. The disruption or continuous straining of the neurofilament subunits within the axonal cytoskeleton due to the sustained acceleration and deceleration-induced forces causes the deficits so often noted in these patients. The microscopic features correspond to Wallerian-type axonal degeneration as the axon disintegrates. This is probably due to metabolic disruption from damage to the internal organelles and a loss of membrane integrity. The mechanism of injury is microscopic, and TBI patients have minimal changes noted on computed tomography (CT) or magnetic resonance imaging (MRI) scans.

For many years, DAI has been known to be associated with coma of immediate onset after brain injury. Injury to the tracts leading in and out of the hypothalamus, and/or direct injury to the pituitary stalk and gland results in many of the common medical complications noted after TBI including dysautonomia, hormonal changes, disorders of salt and water metabolism, and altered temperature regulation. Cognitive deficits attributable to DAI include disorder of memory for new information and diminished capacity for information processing. Before therapeutic rehabilitation interventions are considered, there must be considerable forethought given to the mechanism of DAI and the proper design of motor vehicles, a development that can be considered one of the most effective forms of prevention. As a second priority to prevention, an acute awareness of the mechanistic and statistical mechanism of injury, as well as its severity will allow the proper customization of treatment of the TBI patient with DAI.

Approximately 500,000 new cases of TBI are admitted to hospitals in the U.S. annually (Frankowski, 1985, Carus, 1993, and Horn, 2000), and the incidence requiring hospitalization is estimated to be approximately 200-225/100,000 population (Frankowski, 1985 and Carus, 1993). Currently it is estimated that brain injuries account for 12% of all hospital admissions in the United States (Sandel, 1993). Transportation-related injuries of all types are responsible for approximately 50% of the traumatic brain injuries and a corresponding 50% of deaths from TBI within the United States (Lehmkuhl, 1993, NIH, 1999, and Fearnside, 1997). The predominant mechanism in most cases of TBI is DAI (McLellan, 1990). As found by found by pathological examination, approximately 30% to 40% of the fatal head injuries involve DAI (McLellan, 1990 and Bennett, 1995).

More specifically, approximately 52,000 to 56,000 people die each year from TBI (Kraus, 1996 and Max, 1991), resulting in direct costs exceeding $50 billion (Max, 1991). The costs of severe TBI to the individual and family are extremely high (McMordie, 1988). The direct hospital charges alone were $117,000 in 1993 within the TBI Model Systems database (Lehmkuhl, 1993).

Another 30,000 to 44,000 people will survive a severe TBI with Glasgow Coma Score scale (GCS) (Jennet, 1981) score <9 in the U.S. each year, and more than 70,000 to 90,000 will suffer long-term substantial functional loss due to TBI's (Whyte, 1993, Kraus, 1996, and NIH, 1999). It is estimated that DAI is the predominant mechanism resulting in a poor outcome or death in approximately half of TBI (McLellan, 1990). Hence, this translates into 26,000 deaths per year due to DAI, with another 20,000 to 45,000 patients suffering significant physical or neurobehavioral sequelae resulting in functional loss (Jannet, 1975, McLellan, 1990, Kraus, 1996, Whyte, 1993 and NIH, 1999). DAI-related TBI is an extraordinary medical care problem within the United States that is closely comparable in morbidity, mortality and economic loss to HIV infection (McLellan, 1990, NIH, 1999, and Fearnside, 1997). One can see this is an understudied mechanism of morbidity and mortality in the United States as well as worldwide.

DAI was initially described by pathologists (Strich, 1956). These findings were noted to occur in patients who died from a severe TBI following a high-speed transportation injury. Upon histological examination, the patients were noted to have neuronal anatomical changes of diffuse axonal degeneration. It has been stated that DAI is a misnomer. It is not diffuse throughout the whole brain but rather is predominant in discreet regions of the brain following high speed, long duration deceleration injuries. Motor vehicle crashes are the predominant cause of DAI. A component of DAI is believed to be present in all motor vehicle crashes where the patient has lost consciousness (Denny-Brown, 1941 and Whyte, 1993). Research has indicated that DAI is most frequently associated with a coma of immediate onset after brain injury, but the diagnosis of DAI could only be established at autopsy (McLellan, 1990, Pounder, 1997, Duhaime, 1998, and Graham, 1996).

The pathology of DAI in humans is characterized histologically by widespread damage to axons in the brain stem, mid-brain and corpus callosum and is a consistent feature of TBI (Adams, 1977, Adams 1989, and McLellan, 1990). The histological evaluations are usually characterized microscopically utilizing neurofibrillar stains for microglia, which are abundant in the degenerating white matter. Subsequently, the microscopic features correspond to Wallerian-type axonal degeneration as the axon disintegrates indicating a loss of membrane integrity.

The Adams classification (Adams, 1977, Adams 1989) is used in human autopsy material to classify the degree of DAI as mild, moderate or severe. In this classification, mild, or grade 1, is characterized by microscopic changes in the white matter of the cerebral cortex, corpus callosum, and brain stem and occasionally in the cerebellum. Moderate (grade 2) is defined based on focal lesions in the corpus callosum. In severe (grade 3), there are additional focal lesions in the dorsolateral quadrants of the rostral brain stem (commonly in the superior cerebellar peduncle). Indeed, the clinical syndrome of coma without any preceding lucid interval, in the presence of decerebration, and autonomic dysfunction was often ascribed to primary brainstem injury. A GCS score of less than eight generally reflects a state of unconsciousness in which the patient demonstrates no eye opening, does not follow simple commands to move muscles, and has vocalizations which are limited to sounds. Such signs are indicative of severe brain injury (Jannet, 1975, Jannet, 1981, and Whyte, 1993). With regard to level of unconsciousness the GCS may more accurately reflect the amount of mid-brain and brain-stem injury than cortical injury and thereby be a more accurate predictor of outcome with regard to DAI than some other measures of TBI such as the amount of cortical contusion noted on CT scans (McLellan, 1990).

However, it is now clear that primary brainstem lesions do not occur in isolation but rather in association with DAI that usually involves the cerebral hemispheres and cerebellum in addition to the brainstem (McLellan, 1990). In addition to DAI, a high-speed transportation accident can involve multiple mechanisms of TBI including contusion, anoxia, intracerebral hemorrhage and penetrating cerebral trauma. Clearly, as one adds additional mechanisms of injury the worse, the outcome (Whyte, 1993).

Similar injuries are noted among football players, soccer players or hockey players who have a so-called "high-speed collision" with an opposing player (Tegner, 1996 and Powell, 1999). It is of interest that these sports place athletes in positions where they may be exposed to high speed collisions with other players. They suffer many of the same medical and neurocognitive deficits as those who have been involved in a high speed motor vehicle crash (MVC) (Tegner, 1996 and Powell, 1999). Permanent deformation may involve other tissues of the body. One classic example is the arterial vessel dissections associated with motor vehicle crashes. More recently it has been demonstrated by Kakulas (1999 and 1999) that DAI is a significant mechanism in cervical spinal cord injury from high speed MVC when there has not been any demonstrated cervical displacement. It is likely that the mechanism of injury is similar to that of TBI. Furthermore, it would offer an explanation to the fact that there is a close relationship between cervical spinal cord injury (SCI) and TBI.

It is difficult to correlate the severity of injury in humans with animal models. The Glasgow Coma Scale (Jannet, 1981), the Disability Rating Scale (Rappaport, 1982) or the length of post-traumatic amnesia (Bishara, 1992) cannot be used to assess the outcomes of animals subjected to a TBI. However, methods to measure balance, spatial memory, and learning acquisition in animals are available. Yet, despite the number of experimental neurotrauma animal models elucidated to date (Lightall, 1994), there has not been a reliable, cost-effective small animal model to mimic mild, moderate and severe DAI and that can cost-effectively provide large statistical samples. Maxwell, Povlishock and Graham (Maxwell, 1997) state that in some of the current animal models of DAI, axonal injury does not occur in the parasagital white matter or corpus callosum, which are the most frequent sites of occurrence in humans.

The two most common animal models of human head injury are the fluid percussion impact acceleration or the weight-drop method. Fluid percussion models produce brain injury by the rapid injection of saline or blood into the closed cranium either at the midline (McIntosh, 1984) or laterally (McIntosh, 1989). Unfortunately, these are not ideal models of human diffuse axonal injury. The models more closely replicate some of the features of contusion and/or subarachnoid hemorrhage. The impact acceleration (Lighthall, 1988) and the weight-drop methods (Shohami, 1994) both involve creating an indentation into the brain. Although a modicum of DAI occurs at the periphery of the lesion in these models, the DAI induced by this model is concentrated in different areas and involves a disproportionately smaller volume of the brain than in humans.

The model that most closely approximates human DAI has been a primate model of DAI in which monkeys were exposed to acceleration and deceleration in the oblique, lateral and sagittal planes (Gennarelli, 1982). While the injury induced was similar to that of humans, primate models are prohibitively expensive. More recently a model of DAI in the rat has been developed (Meythaler, 1998) which creates the cellular morphological characteristics of DAI. While the areas of the brain may be somewhat different than in the human, the cellular injury is widespread and allows for research into the subsequent cellular and biochemical cascade that interventions may be successful in alleviating.

Foundation for Hypothesis and Experiments

The quest for a representative Head Injury Criterion (HIC) with reproducible and reliable results has brought about several concepts that have helped quantify the severity of injury and its relationship to specific vehicle models. Despite the relative acceptability of HIC (FMVSS 208) and an even enhanced possibility for improvements through recent Finite Element Models in quantifying human injury causes, and the establishment of acceptable standards for deceleration rates, none of the models is applicable to a small animal. Defining a HIC appropriate for small animals would allow a relative measure for easy comparisons between animal and human injury, and the establishment of a testing protocol for low cost testing. In the absence of such a measure,

one can perform some "order of magnitude" estimates, in order to determine kinematic parameters necessary for animal testing. Such estimates should take into consideration several factors, including:

1) Geometric attributes, topology, and orientation between corresponding neurological structures such as the corpus callosum where DAI is easily detectable,
2) Differences between the human and animal brain masses,
3) Dynamics of the acceleration or deceleration spike,
4) The similarity between the rotational motions that become the cause of shear strain.

More specifically, in considering the geometry of the human brain one has to take into account the near-spherical shape of the mid-surface defining the corpus callosum, and its location and orientation relative to the brain stem and spinal cord, that can function as inelastic foundations. Furthermore, the positioning of the cerebrum as a lamped mass that encircles the corpus callosum has to be properly considered and matched with an animal with a similarly structured complex.

Because of mass differences, driving accelerations and decelerations have to be selected based on the scaling factor:

$$A_{animal} = A_{human}\left(\frac{m_{human}}{m_{animal}}\right)^{2/3}, \tag{1}$$

where A_{animal} is the linear or angular deceleration of an animal brain,
A_{human} is the linear or angular deceleration of a human brain,
m_{animal} is the mass or inertia of the animal brain, and
m_{human} is the mass or inertia of the human brain.

This scale factor considers the effects of a constant angular deceleration on smaller and larger brain masses. It is, though, limited to a constant value of acceleration or deceleration, or the assumption of average deceleration over a range of time within the total deceleration pulse.

Similarly, in considering the acceptable width of a pulse, one has to resort to an order of magnitude estimate. The assumption for this estimate is based on the fact that, angular deformations that cause equal shear strains in the animal and the human are the same order of magnitude or equal. Thus, for the angular displacements a_h and α_a to be equal,

$$a_h \frac{t_h^2}{2} + w_{0h} t_h + \Theta_{0h} = a_l \frac{t_a^2}{2} + w_{0a} t_a + \Theta_{0a} \tag{2}$$

where a_h and α_a are the angular accelerations for human and animal brain masses, respectively,
t_h and t_a are the time values for human and animal brains to cover the same rotation angles Θ_h and Θ_a, respectively,
ω_{0h} and ω_{0a} are the initial velocities for the two masses, and
Θ_h and Θ_a are the initial angular displacements for the two masses.

For zero initial conditions, designating a initial moment of an experiment, equation (2) reduces to

$$a_h \frac{t_h^2}{2} = a_l \frac{t_a^2}{2}. \tag{3}$$

After application of Equation (1) and an implementation of the assumption that "similar strains are derived from similar large-scale angular rotations" Equation (3) reduces to

$$\frac{t_a}{t_h} = \left(\frac{m_a}{m_h}\right)^{1/3} \tag{4}$$

For a rodent with an approximate brain radius of 1/8 of a human and a brain density similar to a human, the expected ratio of accelerations predicted by Equation (1) is 64. Similarly, the expected ratio of relevant deceleration pulse widths resulting from Equation (4) is 0.125. Thus, a deceleration has to increase 64 fold, while its duration has to reduce to one eighth.

Applying the two factors (64 and 1/8) in HIC to illustrate the differences between human and small animal injury criteria, can result in an illustration of how incomparable HIC values for the human and the small animal brain are. In fact, the ratio of criteria corresponding to the two species would be a constant coefficient of 32,768. Despite the fact that HIC was not used as a reasonable guide for design of test equipment, it became obvious that the method would require one order of magnitude faster pulses and nearly two orders of magnitude higher acceleration levels than the 60-100 G's expected in MVC.

METHODS

A testing platform was designed and built with the capability to deliver injury to small animals that undergo motion constraint along specific degrees of freedom. Figure 1 illustrates the main components of the system in an exploded view. In the translating periodic configuration of the machine it consists of a planar slider-crank mechanism. In the angular periodic configuration it also becomes a spherical 4-R mechanism.

Gear 1, the driver link (crank), is slotted, and the pivot mounted in such a manner that the arm length of the gear can be increased or reduced to alter the amplitude of displacement or rotation. The arrow on the photograph of Figure 2 indicates the direction of adjustment. Gear 2 is supported to the end of an input drive-axle, and provides motion to the system. The drive axle passes through hole (a) on cover plate 3 and plugs into an 1.125 KW infinitely variable speed gear motor. Slider 4 moves within rails 5 and carries hole (b) inside which containers with foam padding are inserted to support anesthetized animals. Slider 4 is replaced by an oscillating arm and rails 5 replaced by a pivot support when the device is used in a rotating configuration. Four legs 6 of similar length and solid cylindrical shape are mounted through concentric bolts to an 1 cm thick plate that is permanently fixed to a table of a milling machine. Spacers 7 are used to create proper working clearances for moving components and a containment space for lubricants.

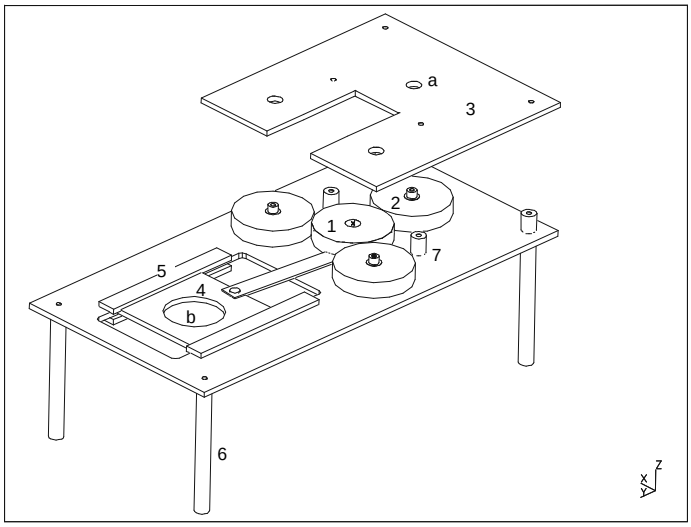


Figure 1: Exploded View of Planetary Gear Drive Used in Periodic Loading Device.

Figure 2: Photographic View of the Amplitude Adjustment Feature on the Testing Platform.

The testing platform was designed with a quick return feature in order to increase the forward deceleration component at the expense of the acceleration component. Within each period of revolution, two events occur, each consisting of one acceleration and one deceleration load. The machine starts from rest, accelerates the container to a maximum speed, decelerates to a zero velocity, and accelerates backwardly towards the initial position where it decelerates to zero at the initial rest position. The quick return feature results in a deceleration peak that is 44% higher than the acceleration peak. The translating platform is uses for testing in purely translating motion with a driving frequency of up to 32 Hz, resulting in a deceleration peak of 180 G's and an acceleration peak of 125 G's. The half-period of each event (one acceleration one deceleration) is 15.6 milliseconds or a complete event period (2 accelerations two decelerations) of 31.2 milliseconds. Figure 3 illustrates the acceleration history for one complete cycle.

Of the periodic motion characteristics of this platform, one that is worth noting is the nearly-linear dependence of acceleration/deceleration peaks on the amplitude. Increasing amplitude resultes in a nearly proportional increase in the deceleration peaks for a particular setting. Figure 4

illustrates this relationship. This behavior is expected in rotors, but in a quick return mechanism, a more drastic deviation from a linear trend could be expected. The second observation is that the platform only produces deceleration peaks 2-3 times, but not two orders of magnitude higher than the ones commonly found in MVC. Furthermore, the width of the deceleration event is about one half to one third of the one in MVC.

The periodic application of the load cycle was considered as a remedy to the lower than desired peak and greater than desired pulse duration. It was expected that fewer axons would be subjected to high strains, therefore repetition of the event was expected to increase the number of damaged axons to a statistically significant number, so that they can be easily detected by histology techniques and to cause behavioral deficits. Tests were performed for a wide range of numbers of repetitions, to assess the effect of the cumulative damage. The lowest number of repetitions was 50 and the greatest was 900.

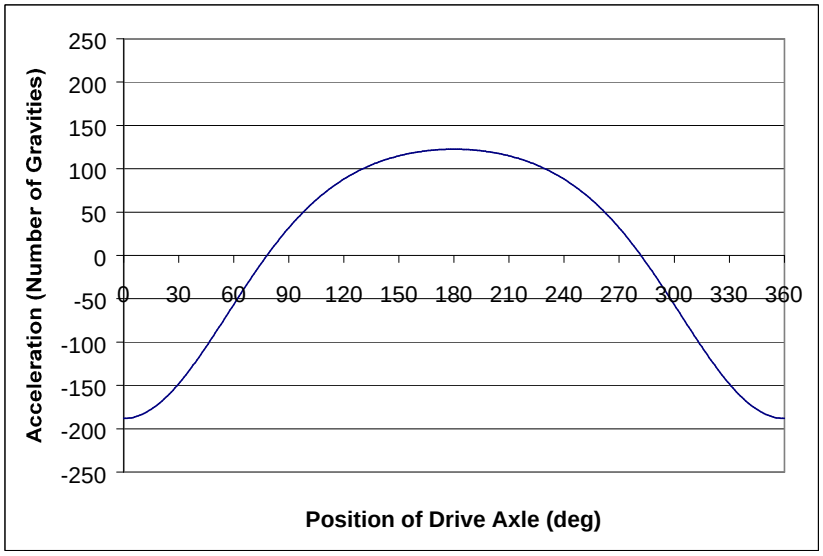


Figure 3: Acceleration Deceleration History Through One Cycle of Periodic Loading.

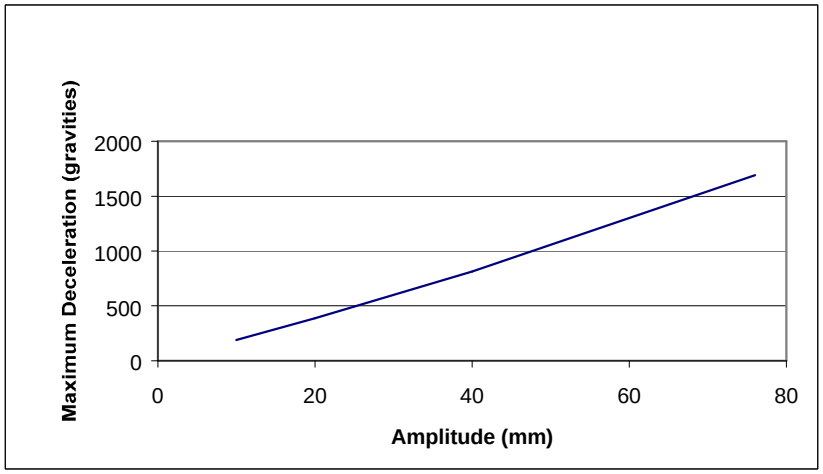


Figure 4: Nearly Linear Variation of Deceleration Peak With Amplitude.

The power drive of the platform is a contributor to flexibility needed in conducting multiple types of experiments, but also to the physical limitations of its dynamic response. One of the critical questions to be addressed, is "how many cycles does the input axle undergo, before it reaches steady-state angular speed." This question was addressed in a drive qualification phase, and is repeated periodically during calibration experiments. Prior to performing experiments on small animals, the machine is "calibrated" by using a high speed camera to ensure that it can reach steady-state speed within the first 5 cycles of revolution. The error caused during this "ramp-up" response is affecting 10% of the testing time for the test with the smallest number of repetitions, and 0.56% of the test time for the greatest number of repetitions. Figure 5. illustrates the ramp-up response of the power drive during a 22 Hz test, the highest speed in which it was used in animal test.

During a range-finding series of experiments with the test platform, high-speed video recording was used to investigate whether the acceleration and deceleration peaks could be increased by constraining only the body of the animal to move with rigid displacement. The head was allowed to freely pivot about the neck. Figure 6. illustrates the absolute trajectory of the animal's eye, while Figure 7. illustrates the same displacement relative to the body constraint-container. This test was performed at 11 Hz axle speed.

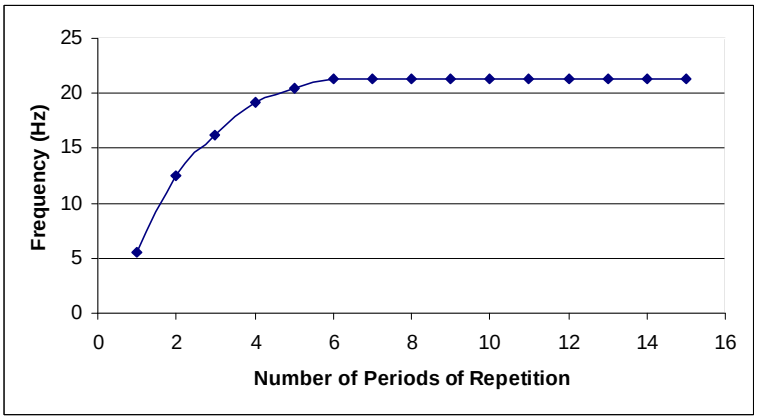


Figure 5: Results of a Ramp-Up Qualification Test Performed on the Power Drive of the Test Platform.

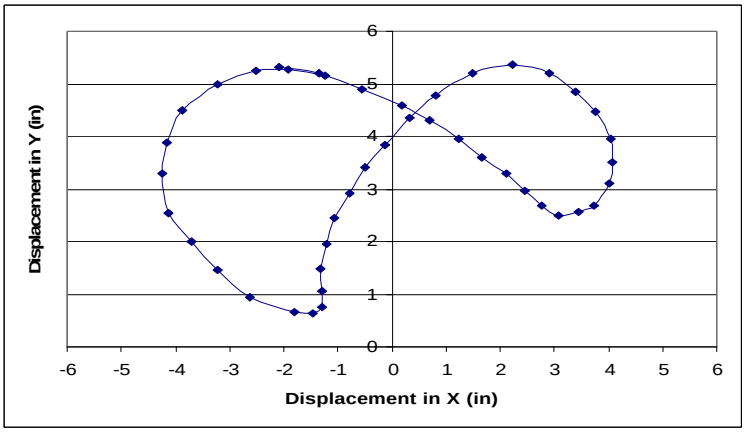


Figure 6: Absolute Trajectory of an Animal's Eye During Test With Unconstrained Head.

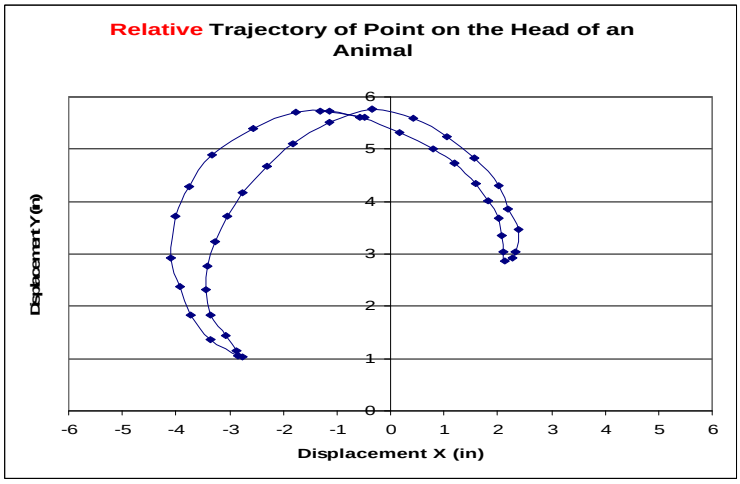


Figure 7: Trajectory Relative to Rigid Displacement of Slider on Test Platform.

Based on absolute displacement of the head, the velocity and acceleration magnitudes can be calculated from imaging information after minor smoothing of the data. The peak value of deceleration for this particular test is 260 G and the peak value of acceleration is 210 G. These peaks are nearly twice as high as the rigid body motion of the test platform would impose if the animal was fully constrained to move with the support tube. Also notice that the platform cannot be driven at substantially higher frequencies than 11 Hz with the head mass not traversing though a critical speed where it transitions to a different mode of oscillation with the head being nearly stationary while the body moves.

A modification of the test platform through a replacement of the slider with a pivoted rocker allowed the angular oscillation of an animal through the motion of the output link of a spherical 4R mechanism. The photograph in Figure 8. displays this configuration of the test platform. The flat cup (A) can be removed to accommodate the conventional tube that rigidly supports an animal. It can also be supported on the rocking output link with a mouthpiece and a support cone (B) for the maxillary bone. The support cone is threaded onto a mouthpiece that carries apertures inclined by about 45 degrees, through which the front teeth pass for alignment, repeatable locating, and support purposes. When the oscillating link is equipped with the tube, experiments can be performed with the entire animal undergoing acceleration and deceleration through periodic motion. When the mouthpiece is installed, experiments can be performed with the body stationary and the head only moving.

During range-finding experiments it was discovered that at operating the platform at 22 Hz induces adequate acceleration and deceleration peaks for multiple organs to catastrophically fail prior to any appreciable brain damage. Obviously, the near 20 cm length of the average animal's length allowed linear shock of destructive value to most internal organs, but not quantification investigations were conducted to derive finite conclusions. The experiments, though, were convincing on the need for testing devices capable of subjecting the head to shock without the entire body moving. Some qualitative dissections showed that most internal organs including the heard muscle suffered sever lacerations. The Abdominal Aorta indicated a tendency to fail even at moderate shock levels. Some motion constraints could also be derived without quantitative assessment of internal organ failures. For example, constraining the rotation of the brain mass about the center of gravity became an obvious and necessary restriction. For that reason, the mouthpiece was designed such that the pivot axis (C) passed through the center of gravity of the brain, located 5 mm behind and 5 mm above the ear canal. Both of these rotational motions were periodic, and constrained within the sagittal plane. The body of the animals was constrained in an

inclined tube (D) such that the angle of oscillation was approximately divided to a positive and a negative half angle.

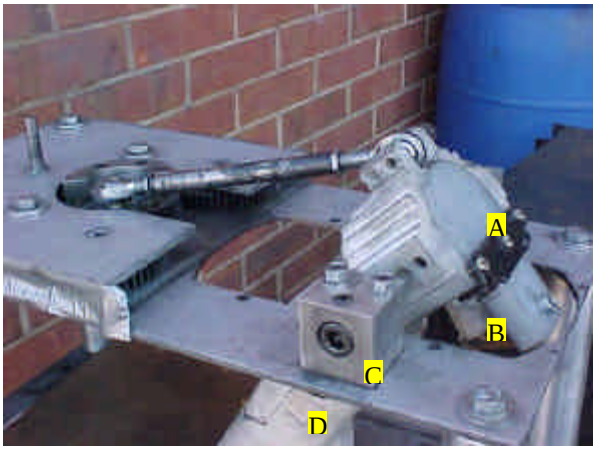
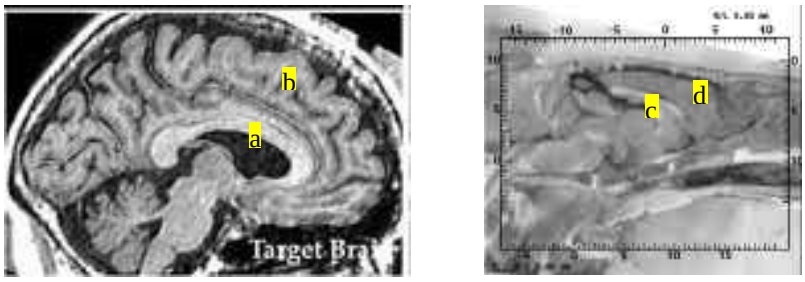


Figure 8: The Test Platform in the Periodic Angular Oscillation Configuration.

One additional configuration of the test platform was developed in order to enable periodic loading within the coronal plane. Motivated by the fairly flat geometric configuration of the corpus callosum in the small brain relative to the rounded one in the human, range-finding experiments were performed with periodic acceleration/deceleration experiments, these tests were intended to cause relative motion of the main mass of the brain about the center of gravity. Figure 9(a) shows a radiogram of the human brain, and Figure 9(b) a section through a small animal brain (rodent). Designation (b) on the human brain illustrates the cerebrum surrounding the corpus callosum (a), and similarly designation (d) illustrates a cerebrum in the rodent that is more highly conformal to a narrow and longitudinal cranial cavity. Coronal periodic motion was not found to cause sever injury even though tests were performed at peak deceleration values similar to the ones performed in the sagittal plane.



(a) (b)

Figure 9: Section Trough Corpus Callosum (a) Human, (b) Rodent.

Periodic application was intended to cause linear accelerations of up to 400 G and angular accelerations of up to 3000 rad/sec^2. The main goal was to increase the statistical incidence of axonal injury. Single acceleration/deceleration events were also investigated at higher magnitudes and with narrower bandwidths. Snapback excitation through spring-loaded cam and linkage

mechanisms was used to generate translating as well as angular acceleration peaks that far exceed the fourfold increase accomplished with the periodic platform.

Figure 10 illustrates the snapback excitation configuration of the testing platform. The mouthpiece (C) was transfered to a lightweight arm (B) pivoted about hinges (C). Two torsional springs were used to actuate the device, at a maximum acceleration of approximately 200,000 rad/sec^2 and a deceleration rate of the same order of magnitude, but that has not yet been quantified. Bandwidths smaller of about 2 miliseconds have been indirectly estimated. Exact measurement of time and acceleration deceleration was not possible with available equipment, and it is believed possible only via high speed video with high framing rates.

An additional 2 configurations of the device have been developed based on linear spring snapback excitation. In both cases, calibrated springs were used to apply initial forces in excess of 3 KN to lightweight links that induced controlled motion on a mouthpiece. Each of these configurations was constrained to motion within the coronal or sagittal plane. In all spring loaded devices, multiple springs were used in testing in order to study the effects of fast-narrow aceleration deceleration curves.

A testing protocol was established according to standard animal care and animal testing facilities. Range-finding experiments were performed with a series of anesthetics to ensure that animal loss to apnia or overdosing was limited. All experiments were performed by the investigators in the presence of a veterinarian with primary appointment in the animal care facility and clinic.

Animals were behaviorally assessed for indications of DAI before being sacrified and examined histologically. The three tests to which animals were subjected, monitor their progress after injury for up to 10 contiquous days. The three tests most broadly used were the beam balance test, the incline plane test, and the Morris Water Maze. Several standard stains were used and experimental ones developed for observation of swollen axonal bulbs and disrupted axons.

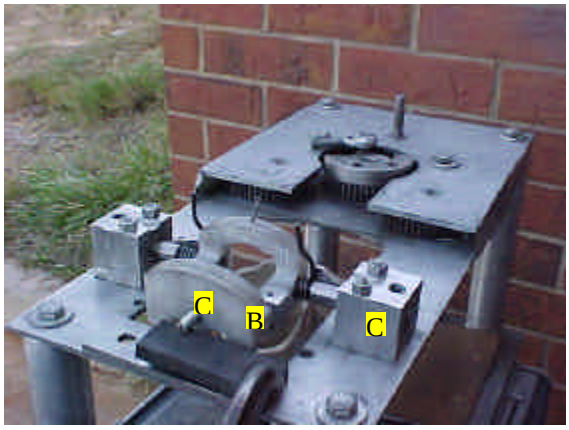


Figure. 10: Snapback Excitation Configuration of Testing Platform.

The animals were anesthetized then surrounded in foam padding and placed in the testing device. The foam padding was fabricated by the UAB Department for Prosthetics and Orthotics, from materials used in prosthetic limps. During range finding experiments that assessed anesthetics, Pentobarbitol was used as the anesthetic. The outcome was not as repeatable as the investigators expected, resulting in a variable duration of unconciousness even within controls. Halothane (2% in 1.5 l/min O2) was foun to provide more reliable measurements of the duration of unconsiousness, and subsequently implemented for all tests.

Rats were behaviorally tested at day 1-5 after injury using 3 functional tests. The Morris Water Maze was used to evaluate spatial learning. In this test, the length of time for the rats to find a clear platform submerged below water level is measured from the 4 quadrants of circular pool. In the von Euler beam test, performance is measured in successively narrow beams (7.7 -1.7 cm beams). The inclined plane test measures the greatest angle that a rat can maintain its position for 5 seconds. Rats were sacrificed in 4 days, 1 week or 2 weeks after injury and the brains and eyes were sectioned for histological analysis. Half of each brain was cut on a freezing, horizontal-sledge type microtome at alternating thickness (15 μm or 30 μm). The other half of each brain was cut at 10 μm on the cryostat or at 2-3 μm after JB-4 (plastic) embedding. The following stains were used on the sections: hemotoxylin & eosin, Richardsons, toluidine blue with pararosaniline, and modified Bielschowsky silver stain. Silver stain and hemotoxylin and eosin are reported to be particularly useful after DAI.

Immunohistochemical staining was also performed using several antibodies because the swellings and retraction bulbs following DAI are known to be immunopositive for ubiquitin, neurofilament and amyloid precursor protein. For the immunohistochemistry, sections or slides were washed 3 times in phosphate-buffered saline (PBS, 0.1 M phosphate buffer, 0.9% saline, pH 7.4). Blocking was accomplished by using 10% normal serum in PBS with 0.3% triton-X. For the neurofilament staining, goat serum was used, and rabbit serum was used for the ubiquitin and the APP. Sections were placed for 2 days at 4 degrees C in a 1:2000 dilution of the following primary antibodies: Sigma rabbit anti-neurofilament 200, Chemicon goat anti-amyloid precursor protein, Chemicon sheep anti-ubiquitin antibody, Chemicon rabbit anti-neurofilament 200, Chemicon rabbit anti-neurofilament 150. Control sections were not incubated in primary antibody but were processed with all of the remaining steps. After washing 3X in PBS, sections were placed in horseradish peroxidase conjugated to affinity purified secondary antibody (Chemicon goat anti-rabbit or Chemicon rabbit anti-sheep or rabbit anti-goat). After washing 3X in PBS, sections were reacted using Pierce enhanced DAB substrate kit.

RESULTS

This investigation has led to a large number of experiments and has pointed to the feasibility of small animal testing in inducing DAI in small animals. Despite the success of several modes of loading to induce the injury, it is considered essential that other low-cost, small animals are tested for an increased sensitivity, and improved repeatability. More specifically, the results have demonstrated a characteristic insensitivity of the rat to shock, and not all tests were successful in producing DAI.

Device (A) (Translating Periodic Motion With Unconstrained Head) Reciprocation with this configuration proved that a large proportion of the rats to die from the injury (19 out of 38). Mortality rate in this dataset is somewhat elevated by range-finding experiments conducted to assess the effects of anesthetics. Deaths, though, did occur with the most successful anesthetic, and believed to be the cause of severe head injuries that sometimes occurred before the animal was removed from the device. Certain frequencies and certain numbers of even repetitions produced more behavioral deficits. In certain cases, the rats appeared completely normal based on appearance but could not balance on a beam that was wider than the rat. Neuropathological changes were also prominent in these animals. Figure 12(a) and (b) show photomicrographs at two different magnifications and with two different stains. Arrows point to examples of axonal swellings compared to axons from uninjured brains found in the dorsolateral brainstem.

Device (B) (Angular Periodic Oscillation of the Entire Body) In this configuration, the animal was supported by being confined in a compartment that undergoes motion along a single rotational axis, but the position of the animal is adjusted to induce a gradually increasing linear component of acceleration relative to the rotational component. The motion induced allowed rotation of the body as well as the head. The pivot around which the animal was oscillated passed through several anatomical regions. A position designated as (0) allowed an extension of the pivot axis to pass through the center of gravity of the brain. A position designated as (+1) allowed the axis to pass through a location 1 inch below the center of gravity, close to the neck. Position (-1) allowed the axis to pass over the head.

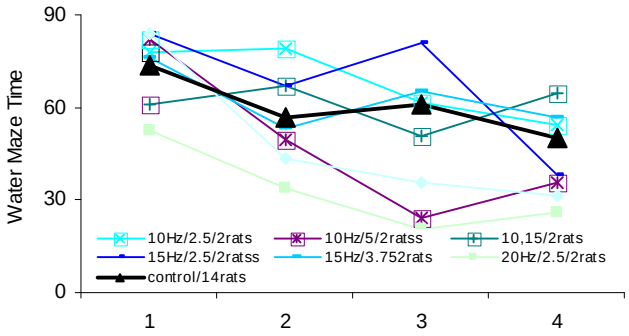


Figure 11: Water Maze Performance of Animals Tested in Device (A) Over a Period of 4 Days.

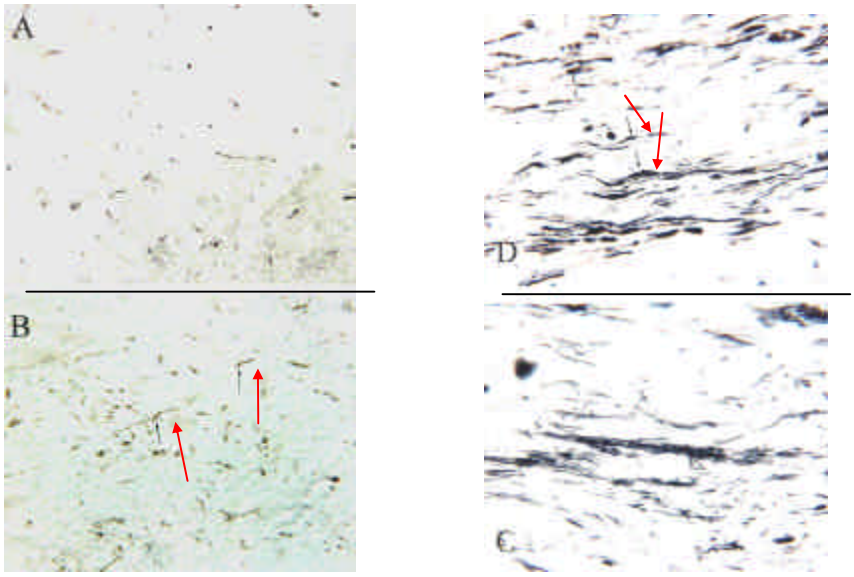


Figure 12: Photomicrographs of (A, C) Uninjured Brain, (B, D) Injured Brain; Dorsolateral Brainstem.

Device (B) resulted in the greatest number of deaths, and an unreasonably low number of brain injuries. Most animals died after internal organs disintegrated under unexpectedly low levels of acceleration and deceleration peaks. In most animals, the lungs survived the shock, while the heart muscle and abdominal aorta suffered significant damage, as did other internal organs. Subsequent histological examination and observation for identification of brain contusions and

DAI proved that none of the dead animals suffered brain injury. Animals that survived the test for subsequent testing were rotated in positions where the pivot point passed through the chest, rather than the center of gravity of the brain. Figure 13 illustrates the outcome of testing in the water-maze from various tests.

The first indication from this sample of injured animals is that at higher frequencies, animals tend to not sustain as severe an injury as at somewhat moderate frequencies. From Figure 13 it is clear that anesthetized controls performed only slightly differently than animals subjected to 100 events. The most profound differences were encountered in animals that had been injured with acceleration deceleration events as wide as 30-50 miliseconds, but remained fairly uninjured below 25 miliseconds. Obvious is also the fact that their deficits were dependent on time, and their ability to learn diminished with time.

Device (C) (Angular Periodic Oscillation of the Head, While the body is Held Stationary)
The body was held stationary in a cylindrical compartment filled with polyurethane foam and inclined by an angle of 60 degrees from the horizontal. The head was supported on a metal structure and aligned through the front teeth in a repeatable manner. The maxillary bone was compressed by a hollow support cone against the alignment mouthpiece. The head underwent a single degree of freedom of motion about a pivot located 5 mm behind and 5 mm above the animal's ear. This particular location was empirically determined to be the center of gravity of a rat's brain by inserting pins through the cranium of sacrificed animals, and subsequent removal and examination of the brain.

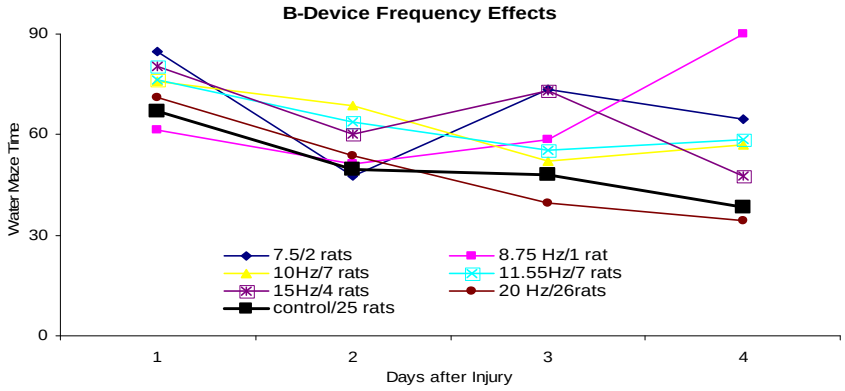


Figure 13: Water Maze Performance of Animals Tested in Device (B) Over a Period of 4 Days. All Tests Performed at the Time Required for 100 Events.

Device C resulted in substantial deficits in most animals and at most frequencies. One remaining question investigated through this sample, is whether the number of repetitions created increased behavioral deficits that are independent of frequency (independent of acceleration deceleration level). This question was addressed with a sample of tests and controls for a period of time extending to 10 contiguous days. As illustrate in Figure 13. the analysis of variation of learning ability does not conclusively determine that animals are receiving an injury that is independent of acceleration. The level of injury indeed varies among various test intensities, but no artificial side effects are created by numbers of repetitions that are independent of the shock event.

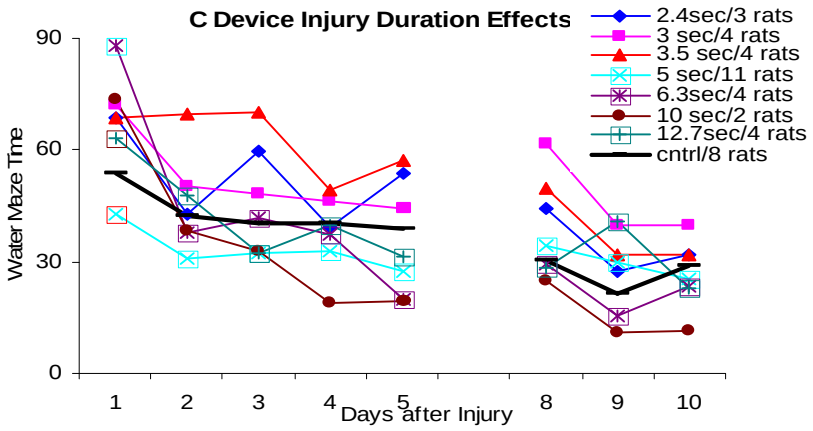


Figure 13: Water Maze Performance of Animals Tested in Device (C) Over a Period of 10 Days. Tests Were Performed at Various Frequencies and Various Numbers of Periodic Repetitions.

Device (D) (Angular Snapback Oscillation of the Head, While the Body is Held Stationary) The body was held stationary in a cylindrical compartment filled with polyurethane foam and inclined by an angle of 30 degrees. A mouthpiece attached to a lightweight link was preloaded with torsional springs and displaced upwardly by 60 degrees. When released, it accelerated under the influence of the spring until intercepted by a Buna Rubber cushion of 1 cm thickness. The subsequent deceleration cycle occurred at an instant that the torsional springs were nearly at nearly zero pre-strain torque.

Despite the short duration of the shock, (about 2 milliseconds) a substantial deficit was induced through the single event injury. As shown in Figure 14, injured animals required more than twice the time that anesthetized controls did in completing a test.

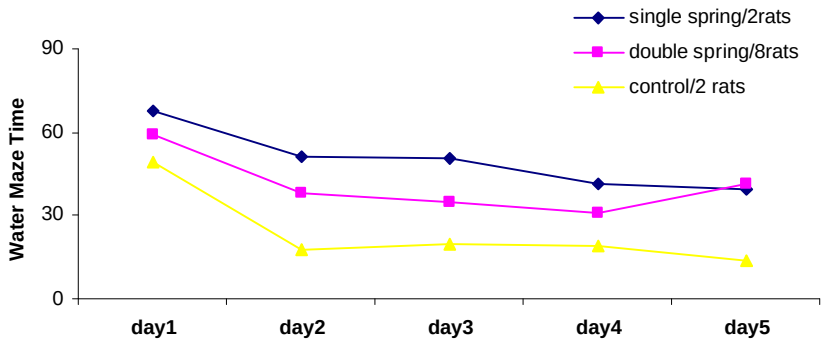


Figure 14: Water Maze Performance of Animals Tested in Device (D) Over a Period of 5 Days. Tests Were Performed at Two Values of Input Torque Through Addition of a Second Calibrated Spring.

Devices (E and F) (Angular Periodic and Snapback Oscillation of the Head, in the Coronal Plane) The body was held stationary in a cylindrical compartment filled with polyurethane foam and tested with periodic motion as well as snapback excitation. Snapback devices were pre-loaded with linear springs and induced acceleration deceleration strokes of less than 2 milisecond

duration. The deceleration cycle did not initiate until the springs had lost contact with the linkage, so inertia was the only actuating force at the moment of impact with a Buna Rubber cushion.

Despite relatively higher acceleration deceleration peaks, no measurable injury was recorder by behavioral or histological techniques. Apparently, testing within the coronal plane does not result in the severe results that testing within the sagittal plane. A total of 24 animals and anesthetized controls were tested with no single death or injury recorded.

CONCLUSIONS

As the results have shown, DAI can indeed be mechanically induced to a small animal, despite the narrow bandwidth required for an acceleration deceleration pulse to reach high enough levels to cause injury. Clearly, there are frequencies that are more likely to cause injury, indicating that faster events do not necessarily cause more damage, even if peak values are higher.

- Both periodic and snapback excitation methods can be used within a sagittal plane to cause injury but neither was found effective within the coronal plane.
- Neither periodic, nor snapback excitation was found to cause contusive injuries.
- Sagittal plane sections of eyes revealed profound changes to the eye, especially when device (D) was used with moderate acceleration values. This Observation was not pursuit and quantified, and remains a future goal to pursue. It is interesting to observe, though, that not all acceleration deceleration tests resulted in structural changes within the eye.
- The brain topology of the rodent used in this study is substantially different than the human, and perhaps a small animal with more comparable structure should be used for future testing.

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DISCUSSION

PAPER: **Mechanically Induced Diffuse Axonal Injury: Brain Injury Due to Periodic and Indicial Traumatic Events**

PRESENTER: ***Jean Peduzzi, University of Alabama***
Evangelo Eleftheriou, University of Alabama

QUESTION: I have an infinite amount of questions, but I'll restrain myself to one. Okay. Sagittal versus coronal plane. Did you have the same kinematics that you measured, linear and angular?

ANSWER: We performed that particular test in, at levels that were extremely high but not at the low levels. The same kinematics being angular, displacement, the level of velocity, the level of acceleration.

Q: Because what I see–I don't actually see that when you attach the neck you have the angle and kinematics, especially in coronal plane.

A: The neck is irrelevant to the test because we only displaced the head. The body, the rest of the body is stationary.

Q: Oh, you forced the head to rotate.

A: Yes.

Q: Oh, okay. Why do you think that is?

A: We displaced the head. The rest of the animal is stationary.

Q: Why do you think in coronal plane it's less?

A: Because of the encapsulation of the brain. That particular animal has a configuration in his brain where there is extreme site support. If there is a sheer action happening within the rodent's brain, it is one that you can describe as a fountain lobe; in other words, a very constrained tube double-wall support, an internal flow that is highly accelerated relative to the boundaries. While in the human, it would be the one-sided support.

Q: Okay. Thanks. I'll ask my, the rest of my questions late.