

Characterization of Traumatic Brain Injury Mechanisms and Development Studied Via Immunohistochemistry and Magnetic Resonance Spectroscopy

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ABSTRACT

Traumatic brain injury remains a serious issue in the United States despite a large research effort over the years. Uncertainty regarding injury mechanisms and cascades drives the current study, which seeks to address many unknowns. The first phase of this study focuses on determining a direct relationship between metabolites via magnetic resonance spectroscopy (MRS) and axonal damage assessed by immunohistochemistry in a Göttingen minipig in vivo model. Two injury devices were designed and fabricated. One imparts rotational acceleration in the median plane while the other imparts purely translation acceleration. The animals (n=15) undergo baseline 7T MR scans prior to injury, immediately post-injury, and twenty-four hours post injury. MRS is performed within ~200 mm³ voxel placed in the genu of the corpus callosum. Relevant metabolites include glutamate, N-acetylaspartate, myoInositol, creatine, and lactate. No clear trends were found for any of the metabolites for either time point for these few tests. Further testing is being conducted to see the meaning of the metabolite differences in terms of underlying damage characterized by immunohistochemistry. This work will be adapted to characterize longitudinal TBI development. High-speed biplane x-ray studies looking at head kinematics, brain response, intracranial pressure, and brain injury will support the development of an FE model of the minipig head. Once validated, the model can be used to scale graded injury metrics for prediction of brain injury in the human. Ultimately, a new head injury criterion will be developed for better prediction of the potential for traumatic brain injury.

INTRODUCTION

Traumatic brain injury (TBI) continues to be a leading cause of death and disability in the United States, accounting for 30.5% of all injury-related deaths ("Traumatic Brain Injury in the United States" 2002-2006). Despite the large body of research dedicated to head injury, there is still a great deal of uncertainty regarding the mechanisms and pathogenesis involved. Some of the classic experimental brain injury models are fluid percussion (Lindgren and Rinder 1966), inertial rotation (Unterharnscheidt and Higgins 1969), controlled cortical impact (Lighthall, 1988), and weight drop (Marmarou et al., 1994). These models have limitations such as some of the animals used, especially rodents, do not have brain morphology similar to humans, and some models do not use physiologically relevant injury methods.

This study seeks to answer many of the unknowns surrounding TBI. Specifically, a mild traumatic brain injury threshold needs to be established that relates input kinematics to underlying damage and metabolite concentration changes. A purely linear impact is being used to avoid brain stem injury by limiting neck flexion. After short term injury development is characterized, longitudinal development will be observed up to two weeks after injury. Sibling studies will be used to relate input kinematics, neuronal damage, and metabolite change in one animal to the relative brain/skull kinematics in the other. High speed bi-plane x-ray with neutral density targets in the brain will be used to track relative motion. Ultimately, the current study will produce an FE model of the minipig head that can be used in conjunction with a model of the human head, such as SIMon, to scale graded injury metrics relating linear and angular head accelerations to levels of neuronal damage to be expected in the human. A new injury criterion will be developed, or perhaps an existing modified, to relate impact conditions to the level and risk of injury.

In order to address criticisms of previous experimental head injury models, a Göttingen minipig is being used. As shown in Figure 1, the brain of the Göttingen minipig is similar to a human's in terms of exiting angle of the brain stem into the spinal cord and brain morphology. It has a pronounced falx cerebri and distinct hemispheres, which do not exist in rodent models, and even other minipig models. The brain also has well developed convolutions. Further, the skull of this minipig is not as thick as other swine models.

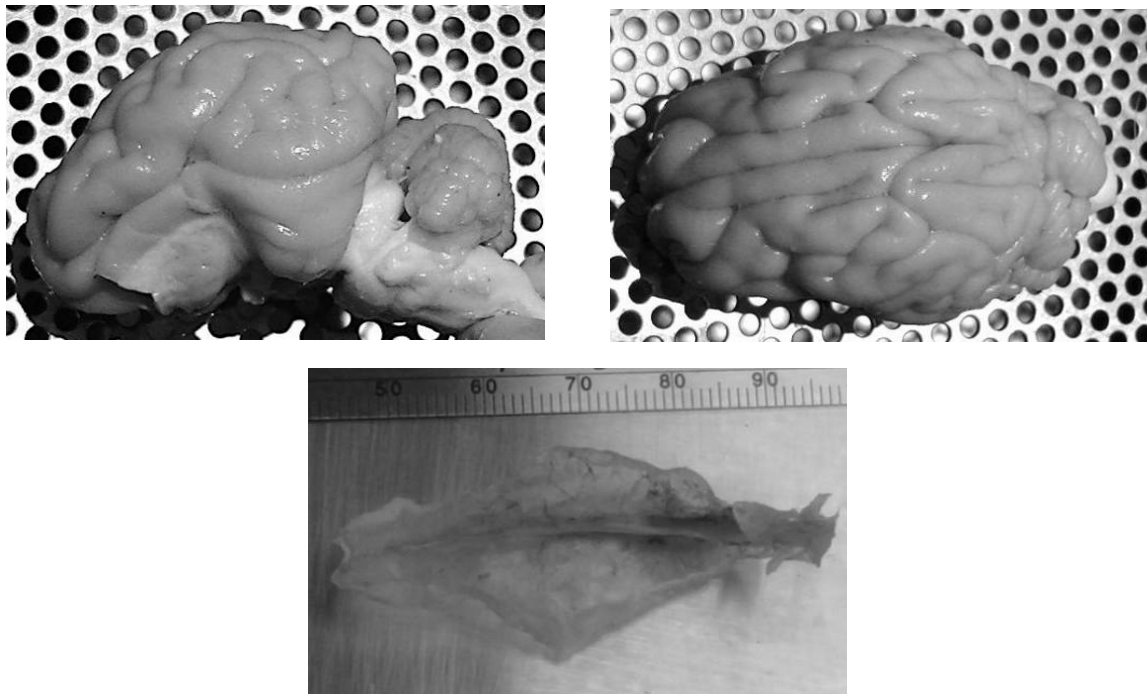


Figure 1: Göttingen minipig brain after perfusing with 4% paraformaldehyde (top). Falx cerebri (bottom).

The first phase of this study focuses on determining a direct relationship between short term neuronal damage as assessed using magnetic resonance spectroscopy (MRS) and immunohistochemistry in the Göttingen minipig model.

METHODS

Input acceleration thresholds:

Thresholds were estimated for the Göttingen minipig based on the literature. For the angular acceleration threshold, Ommaya 1984 (Ommaya, 1984) was referenced because proposed tolerances for rotation in the sagittal plane for sub-human primates were developed and scaled to the human. Brain density and material properties were

assumed to be equal. Both brain radii and brain mass scaling were done in order to create a range for the minipig. The calculated thresholds for AIS 3 are shown in Table 1.

For linear acceleration, the current Head Injury Criteria (HIC) threshold for the National Highway Traffic Safety Administrations' New Car Assessment Program for a 50th percentile male anthropomorphic test device was taken as a starting point. A value of 700 for 15 ms was used because it corresponds to a 5% risk of AIS 4. As mild traumatic brain injury is of interest, this value was used as an upper boundary for linear acceleration. This value was scaled based on a method proposed by Mertz and Irwin, 2003 that uses length scaling to calculate HIC for different age and gender groups. Brain radius was used to determine the length scaling factor. The linear acceleration threshold corresponding to scaled HIC is shown in Table 1.

Table 1. Acceleration and speed thresholds for the minipig based on scaling from the literature. AIS 3 was used for angular thresholds an AIS 4 was used for linear threshold. Since an initial objective is to conduct tests near threshold (below AIS 3), values less than a quarter of those shown in Table 1 were selected as starting-level inputs.

	Angular	Linear
Speed	>30 rad/s	-----
Acceleration	24-31 krad/s ²	300G or 2943 m/s ²

Animal model:

Göttingen minipigs were divided into three main groups: control, rotational injury, and linear injury (n=20 total). There are one hour and twenty-four hour survival animals so that injury progression can be observed. In some cases, injury might be seen at the 24 hour interval, but not within the first hour. The overall division of the animals will be decided once injury results are observed. The division of animals tested as of November 2011 is shown in Table 2.

Table 2. Minipig division to date.

	# of Minipigs
24 hr rotational injury	1
24 hr control	2
1 hr rotational injury	3
1 hr control	1
Scanning, staining, and perfusion technique	5

Magnetic Resonance Spectroscopy:

Magnetic resonance spectroscopy (MRS) quantifies the metabolite concentrations inside a 5 mm³ voxel determined by the user. The minipigs undergo baseline scans prior to injury in the 7T Bruker Biospin MRI Scanner in the Center for Biomolecular Imaging at the Wake Forest University Baptist Medical Center, then immediate post-injury (one-hour), and twenty-four hours post injury. A custom MRI coil (two-channel quadrature coil, 15 cm diameter) was purchased to facilitate proper tuning with the large tissue volume of the minipig head within its bore. Also, this coil was designed to be short enough to allow proper positioning of the minipig head within the isocenter by avoiding interference with the sternum. MRS is carried out on a roughly 200 mm³ voxel placed in the genu of the corpus callosum (Figure 2). The metabolites quantified are alanine, aspartate, phosphocreatine, creatine, γ -

aminobutyric acid, glucose, glutamine, a combination of glutamate and glutamine, scyllo, taurine, guanidinoacetate, glycerophosphocholine, phosphocholine, N-acetylaspartate, N-acetylaspartylglutamate. Those of particular interest include glutamate, the combination of N-acetylaspartate and N-acetylaspartylglutamate (NAA), a combination of glycerophosphocholine and phosphocholine (choline), myoInositol, a combination of phosphocreatine and creatine (creatine), and lactate.

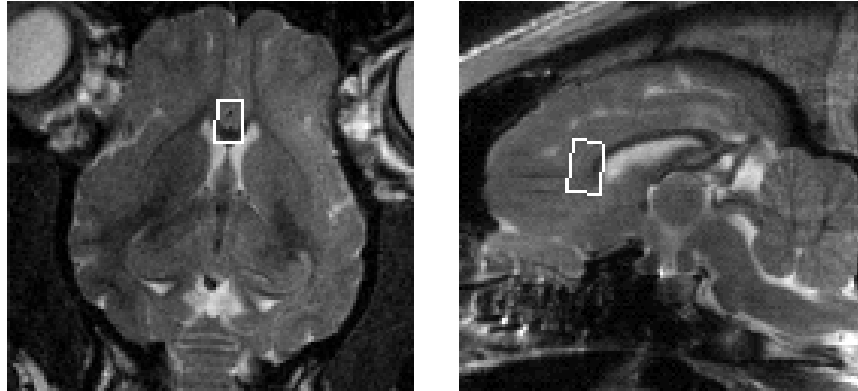


Figure 2: MRI scans showing the voxel placed in the genu of the corpus callosum.

Head Surgical Preparation:

Head surgical preparation is conducted on sham animals to confirm that the surgery does not have any adverse effects on the outcome. The incision site is shaved and scrubbed with alcohol and iodine before a last scrub with sterile iodine. A local anesthetic (Bupivacaine 0.25%) is given subcutaneously around the incision site and analgesia (Buprenorphine 0.01-0.05 mg/kg, intramuscular) is administered before incision. A midline incision is made so that expanding bone screws can be inserted into the skull. These screws are positioned approximately 2 cm below the nuchal crest and 3 mm below the coronal suture so that a steel slug (1 in diameter, 1 in long) can be attached using Bosworth Trim® dental cement (Bosworth® Company, Illinois). Figure 3 shows the steel slug attached to a minipig skull and then directly bolted into the injury device, therefore, forming a rigid attachment between the minipig and the device. The minipig is then transferred to the injury device where the steel slug is bolted in place. Canvas is wrapped around the body and tightened with straps to secure the minipig in place.

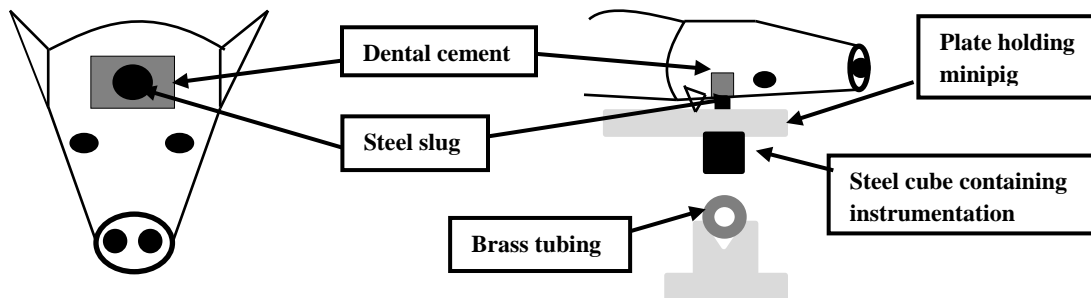


Figure 3: Schematics showing the rigid attachment of the minipig to the injury device and how the steel cube impacts brass tubing.

Rotational Injury:

The rotational injury device (Figure 4) consists of two aluminum platforms that can rotate with respect to one another. There is a winch to lift the two platforms and there is also an aluminum block holding brass tubing that is crushed at impact. The minipig is attached to the platform that is the furthest away from the main pivot point. Rotation between the two platforms needs to be eliminated. This is accomplished by inserting a copper tube between the two platforms at the hinge point. With the copper tube in place, the platforms rotate at the same angle until

impact. At impact, the copper tube is crushed and the platform holding the minipig is allowed to rotate with respect to the other plate, adding extra rotational motion. The minipig is rigidly attached the injury device, as explained previously, by bolting the steel slug into the device. The steel slug is bolted to a steel cube on the underside of the platform. This cube hosts two linear accelerometers. An angular accelerometer and angular rate sensor are bolted to sides of the cube. The cube impacts brass tubing that absorbs some energy of the fall and causes rapid deceleration of the minipig. High speed video is used for overall observation of the event.

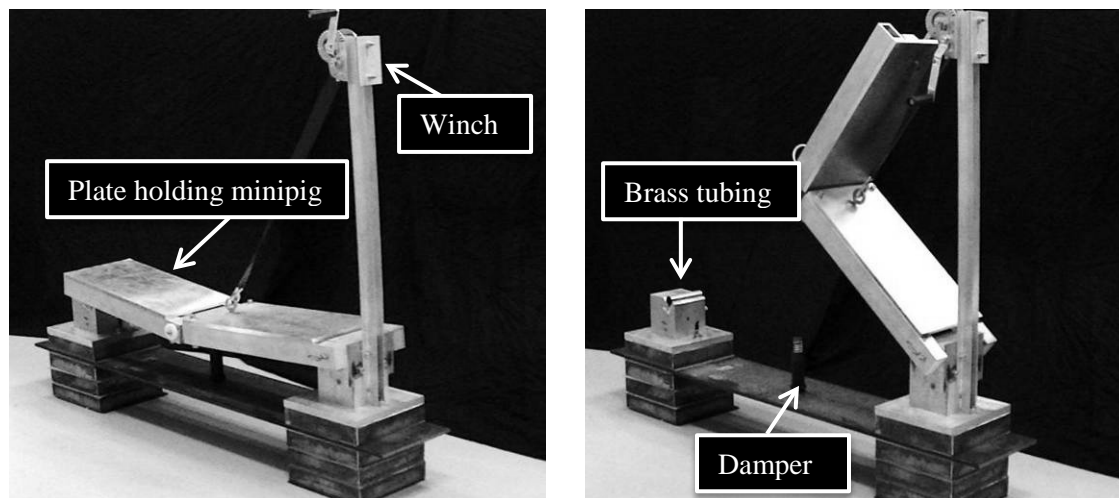


Figure 4: Rotational injury device.

Repeatability tests were conducted using the rotational injury device. These were carried out by dropping the device from the same height and impacting the same brass tube configuration.

Linear Injury:

The linear injury device (used currently) was designed to produce a purely linear impact. This is achieved by dropping the minipig and abruptly decelerating it with brass tubing similar to the rotational injury device, but motion is limited to one plane, using linear rails, which allows no rotational motion and therefore eliminates rotational acceleration.

Immunohistochemistry:

At the time of sacrifice, the minipigs are euthanized using Beuthanasia-D (90 mg/kg Sodium Pentobarbital or greater, IV), flushed with heparinized saline, and then perfused with 4% paraformaldehyde to fix the brain. The brains are extracted and stored in paraformaldehyde for transport to the Virginia-Maryland Regional College of Veterinary Medicine where they are sectioned and stained. Staining is carried out using β -amyloid precursor protein, Fluoro-Jade C, pan neurofilament or Neu-N, glial fibrillary acid protein and vimentin, ionized calcium binding adaptor molecule 1, and albumin. This combination of stains will look at diffuse axonal injury, neuron damage, astrocyte response, microglia activation, and blood brain barrier leakage, respectively.

RESULTS

Angular and linear acceleration and angular speed were repeatable over four similar tests (Figure 5).

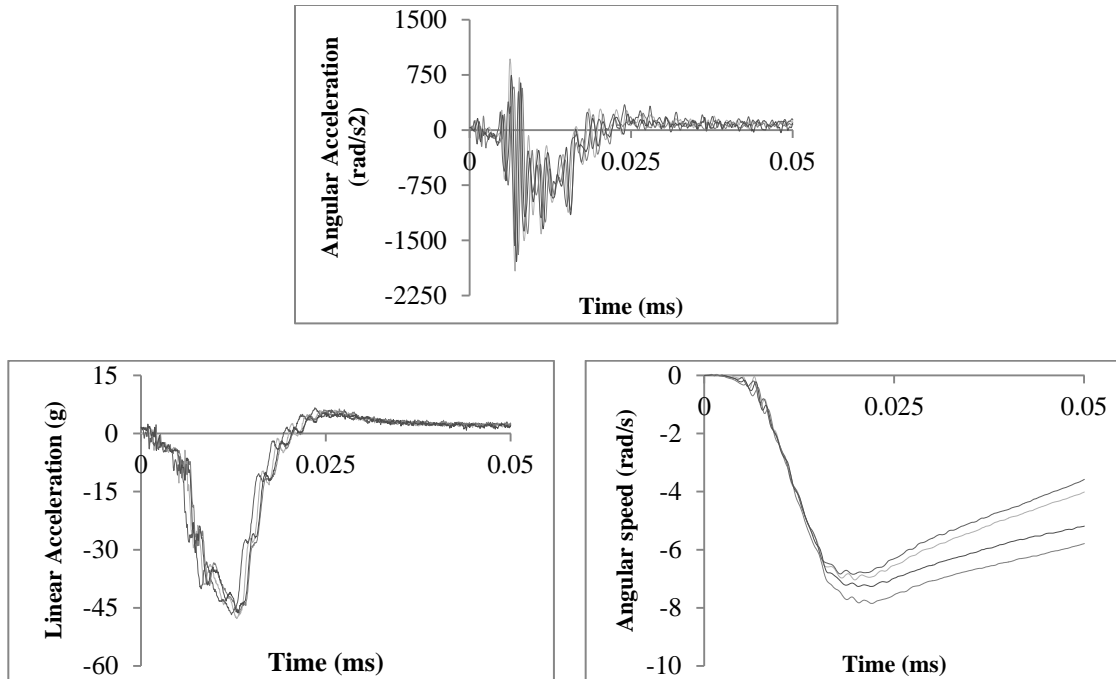


Figure 5: Repeatability test results showing angular acceleration (top), linear acceleration (bottom left) and angular speed (bottom right).

Three minipigs have been dropped at different heights using the rotational injury device. Figure 6 shows still images of a rotational injury event. Two animals have been dropped at 10 degrees. There was a design change to the device so another animal had to be dropped at the same height again. One survival animal has been dropped at 15 degrees. The resulting peak linear acceleration, rotational speed, and rotational acceleration are shown in Table 3.

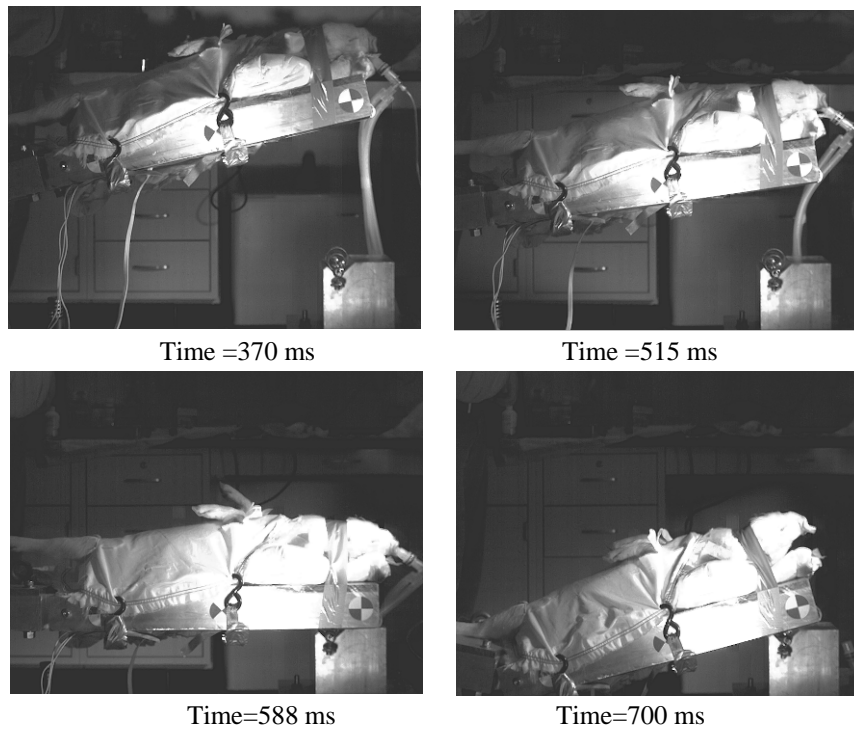


Figure 6: Still images of a video taken during a rotational injury event.

Table 3. Measured linear acceleration, rotational speed, and rotational acceleration from two one hour survival and one 24 hour survival rotational injury tests with data channels filtered at SAE Channel Frequency Class 1000 Hz [SAE Standard J211-1, 2000].

Test #	Test Description	Peak Linear Acceleration (G's)	Peak Rotational Speed (rad/s)	Peak Rotational Acceleration (rad/s ²)
1	1 hour: 10 degree drop	32	6	1300
2	1 hour: 10 degree drop	60	6	1700
3	24 hour: 15 degree drop	42	8	1800

Table 4 summarizes the percent differences between baseline scans and 1 hour post injury scans and differences between 1 hour post injury scans and 24 hour post injury scans for the 24 hour survival animals.

Table 4. Percent differences of metabolite concentrations for all tests to date showing the peak rotational acceleration with 1 hr/base meaning difference between 1 hour survival and baseline scans and 24 hr/base meaning differences between 24 hour survival compared to 1hr scans. Glu: glutamate; Ins: myoInositol; Cho: choline; NAA: N-acetylaspartate; Cr: creatine.

	1	2	3	
	1300 rad/s ²	1700 rad/s ²	1800 rad/s ²	
	1 hr/base	1 hr/base	1 hr/base	24 hr/1hr
Glu	-	-	38%	-1%
Ins	-	-48%	-21%	-13%
Cho	-62%	8%	-15%	3%
NAA	73%	-20%	4%	21%
Cr	41%	34%	-2%	-9%

DISCUSSION

Other metabolites can be quantified, but only those of interest were analyzed for this study so far. Lactate can be quantified, but the standard deviation measured during scanning was too high to be considered meaningful.

As shown, there are no clear trends in the metabolite concentrations changes. Therefore, the next step in this study is to compare the metabolite differences to immunohistochemistry so that the differences can be compared to actual damage. This will give understanding into the exact meanings of the metabolite concentration differences. In addition, higher-energy impact levels are being examined.

CONCLUSIONS

Future steps include making the linear injury device and testing it. Histology will be used to see what severities of injuries were produced thus far so that the remaining animals can be utilized efficiently.

Once the mapping of neuronal damage to metabolite concentration changes is completed, longitudinal development of injury can be characterized by metabolic changes observed with MRS at different time points, and related to input head kinematics. High-speed biplane x-ray studies looking at head kinematics, brain response, intracranial pressure, and brain injury will support the development of an FE model of the minipig head. Once validated, the model can be used to scale graded injury metrics for prediction of brain injury in the human.

Ultimately, a new head injury criterion will be developed for better prediction of the potential for traumatic brain injury.

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