Changes in the Mechanical Response of Brain Tissue Following Primary Blast Injury

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ABSTRACT

Blast induced traumatic brain injury (bTBI) is a serious concern for military personnel exposed to primary blast. Computational models and imaging diagnostics are useful tools that are commonly used to study mechanical response of the brain during trauma, but rely on information about the mechanical property changes due to injury. In this study, thirty adult, male Sprague-Dawley rats were exposed to varying levels of primary blast which were generated using a compressed-gas driven shock tube. Animals were sacrificed at 2 and 24hrs following trauma and their brain tissue immediately removed for post trauma, tissue-level stiffness assessment using a mechanical indenter. Ramp and hold indentation tests with a cylindrical indenter were performed to measure the mechanical response of the tissue. Significantly higher forces were measured in the midbrain inferior region in the blast high 24 hour when compared to sham group (+57.7%, p=0.02). In addition, we observed lower forces in the brainstem region (-65%, p=0.04) of the blast low 24 hour as compared to the sham group. There were no significant changes in the 2 hour groups. The results show a temporal, regionally-dependent, and severity-dependent mechanical response;—stiffening in the blast high 24 hour, softening in blast low 24 hour—to injury. These results provide insight to the effect of bTBI on the mechanical response of brain tissue, which may help improve computational models of the head and imaging diagnostics focused on detecting bTBI.

INTRODUCTION

During modern conflicts, improvised explosive devices (IEDs) have resulted in increasing incidence of TBI associated with blast exposure (Owens et al.). In reviews by the US Army and US Navy and Marine Corps of casualties in Afghanistan and Iraq, 52-63% of all TBI cases were associated with explosions (Bass 2012). Brain injuries resulting from blast typically involve edema in the tissue, alterations in cerebral blood flow, hemorrhaging, and increase in intra-cranial pressure and require immediate medical attention (Ling, 2009). If left untreated, victims of these injuries may suffer from long-term neurological problems which can lead to substantial societal and economic burdens (Meaney 2014). Accurate diagnosis and immediate medical care of blast traumatic brain injury (bTBI) can improve clinical outcome in victims of bTBI (Ling 2009).

A better understanding of the mechanical response of the brain during and after these events may help to improve diagnosis of bTBI in both clinical and battlefield scenarios. Finite Element (FE) models of the head and diagnostic tools that are based on imaging modalities are

well suited to study the effect of TBI on the brain. For example, non-invasive diagnostic techniques, such as field-deployable ultrasound, rely on detecting changes to the mechanical properties of brain tissue after injury; however, these tools require a priori information on how injured tissue correlates with mechanical changes. It is necessary to validate their accuracy with direct measurement of tissue stiffness using mechanical tests. Given the complex nature of the brain, the mechanical response to injury may be coupled to inflammatory response and tissue level changes in blood and fluid perfusion. Moreover, changes to the brain mechanical properties with a blast injury may be a) region-specific, b) time-specific, and c) blast severity-specific.

The goal of this study is to quantify changes in the mechanical response of brain tissue following blast injury. Comparisons between normal and injured brain tissue were made by focusing on studying the force response of tissue to indentation testing. The results of this study provide direct measurement of brain tissue stiffness changes following bTBI and may help improve computational models of the head and imaging diagnostics for assessing injury due to blast.

METHODS

Animal Injury

All animal protocols were approved by the University of Virginia's Institutional Animal Care and Use Committee. Thirty adult, male Sprague-Dawley rats of average weight (284 ± 19.2 gram) were prepared for blast injury in this study. Anesthesia was induced with a mixture of 4% isoflurane and 100% medical grade O_2 for 3-4 minutes in an induction chamber. Vital signs including heart rate, pulse oximetry, temperature, and respiratory rate were monitored during this time. Once the animals were sedated, the torso was wrapped in a protective vest to prevent pulmonary injuries from occurring during blast trauma (Rafaels 2012). The animals were secured to a platform and positioned at the end of a shock tube with the head incident to the oncoming blast wave. Shockwaves were generated using a compressed-gas driven system that was capable of delivering uniform blast waves to the specimen. Animals were then exposed to one of the two following levels of peak incident blast overpressure: low severity; 18-20psi, n=10 and high severity; 25-30psi, n=10. Blast duration for both severities was 5ms. The remaining animals were used as controls (sham). Sham animals were placed briefly inside shock tube but not exposed to blast overpressure. Following blast injury, the animals were monitored for a period of either two hours (acute injury, n=10) or twenty-four hours (n=10) after which they were sacrificed and their brain tissue immediately collected. Five animals were tested per injury group, including sham, and at each time point (Table 1).

Table 1: Test matrix for blast injuries

Treatment	#	Weight
Sham 2 hour	5	296 ± 8.9
Sham 24 hour	5	279 ± 26.2
Blast Low 2 hour	5	284 ± 23.2
Blast Low 24 hour	5	274 ± 20.5
Blast High 2 hour	5	292 ± 14.6
Blast High 24 hour	5	273 ± 7.8
TOTAL	30	284 ± 19.18

Sample Preparation

Whole brain specimens were prepared for indentation tests immediately following tissue collection. To reduce the effects of temperature and level of hydration on the results, specimens were submerged in a physiological buffer (saline) for five minutes at room temperature (19-21) °C. Hydrated specimens were then placed into a coronal slice matrix (Braintree Scientific, Inc.) with incision planes spaced 1mm apart. Three tissue cross-sections, referred to as midbrain, frontal, and brainstem, were cut from each whole brain specimen using 0.23mm thick razor blades (VWR Scientific, Media, PA). To accomplish this, five incisions were made in the coronal plane, parallel to each other (Figure 1). Frontal tissue cross-sections were cut to a 4mm thickness by making incisions at 0 and 4 bregma. Midbrain tissue cross-sections were cut to 6 mm by adding an incision -6 bregma. Brainstem tissue cross-sections were cut to 4 mm by making incisions at -9 and -13 bregma. Samples were then removed from the slicing matrix and placed on an aluminum test stage or in a saline bath. The slices were tested in the following order: midbrain, frontal, brainstem. Indentation direction varied among cross-sections (figure 1). The weight and dimensions of each sample were measured and recorded prior to testing.

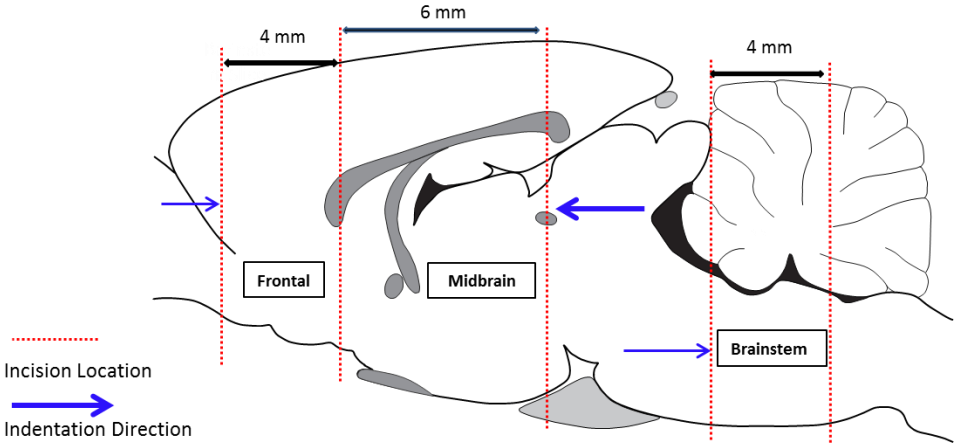

Figure 1: Coronal incisions and indentation directions for the frontal, midbrain, and brainstem cross-sections. Image adapted from (Paxinos, 1986).

Five of eight total regions on the surface of the three tissue cross-sections were selected for indentation testing (Figure 2). The coordinates of these regions were determined using a stereotaxic reference frame (Paxinos, 1986). In the frontal slice, one of two regions was randomly selected for indentation. In the midbrain slice, three of five total regions were randomly tested at the aqueduct, inferior, and superior. In the brainstem region, one region was tested at the center of the pons. Since the blast wave produces a symmetric injury across brain halves, regions tested across the brain in the midbrain inferior, midbrain superior, and frontal were assumed to be homogenous. Evan's Blue Dye was used to mark each coordinate on the tissue cross-section for a visual reference onto which the indenter could be positioned. The amount of dye under the indenter was assumed to have a negligible effect on the tissue properties. A total of 30 minutes were allotted for sample preparation, i.e. from the time of sacrifice to the time indentation testing began.

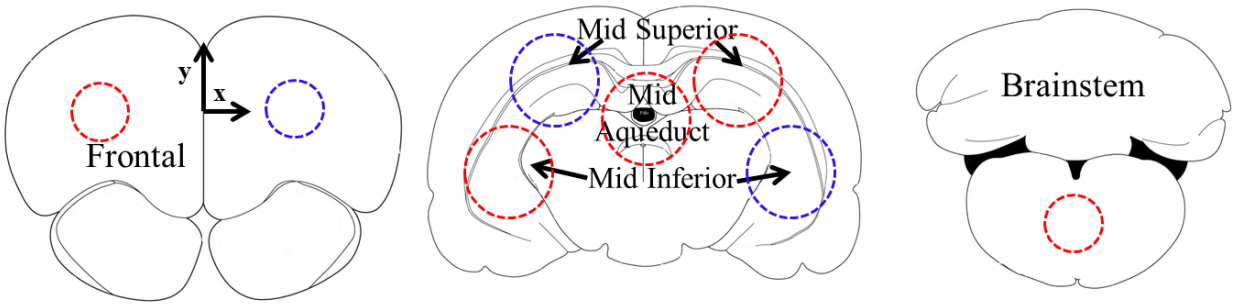

Figure 2: Schematic of a tissue sample showing the coordinate locations of the five indentation regions. The origin is defined differently for each slice. Frontal slice$(\boldsymbol{x}, \boldsymbol{y})$ = (±2.5, 0). Midbrain slice$(\boldsymbol{x}, \boldsymbol{y})$: Aqueduct = (0, 0), Superior= (±2, 1), Inferior = (±3, -1). Brainstem slice$(\boldsymbol{x}, \boldsymbol{y})$ = (0, 0). Dashed red circles represent the cross-sectional area of the indenter overlaid onto the tissue sample. Blue circles represent alternative options for the same region, chosen randomly for each test. Images adapted from (Paxinos, 1986).

Indentation Testing

The aluminum test stage with sample was mounted atop a 50gram load cell (Model 31 Low, Honeywell International Inc., Golden Valley, MN), and beneath a cylindrical indenter mounted to a linear actuator equipped with an LVDT to measure displacement (ElectroForce® 3100 Test Instrument, Bose Corporation – ElectroForce Systems Group, Eden Prairie, MN). Indentations on the midbrain slice were performed using an indenter with a diameter of 3.18mm, while 1.59mm diameter indenter was used on the other tissue cross-sections. Excess compliance in the test frame due to the motion of the actuator induced an inertial based force response in the load cell. A 500g linear accelerometer (Model#: 7264B-500, Humanetics Innovative Solutions, Plymouth, MI) was mounted to the test stage to subtract off this effect. Force, displacement, and acceleration data were acquired at 20kHz (DEWE-2010, Dewetron Inc., Wakefield, RI).

At each coordinate the indenter was centered on the Evan's Blue Dye. This was accomplished by mounting a 1.3mm diameter spherical tip punch to the actuator and positing directly over the dye via visual inspection. The spherical punch was exchanged with the plane-ended cylindrical indenter. The indenter was advanced slowly toward the tissue at a rate of 0.01mm/s until a tare load of 0.3gram was achieved. The indenter tip was then pressed 0.6mm into the tissue, normal with respect to the local surface, in approximately 8ms and then held for 30s to measure the tissue's relaxation. A peak displacement velocity of 120mm/s was observed during the ramp portion of the displacement. After each test the indenter was carefully removed from the tissue. Five minutes were allotted between tests to allow for tissue recovery and instrumentation adjustments. The order in which regions were tested in the midbrain was randomized for each animal. In between each indentation test on the midbrain the tissue was sprayed with saline. The protocol was repeated for the remaining regions and slices and all testing was completed within 60 minutes of animal sacrifice.

Data Processing and Statistics

Force and displacement data were processed to make comparisons across injury severities and time points. Raw data were filtered using a butterworth filter with a low pass cut-off frequency of 400Hz (Matlab, The MathWorks, Natick, Ma). Since displacement time histories

were found to be extremely repeatable, analysis of the mechanical response data was performed by focusing on tissue forces. Force isochrones were extracted at the maximum force at time=0 s, and forces at time=0.01, 0.1, 1, 10, and 29 s. The forces at each isochrones and each region for the six experimental groups were compared using a 1-way ANOVA with post-hoc Tukey's HSD tests to evaluate changes in brain tissue mechanical response due to blast injury.

RESULTS

Twenty-nine of the original thirty animals survived; one fatality occurred following a high blast condition. A total of 145 indentation tests were performed. Large variability was observed in the experimental data, while differences in the force response within a particular region were generally unremarkable (Figure 3). However, significantly higher peak forces were measured in the midbrain inferior region in the blast high 24 hour treatment when compared to sham controls (+57.7%, p=0.02).

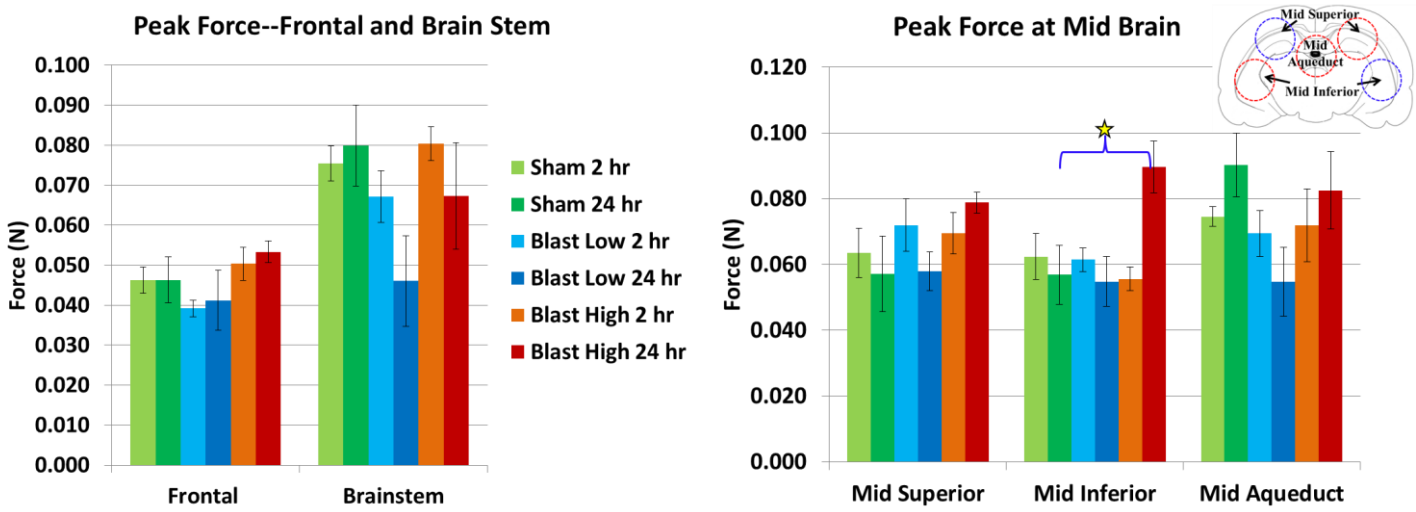

Figure 3: Peak indentation forces in the frontal and brainstem regions (bottom) and midbrain regions (top). Bars are mean ± SE.

In addition to elevated forces measured in the midbrain, we observed statistically significant changes in the brainstem; peak forces were, on average, lower (-65%, p=0.04) of the blast low 24 hour as compared to the sham group at time=0.01s. Although differences between injury treatments were not found to be statistically significant, we observed a general trend of lower mean forces in the blast low 24 hour group in the midbrain aqueduct and brainstem regions. In addition higher mean forces were observed in the midbrain superior and midbrain inferior regions across all isochrones. There were no significant changes in the 2 hour groups when compared to sham controls.

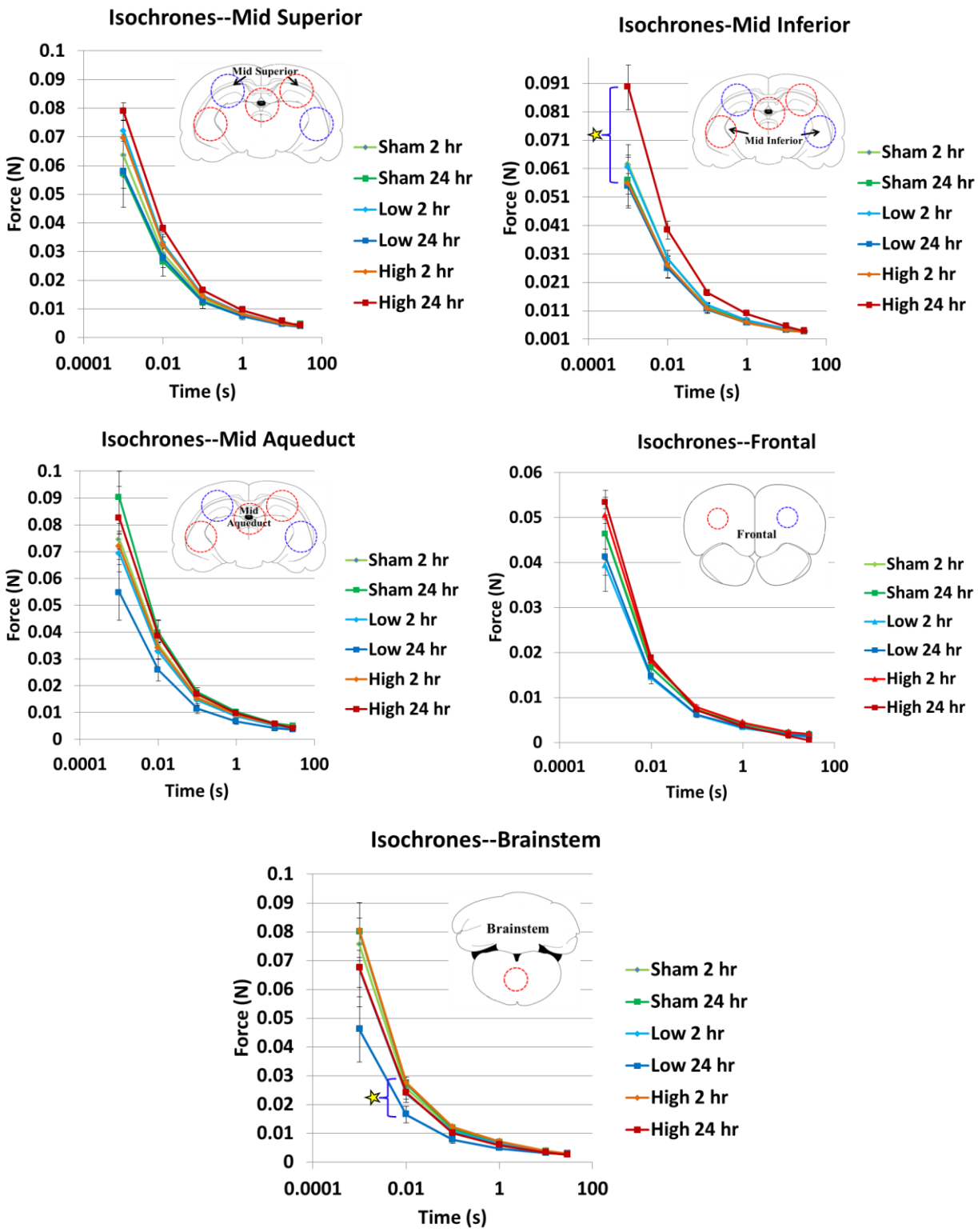

Figure 4: All isochrones forces for the midbrain superior, midbrain aqueduct, midbrain inferior, frontal, and brainstem regions. Points are mean ± SE.

DISCUSSION

This study finds varying changes in mechanical response across regions of the brain due to blast exposure. Specifically, low severity blast causes a softening, while high level blast causes stiffening. These changes vary by region, which could be due to the heterogeneity of the brain tissue and surrounding vasculature and ventricles. Additionally, the injury from the blast wave is assumed to be symmetric, but its interaction with the head and brain may cause preferential damage to certain regions or types of tissue. The mechanical response changes could be due to many factors of blast injury, including acute hyperemia and severe edema in the tissue of the brain, alterations in cerebral blood flow, hemorrhaging, and increase in intra-cranial pressure (ICP) (Ling, 2009). The observed stiffening in this study could be to a combination of these factors, namely edema (Elliot 2008). Edematous tissue has higher water content, so an acute time increase in stiffness with a high blast severity is possible. The softening of the brainstem region due to low blast at 24 hours can be due to changes in cerebral blood perfusion and cerebrospinal fluid flow (Colgan 2010 and Bryan 1995). The specific contribution of these physiologic mechanisms to mechanical response is unknown, but further evaluation is needed to correlate these responses to histological and imaging data.

In a primary blast injury, the insult to the brain is through a propagating shear wave, and force interface with the brain is not fully understood (Ling 2009). Direct shearing or compression of brain tissue may occur, but at very low strain levels (Panzer 2012). Therefore, a strain mediated change in mechanical response is unlikely. Physiologic-mediated changes due to edema and blood flow, however, generally manifest in the time course of a few hours up to a week (Kochanek 1995 and Stroncek, 2008).The mechanical responses in this study are temporally dependent with significant changes observed at a 24 time point, supporting a theory of a physiologically dominated injury. Previous studies using controlled cortical impact (CCI) injuries find similar temporal responses that last from 7-28 days (Boulet 2013 and Xu 2014).

There have been an increasing number of studies that characterized changes in material stiffness due to CCI injury to the brain, using Magnetic Resonance Elastography (MRE) (Boulet, 2011 and 2013), Shear Wave Elastography (Xu, 2014), and indentation testing (Gabler, 2013). Boulet et al. found 20-30% decrease in stiffness in injured hemisphere at acute time points, with gradual recovery over 28 days. Xu et al. also found a similar effect, with ipsilateral softening and contralateral stiffening that causes a 20-25% decrease in relative stiffness. Gabler et al. found a 20% decrease in relative stiffness due to injury in a contralateral comparison of injured tissue after 24 hours. Brain material property changes were also studied for a different mode of injury in a TBI-inducing weight drop model (Shafieian, 2009). Shafieian found a ~25% decrease in stiffness and various changes in viscoelastic properties of the brain in injured versus sham rats. While these studies find significant changes in mechanical properties of the brain, they implemented direct impact to the brain or skull. This mode of injury causes direct damage and higher strains to the tissue, and thus a softening of the brain is reasonable at an acute time stage. In combination with a physiologic response due to injury, including edema, blood flow, and the formation of contusion cavities, the effect of injury on mechanical response past an acute time stage is unknown. The stiffening observed in this study could be a result of one or a combination of these factors.

This study had several limitations including: small sample size, inherent variability of the material properties of the brain, surface effects of the contact between the indenter and brain tissue, variability in indentation depth and rate associated with the test machine, and repeatability of region location and cross-section size. Additionally, assumptions were made about the

homogeneity of brain tissue within regions and across brain halves but they are assumed to contribute to the overall variability observed in the data. There are also limitations associated with the mechanical indentation, as substrate effects between the indenter and cross-section heights as well as the boundary condition between the indenter and tissue edges were not always maintained. Further, the indentations made on the midbrain regions were within 2 mm of each other, but sufficient time was allotted for the tissue to recover between tests. Finally, the mechanical response data doesn't give absolute material properties, but stiffness changes of the tissue can assumed from the force-displacement data. It is assumed that a higher force corresponds to a stiffening of the tissue, while a reduction in force corresponds to a softening. These assumptions are valid within the boundaries of the experiments, namely the same regions tested for each animal with the same indenter diameter, indentation depth, indentation rate, pre-compression, and ramp-hold time.

Future studies are needed to correlate cellular level changes or functional deficit with tissue level mechanical response. Ongoing parallel test series are being conducted using shear wave elastography and histology to improve our understanding of physiologic effect on tissue mechanical response due to bTBI. Additionally, mathematical modeling within a quasi-linear viscoelastic (QLV) framework is planned for the data to compare material stiffness parameters with published data.

CONCLUSIONS

This study presents an experimental framework for a closed-head bTBI injury model and tissue mechanical characterization. The results show a temporal, regionally-dependent, and severity-dependent mechanical response; stiffening in the blast high 24 hour, softening in blast low 24 hour post injury. To the authors' knowledge, this is the first study to examine the mechanical properties of the brain following bTBI. These mechanical changes can serve as correlates to injury for improving early diagnosis of bTBI and modeling tissue injury, as well as elucidate the dependence on injury mechanisms and physiologic response.

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REFERENCES

BASS, C. R., PANZER, M. B., RAFAELS, K. A., WOOD, G., SHRIDHARANI, J., & CAPEHART, B. (2012). Brain injuries from blast. Annals of biomedical engineering, 40(1), 185-202.

BOULET, T., KELSO, M. L., & OTHMAN, S. F. (2011). Microscopic magnetic resonance elastography of traumatic brain injury model. Journal of neuroscience methods, 201(2), 296-306.

BOULET, T., KELSO, M. L., & OTHMAN, S. F. (2013). Long-term in vivo imaging of viscoelastic properties of the mouse brain after controlled cortical impact. Journal of neurotrauma, 30(17), 1512-1520.

BRYAN RM JR, CHERIAN L, ROBERTSON C. (1995). Regional cerebral blood flow after controlled cortical impact injury in rats. Anesth Analg; 80:687–695.

CHATELIN, S., CONSTANTINESCO, A., & WILLINGER, R. (2010). Fifty years of brain tissue mechanical testing: from in vitro to in vivo investigations. Biorheology, 47(5), 255-276.

COLGAN NC, CRONIN MM, GOBBO OL, ET AL. (2010). Quantitative MRI analysis of brain volume changes due to controlled cortical impact. J Neurotraum; 27:1265–1274.

ELLIOTT MB, JALLO JJ, TUMA RF. (2008). An investigation of cerebral edema and injury volume assessments for controlled cortical impact injury. J Neurosci Methods; 320–324.

GABLER, L. F., STONE, J. R., MOURAD, P. D., CRANDALL, J. R., & SALZAR, R. S. (2013). Region Specific Viscoelastic Properties of the Adult Rat Brain under Indentation following Traumatic Brain Injury. IRCOBI Conference on the Biomechanics of Impact, Gothenburg, Sweden.

KOCHANEK, P.M., MARION, D.W., ZHANG, W., SCHIDING, J.K., WHITE, M., PALMER, A.M., CLARK, R.S., O'MALLEY, M.E., STYREN, S.D., HO, C., ET AL. (1995). Severe controlled cortical impact in rats: assessment of cerebral edema, blood flow, and contusion volume. J. Neurotrauma 12, 1015–1025.

LING, G., BANDAK, F., ARMONDA, R., GRANT, G., & ECKLUND, J. (2009). Explosive blast neurotrauma. Journal of neurotrauma, 26(6), 815-825.

MEANEY, D. F., MORRISON, B., & BASS, C. D. (2014). The mechanics of traumatic brain injury: a review of what we know and what we need to know for reducing its societal burden. Journal of biomechanical engineering, 136(2), 021008.

OWENS, B. D., KRAGH JR, J. F., WENKE, J. C., MACAITIS, J., WADE, C. E., & HOLCOMB, J. B. (2008). Combat wounds in operation Iraqi Freedom and operation Enduring Freedom. Journal of Trauma and Acute Care Surgery, 64(2), 295-299.

PANZER, M. B., MYERS, B. S., CAPEHART, B. P., & BASS, C. R. (2012). Development of a finite element model for blast brain injury and the effects of CSF cavitation. Annals of biomedical engineering, 40(7), 1530-1544.

PASCRELL JR(NJ-08), C. B. (2009). Introduction to the report of the international conference on behavioral health and traumatic brain injury. The Clinical Neuropsychologist, 23(8), 1281-1290.

PAXINOS, G., & WATSON, C. H. (1986). The rat brain in stereotaxic coordinates 2nd edn. Academic Press, New York.

RAFAELS, K. A., CAMERON, R., PANZER, M. B., SALZAR, R. S., WOODS, W. A., FELDMAN, S. H., ... & TOMAN, A. (2012). Brain injury risk from primary blast. Journal of trauma and acute care surgery, 73(4), 895-901.

SHAFIEIAN, M., DARVISH, K. K., & STONE, J. R. (2009). Changes to the viscoelastic properties of brain tissue after traumatic axonal injury. Journal of biomechanics, 42(13), 2136-2142.

STRONCEK, J. D., & REICHERT, W. M. (2008). Overview of wound healing in different tissue types. Indwelling neural implants: strategies for contending with the in vivo environment.

SVETLOV, S. I., LARNER, S. F., KIRK, D. R., ATKINSON, J., HAYES, R. L., & WANG, K. K. (2009). Biomarkers of blast-induced neurotrauma: profiling molecular and cellular mechanisms of blast brain injury. Journal of neurotrauma, 26(6), 913-921.

XU, Z. S., YAO, A., CHU, S. S., PAUN, M. K., MCCLINTIC, A. M., MURPHY, S. P., & MOURAD, P. D. (2014). Detection of Mild Traumatic Brain Injury in Rodent Models Using Shear Wave Elastography Preliminary Studies. Journal of Ultrasound in Medicine, 33(10), 1763-1771.