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14. ABSTRACT

Injury severity in blast induced Traumatic Brain Injury (bTBI) increases with blast overpressure (BOP) and impulse in dose-dependent manner. Pure primary blast waves were simulated in compressed gas shock-tubes in discrete increments. We evaluated 24 hour survival of rats in 0-450 kPa (0-800 Pa·s impulse) range at 10 discrete levels (60, 100, 130, 160, 190, 230, 250, 290, 350 and 420 kPa) and determined the mortality rate as a non-linear function of BOP. Using logistic regression model, predicted mortality rate (PMR) function was calculated, and used to establish TBI severities. We determined a BOP of 145 kPa as upper mild TBI threshold (5% PMR). Also we determined 146-220 kPa and 221-290 kPa levels as moderate and severe TBI based on 35%, and 70% PMR, respectively, while BOP above 290 kPa is lethal. Since there are no standards for animal bTBI injury severity, these thresholds need further refinements using histopathology, immunohistochemistry and behavior. Further, we specifically investigated mild TBI range (0-145 kPa) using physiological (heart rate), pathological (lung injury), immuno-histochemical (oxidative/nitrosative and blood-brain barrier markers) as well as blood borne biomarkers. With these additional data, we conclude that mild bTBI occurs in rats when the BOP is in the range of 85-145 kPa.

15. SUBJECT TERMS

Blast Induced Neurotrauma, Blast TBI, Primary blast brain injury, Blast overpressure, Risk injury function, Survival dose-response curve, Mortality rate

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1. INTRODUCTION

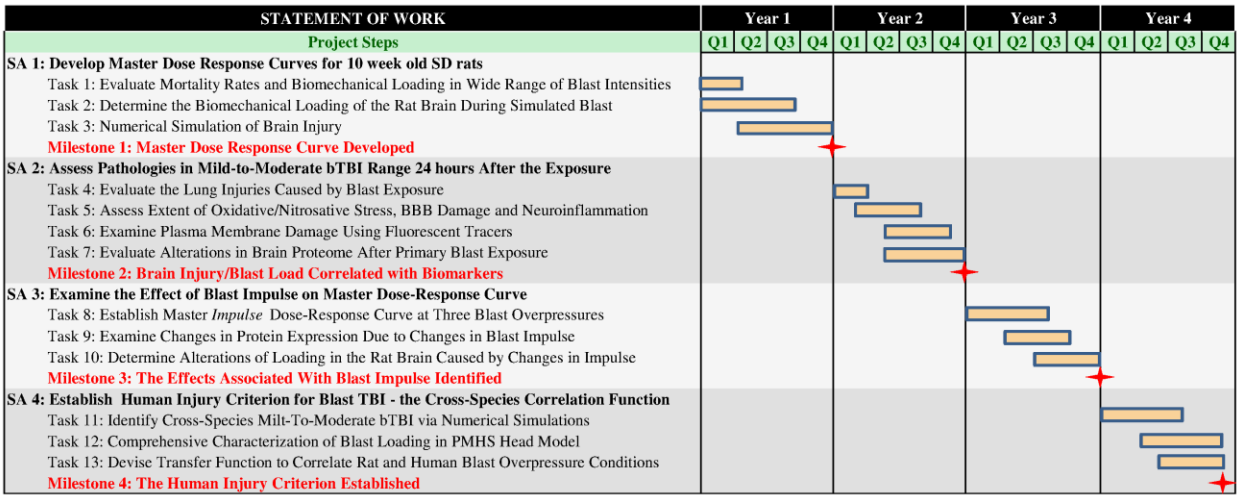
The main goal of this research effort is to establish a ***master*** dose response curve correlating blast overpressure with mortality rate using a field validated blast injury animal model. This curve will map the probability of 24 hour survival over a wide range of field-relevant blast over pressures. We used peak overpressure (0-450 kPa) and impulse (0-800 Pa·s) as two predictors brain injury, and based on these values established the thresholds for blast TBI in rat model. This model was further correlated with systemic pathophysiological changes: bradycardia and extent of lung injury, to determine if these changes can be used as convenient markers of injury which can be used in the field and inform affected personnel immediately upon the exposure to shock wave. We used experimental measures to probe the response of the body to blast loading via implantation of intracranial pressure (ICP) and carotid artery (CA) sensors in anesthetized live animal model. The relationship between the blast over pressures (external loading to the head) and the resulting waveforms of intracranial and arterial pressure (internal loading to the brain and torso) indicates wave is traveling in the body gradually losing its potential. We have initiate the development of Rat Testing Device (RTD), a prototype surrogate model equipped with variety of pressure sensors and accelerator to precisely determine loading conditions and facilitate comparison in different blast loading scenarios. Developed numerical model of the shock wave propagation in the shock tube indicates excellent correlation with experimental data. Numerical model of the rat helped identify loading pathways through which blast overpressure 'leaks' into the brain leading to differential loading of the tissue in various discrete regions.

2. KEYWORDS

Blast Induced Neurotrauma, Blast TBI, Primary blast brain injury, Blast overpressure, Risk injury function, Survival dose-response curve, Mortality rate

3. ACCOMPLISHMENTS

Major Goals of the Project (Statement of Work with Timeline):



Completion: Task 1 and 2 completed; Task 3 is in progress (50% complete), Tasks 4 & 5 (25% complete)

Task 1: Evaluate Mortality Rates and Biomechanical Loading in Wide Range of Blast Intensities

Optimization of 9-inch shock tube operations

The comprehensive calibration of the 9-inches square cross section shock tube was completed, including data analysis and manuscript preparation. The new 9-inch square cross section shock tube was designed and fabricated based on extensive testing experience from the past, but there is still room for improvement. For example, during recently performed experiments we have optimized the pressure sensor mounting, which otherwise might sometimes introduce artifacts into the recorded pressure history. Armed with this new experience we are constantly improving our pressure measurement procedures to get as accurate measurements as possible.

Additionally, the modifications introduced during manufacturing process as well as environmental factors which might influence the profiles of generated shock waves compared to original design and it is more than likely due to manufacturing process some parts critical for shock tube operation are different, which might influence its performance. Thus the comprehensive calibration gave us an insight into the performance of the shock tube and helped establish a basic set of working parameters. In the same time there were a number of parameters and new sensors which were tested and optimized in order to make these measurements as reliable as possible. For this purpose we performed additional test shots to answer questions regarding the source of observed overpressure noise and aberrations adversely affecting recorded waveforms.

Dose-response logistic regression model

All the test animals were in a prone position with a head restrained and thus no artifacts associated with uncontrolled head acceleration were present in this bTBI model, as demonstrated elsewhere.[1] The net movement of the specimen did not exceed 3 mm as determined by analysis

of captured high speed video. We exposed 13 rats per discrete incident peak overpressure to develop a dose-response linear regression model for predicted mortality rate (PMR) as a function of BOP and corresponding impulse values (fig. 1). We did not observe any mortality of animals exposed to blast intensity lower than 170 kPa (fig. 1, left) or impulse 300 Pa·s (fig. 1, right). However mortality rate gradually increased from 170 kPa to 300 kPa, and mortality rate was 100% above 300 kPa (or 500 Pa·s impulse). These accounted for an immediate loss (not delayed deaths, i.e. animals were removed from the shock tube in less than a minute after the shock wave exposure) of 45 rats as a consequence of shock wave exposure, consistent with our previous work.[1, 2] The insets in fig. 1 present receiver operating characteristic (ROC) curves for respective logistic regression fits with the following areas under ROC curves: 0.878 (left) and 0.882 (right), for BOP and impulse, respectively. Analysis from these models indicated good predictive power of mortality rate using peak overpressure and impulse as metrics to gauge injury risk under primary blast. The McFadden pseudo-R^2 values of 0.389 and 0.412 were obtained for fits using peak overpressure and impulse as independent variables, respectively. These values indicate a highly satisfactory quality of fit.

The logistic regression models were developed and evaluated using Systat 13.0 software (Systat Software, Inc., San Jose, CA). Dose-response models for heart rate and pulmonary injury were fitted with Origin 9.0 software (OriginLab Corp., Northampton, MA) using dose-response function:

$$y = A_1 + (A_2 - A_1)/(1 + 10^{(\log x_0 - x)p}) \quad (1)$$

Where A_1, A_2 are asymptotes, $log\ x_0$ is an inflection point, and p is a slope value.

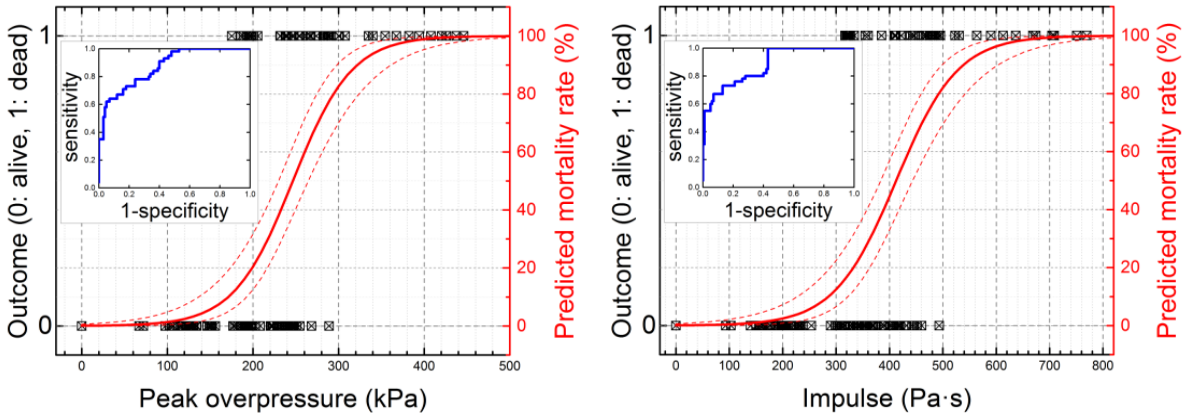


Figure 1 The logistic regression dose-response model for rats exposed to single blast with intensity in the range of 60-450 kPa peak overpressure (left) and corresponding impulse (90-780 Pa·s, right). The insets present receiver operating characteristic (ROC) curves for respective logistic regression fits with areas under ROC curves: 0.88 and 0.88. McFadden R^2 = 0.39 and 0.41, for fits using peak overpressure and impulse as independent variables, respectively. Dashed lines indicate confidence intervals of the model.

Primary blast impacts mild pulmonary injury

Animals tested in prone position have their abdomen partially protected from the blast wave by the aluminum holder used in our experiments.[1] The observed levels of pulmonary injury expressed using Yelveton's scoring system[3] revealed a low level of injury (fig. 2 A, B). However, we observed an increasing trend of injury score with increasing peak overpressure and impulse. We observed only a few cases where pathological score exceeded 21 for the blast

strength higher than 300 kPa BOP with high standard deviations (fig. 2 C). A score of 21 is considered as a cut-off threshold for mild pulmonary injury.[3] The pathological score at 50% PMR (at 260 kPa) was found to be less than 10, while the score was less than 4 in the 60 - 190 kPa range. Moreover, there are six animals which died as a result of blast exposure and had no lung injury (score of zero, fig. 2C, D). These results suggested minimal pulmonary injuries and thus, we conclude lung injury is not a viable indicator of PMR.

Blast induced bradycardia

We evaluated the functional changes in the heart rate, blood oxygen saturation (spO_2) and perfusion index in the 60-250 kPa peak overpressure range over period of 30 minutes before and after blast exposure. We found that the onset of bradycardia occurred immediately after the blast exposure even at 60 kPa (fig. 4, p=0.01, power: 0.85 vs control). The values of the differential

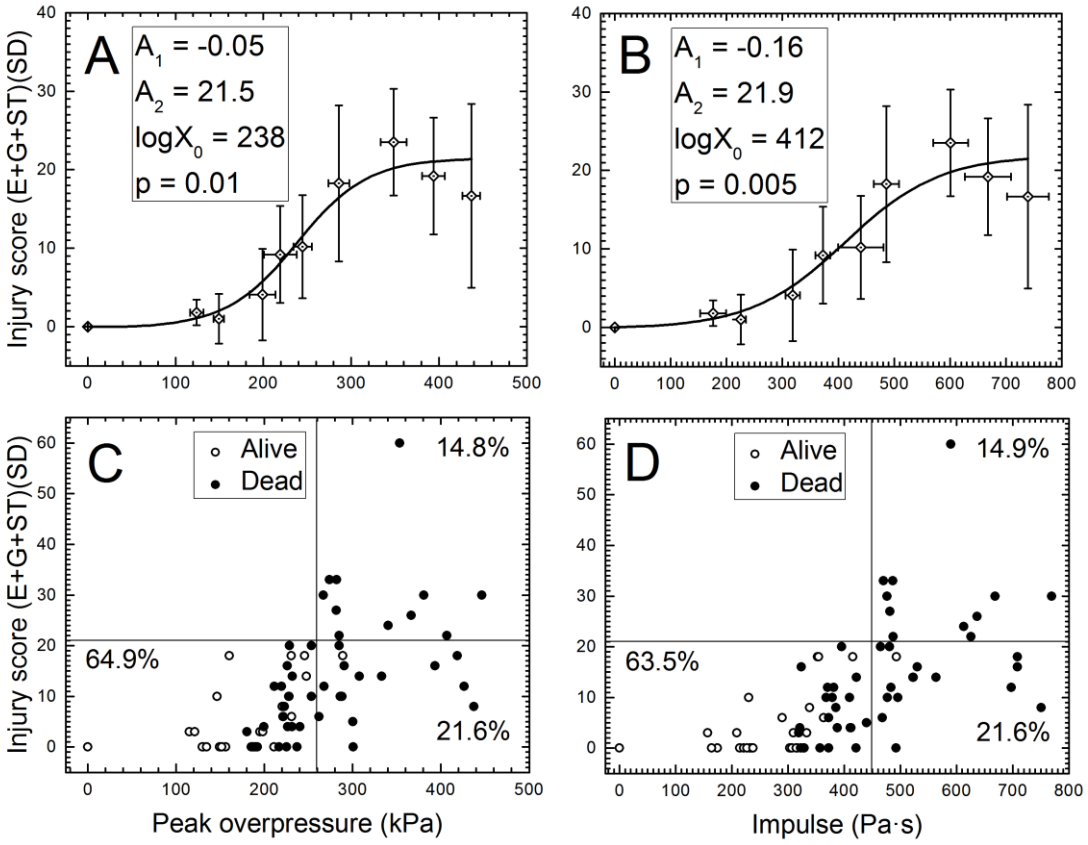


Figure 2 Lung injury scores for rats exposed to a single blast. The dose-response model was used to fit the IS as a function of peak overpressure (A, C) and impulse (B, D). The scattergrams (C, D) illustrate individual scores and their distribution among the cohort of 75 rats evaluated in this test. The value of 21 is the upper limit of the slight lung injury level as defined by Yelverton. The vertical lines (peak overpressure of 260 kPa (C), or impulse of 450 Pa·s (D)) correspond to 50% predicted mortality rate according to the dose-response model in fig. 1. There are six animals which died after the blast and had no apparent lung injuries (score of zero).

average heart rates (ΔHR) decreased gradually with increase in blast intensity: -29±10 (60 kPa), -26±20 (100 kPa), -43±26 (130 kPa), -62±21 (190 kPa), -62±43 (230 kPa) and -62±24 (250 kPa) bpm. These data were modeled using a simple dose-response function to quantify the characteristics of ΔHR as a function of two blast parameters, the peak overpressure and the

impulse. This mathematical modeling generated asymptotic values of A_1 and A_2: -13.9 and -59.5 (for peak overpressure, fig. 2A), and -12.7 and -67.1 (for impulse, fig. 2B), respectively. The calculated inflection points ($\log(x_0)$) are 109.3 kPa and 186.9 Pa·s for both blast parameters (fig. 3). The control group was not correlated with any of the blast exposure groups ($p < 0.05$).

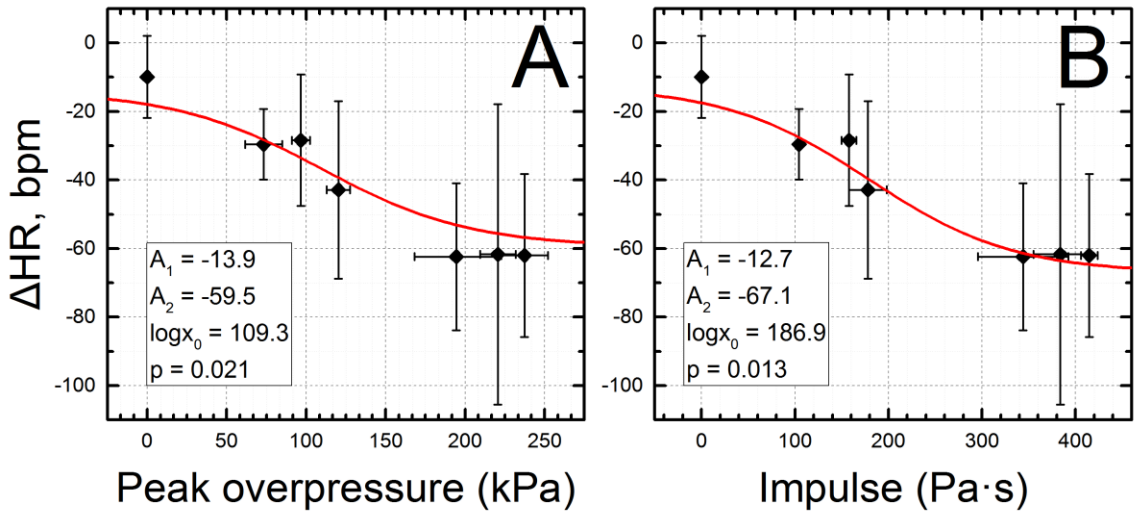


Figure 3 Blast induced bradycardia as a function of peak overpressure (A) and impulse (B). In both cases, the dose-response function (1) was used to model the pathological response and resulting parameters are listed in respective insets in both figures. All blast exposed groups have statistically significant heart rate decrease versus control ($p < 0.05$).

Induction of oxidative/nitrosative stress markers

Using our logistic regression risk injury model, we defined the upper level of mTBI at 145 kPa

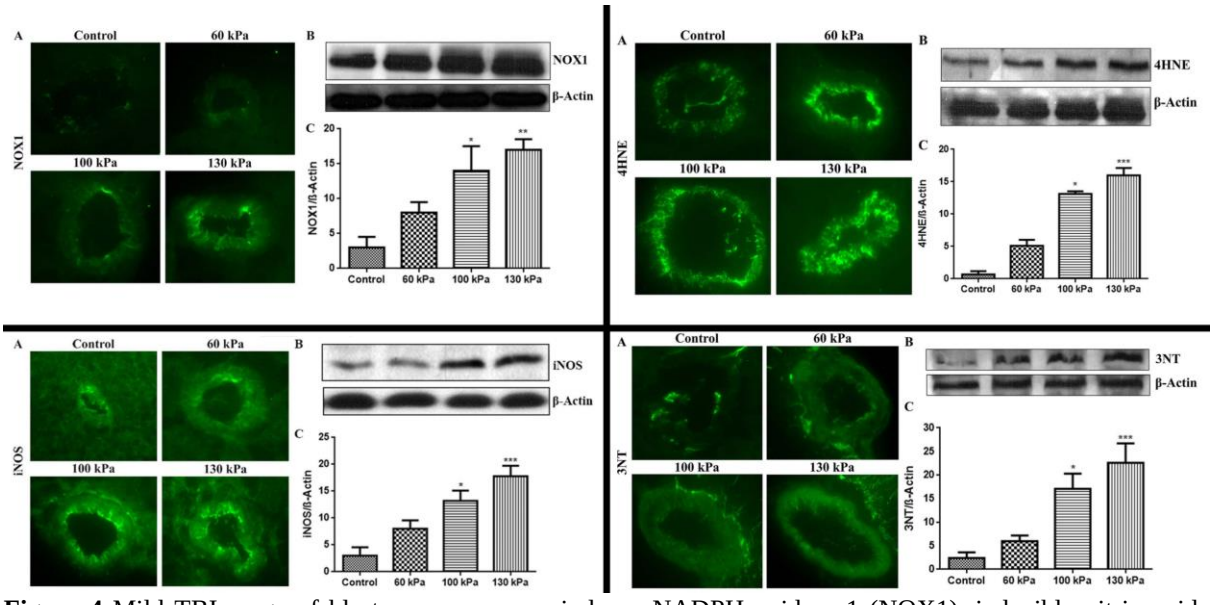


Figure 4 Mild TBI range of blast-wave exposure induces NADPH oxidase 1 (NOX1), inducible nitric oxide synthase (iNOS), 4-hydroxynonenal (4HNE) and 3-nitrotyrosine (3NT) expression in rat brain microvessels. (A) A representative of immunofluorescent staining of specific marker as indicated on the Y-axis in intact microvessels of brain cross sections from rats subjected to a single exposure to 60, 100, or 130 kPa peak overpressure, and control. (B) Corresponding Western blot specific marker (as indicated on the Y-axis) and housekeeping protein, β-actin. (C) The quantification of the results of the immunoreactive fluorescence intensities. Values are mean ± SEM (n = 4) with p-value ≤ 0.01 compared with control.

BOP as 5% PMR. We examined the oxidative/nitrosative injury and the BBB integrity in the range of 60 kPa to 130 kPa peak overpressures and determined lower threshold of mTBI at 80 kPa. These markers were evaluated 24 hours after the injury, at a time point which proved in our previous study to yield their maximum levels in analogous bTBI model.[4] We first evaluated the induction of free radical generating enzymes NADPH oxidase (NOX1) and inducible nitric oxide synthase (iNOS) in the brain capillary cross section tissues. We found that blast-wave exposure significantly up-regulated the induction of NOX1 with 100 kPa (p= 0.02) and 130 kPa (p= 0.001) BOP (fig. 4, left upper panel). Similarly, iNOS expression was increased at 130 kPa BOP (p= 0.0005) and at 100 kPa BOP (p=0.04) (fig. 4, left lower panel). Induction of NOX1 and iNOS produces superoxide and nitric oxide respectively, which will also react together to form peroxynitrite, a more reactive free radical. The oxidative/nitrosative damage is a post-oxidant production event. Proteins adducted with 4-hydroxynonenal (4HNE, oxidative stress marker) or 3-nitrotyrosine (3NT, nitrosative stress marker) are used for assessing the extent of injury in the tissue. In parallel with the induction of NOX1 and iNOS enzymes, we found that the level of oxidative damage signature 4HNE (fig. 4, right upper panel) is increased in mTBI range exposure with 100 kPa (p= 0.05) and 130 kPa (p= 0.0004). Similarly, 3NT (fig. 4, right lower panel) was found to be increased with the mTBI range exposure (100 kPa BOP, p= 0.04 and 130 kPa BOP, p= 0.002).

Disruption of the BBB integrity

The capillary oxidative/nitrosative damage might lead to BBB disruption, and thus we evaluated the alterations of tight junction (TJ) proteins claudin-5, occludin and zonula occluden 1 (ZO-1). TJ proteins are the primary functional barrier biomolecules of the BBB. A reduction in TJ protein levels or disruption of the architectural structure of any TJ protein is expected to impair BBB integrity, thereby enhances the chance of immune cell infiltration into the brain for initiation of neuroinflammation.

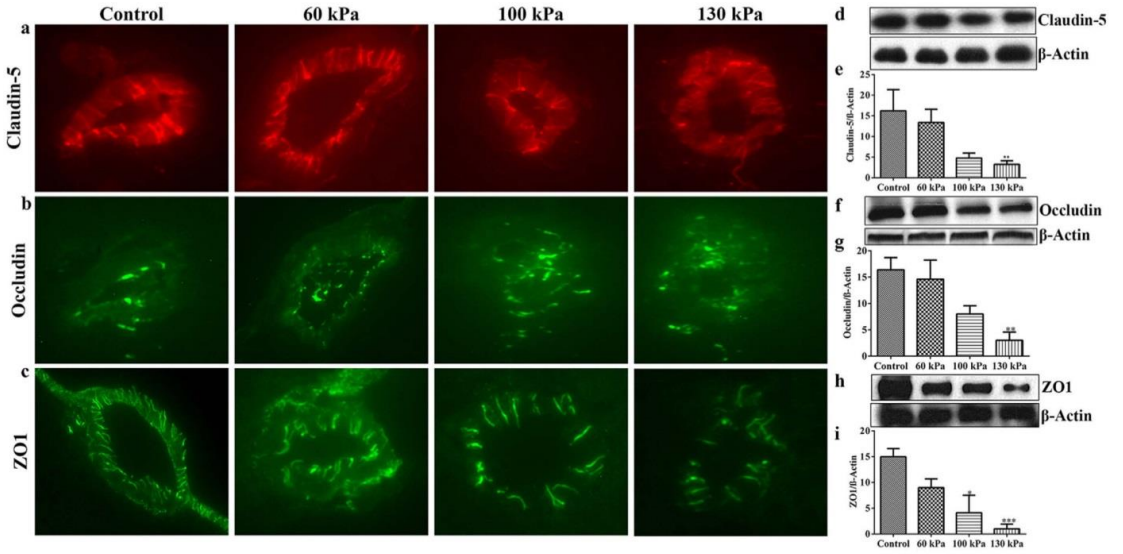


Figure 5 Oxidative/nitrosative damage of capillaries impaired BBB integrity by disrupting the tight junction proteins. (A). A representative of immunofluorescent staining of TJ protein Claudin-5 (a), Occludin (b), and zonula occluden 1 (ZO-1, c) in capillaries of whole brain cross-sections in control and blast exposed animals. (B). Corresponding Western blot and bar graphs showing the quantification of respective immunoreactive fluorescence intensity of Claudin-5 (d, e), Occludin (f, g), and zonula occluden 1 (h, i). Values are mean ± SEM, (n = 4), and asterisk indicates each blast intensity compared with control where level of statistical significance was achieved ($p < 0.05$).

Interestingly, our results showed that mTBI range of blast-wave exposure decreased the levels of claudin-5 (130 kPa, p = 0.001) (fig. 5 a, d, e), occludin (130 kPa, p= 0.002) (fig. 5 b, f, g) and ZO-1 (100 kPa, p = 0.03; 130 kPa, p = 0.0007) (fig. 5 c, h, i) proteins in brain tissue sections. These data suggest possible leakiness of the BBB and it might result in neuronal inflammation around the perivascular region of the brain.

Assessment of BBB leakage

To assess this BBB leakiness and neuronal injury, we examined the leaking out of neuronal-specific enolase (NSE) into the blood samples of tissues exposed at BOP of 60, 100 and 130 kPa versus control animals. In agreement with a decrease in BBB integrity, we observed elevation of NSE levels in blood samples exposed to blast compared with controls (fig. 6). The glycolytic enzyme enolase is a dimeric isoenzymes and also known as neuronspecific enolase (NSE-aa, ag and gg), as these isoenzymes were initially detected in neurons and neuronendocrine cells. However, in other pathological conditions such as small cell lung cancer and neuroblastoma, NSE exhibits a signatory value in disease detection and progression. In the absence of such pathological status or stimuli, as in our experimental setup, NSE detection in plasma samples establishes BBB leakage in primary bTBI.

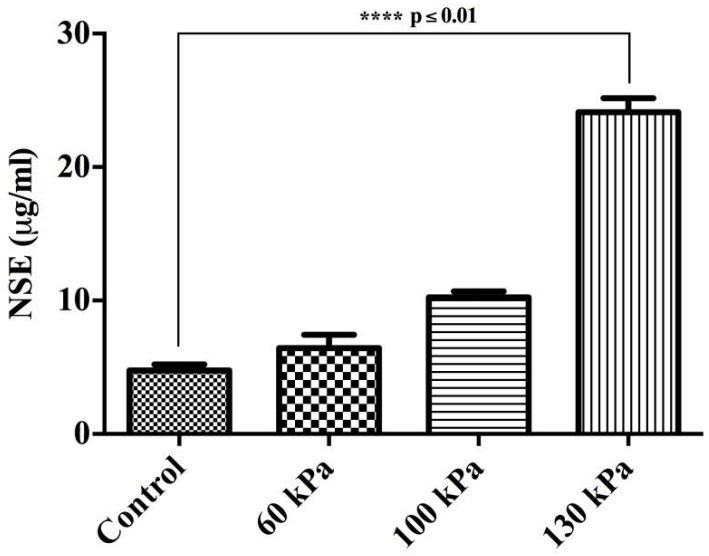


Figure 6 The neuronal damage marker leakage into serum is facilitated by BBB disruption. ELISA results show the levels of neuronal specific enolase (NSE) in rat blood serum collected from a single blast exposure at 60, 100 and 130 kPa. Blood samples were collected at the time of sacrifice after 24 hour post blast exposure. Values are mean ± SEM, n=4. Statistical significance ($p < 0.01$) compared with control was observed at130 kPa as the lowest blast intensity.

Aperio VERSA 200 slide scanner

The installation of the new casework in the biochemistry suite was completed (under the financial support from internal funds of NJIT). We thus proceeded with the installation of the Aperio VERSA 200 slide scanner (fig. 7). The installation of the hardware and related training sessions were performed in March 2016. Subsequently we have acquired the server to run the database and analysis eSlide Manager. The system was ensured to be fully operational and tested before advancing to the next step, i.e. training program utilizing two acquired analysis algorithms: 1) Area Quantification FL, and 2) Genie. These activities were concurrent with scanning of slides prepared by members of the group to familiarize themselves with the system in order to perform planned research tasks efficiently. To date more than 200 slides with coronal sections were digitized using this system and it will be the core of our activities in the future.

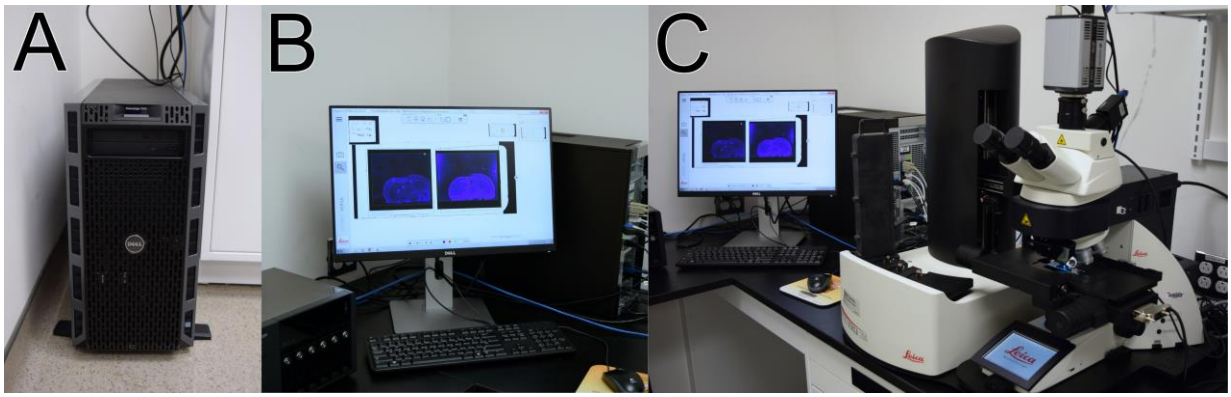


Figure 7 The overview of the new Aperio VERSA 200 slide scanner system: A) Dell PowerEdge T630 server, B) the workstation and NAS used to collect the data, and C) the slide scanner performing data acquisition. The stained and digitized coronal section of the rat brain is visible on the monitor.

Task 2: Determine the Biomechanical Loading of the Rat Brain During Simulated Blast

Correlation of intracranial pressure during blast exposure and brain injury mechanisms is ongoing research topic where many important questions are awaiting to be appropriately addressed. Particularly, due to limited number of studies very little experimental evidence is available in the literature correlating specific brain pathologies with ICP fluctuations. To date most of the experimental work was performed using numerical models and direct ICP pressure measurements. These studies give only limited insight into BINT associated with ICP fluctuations. The contribution to brain injury associated exclusively with skull flexures is a difficult research task aggravated by simultaneous occurrence of other injury factors contributing to increase of ICP during blast exposure.

Experiments performed in our laboratory at University of Nebraska-Lincoln preformed on 10 weeks old Sprague Dawley rats exposed to five discrete blast intensities (130, 190, 220, 250 and 290 kPa peak overpressure) resulted in peak incident-to-ICP ratios in the range of 1.5 to 1.3 for lower and higher peak overpressures (see: Skotak et. al., J. Neurotrauma, 2013, 30, 1147-1160). The FFT analysis of ICP profiles indicated the oscillations have harmonic characteristics, with low (5-10 kHz) and high frequency component (10-20 kHz). The high frequency oscillations were linked to observed mortality (animals exposed to 190, 250 and 290 kPa BOP) and we have also observed the maximum ICP frequency increases with increasing blast intensity: 13 kHz (190 kPa), 16 kHz (250 kPa) and 19 kHz (290 kPa).

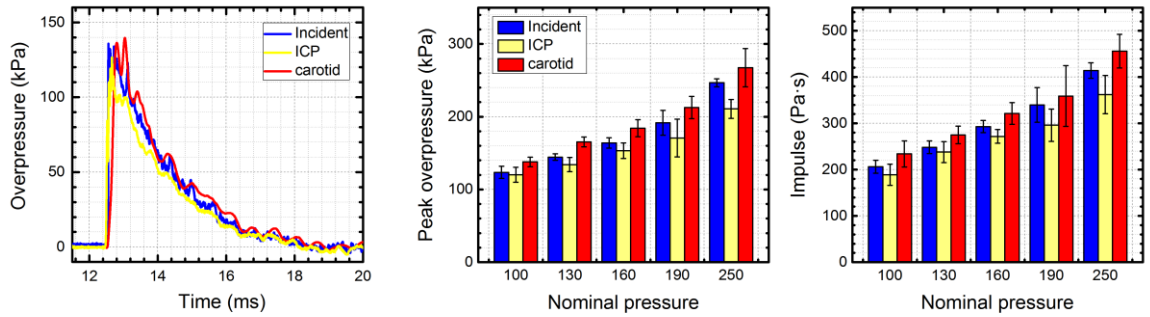


Figure 8 Representative pressure profiles measured by incident (loading), intracranial and carotid artery sensors (response) implanted in a rat exposed to 130 kPa of nominal blast intensity. Biomechanical loading was performed at 5 blast overpressures in the 100 to 250 kPa range and peak overpressure (middle panel) and impulse (right panel) were quantified.

However, our measurements performed on a cohort of 6 animals indicate ICP-to-incident pressure ratio is less than 1, which is a strong contradiction of what has been reported in the literature to date (fig. 8). Most likely the methodology differences are responsible for observed discrepancies. We have initiated use of miniature Millar SPR-671 sensors which are only 0.5 mm OD and typically are suitable for measurements of arterial blood pressure. These sensors have robust construction and easily withstand blast exposure, which was confirmed during our experiments. Currently we are performing implantation of sensors using live animals and this might be a contributing factor responsible for observed differences. The sensor size and mounting strategy might also play important role. In the past the sensor was mounted in the forehead, which is not ideal location considering biofidelity of the measurement, i.e. when the skull structure is affected by craniotomy and application of the cement to keep sensor cannula in place, one would expect the pressure transmission of the head to be quite different than when skull and skin layer remain intact. It is also hard to avoid relative motion of the sensor with respect to skull using this strategy, although it might be a general drawback of any sensor implantation methodology due to specificity of blast experimentation. There is always a minute head displacement independently how well the restrain system is designed and performed.

Task 3: Numerical Simulation of Brain Injury

Rat head and shock tube models

High resolution MRI and CT datasets of a male Sprague Dawley rat were used in combination to generate a three dimensional rat head model. Separate T2-weighted MRI scans for muscle and skin, and brain, along with a CT scan for skull and bones were used to achieve proper contrast and segmentation of various tissues (i.e. muscle, skin, brain, skull and bones). Brain MRI consisted of an isotropic resolution of 256 x 256 x 256 pixels, for a field of view of 30 mm in all three directions. MRI for muscle and skin has an anisotropic resolution of 512 x 512 x 256, for a field of view of 30, 30 and 50 mm respectively. The three datasets where overlapped, registered, segmented and triangulated using Avzio 6.2. The triangulated surface mesh was imported to Hypermesh, and a volume mesh consisting of 10-node tetrahedron element was generated from the imported mesh. The generated interface is shared between the brain, skin and skull regions, which are treated as Lagrangian elements. The model is then imported to Abaqus 6.13, and inserted into the shock tube model. The generation and propagation of blast waves are modeled in the shock tube environment. The air inside the tube, in which the blast wave propagates, is modeled with Eulerian elements. The size of the tube corresponds to the physical dimensions of the shock tube used in experiments, with the cross section 229 x 229 mm. The tube was subjected to biased meshing (mesh size varying from 100mm to 5mm), with the highest density of fine meshes in the region encompassing the rat head (the test section) and coarse mesh elsewhere, to reduce the total number of elements in the model, without sacrificing accuracy.

Loading, Interface and Boundary Conditions

The rat head model is subjected to the blast wave from the frontal direction. A complete model of the shock tube, with the driver, transition and extension section, reproducing the burst, expansion and development of the blast wave would be time consuming and computationally taxing, thus less efficient for this research task. Hence, a partial model with experimentally measured pressure-time profile is used as the pressure boundary condition as well as the input,

with the numerical model including only downstream flow field including test specimen section of the shock tube.

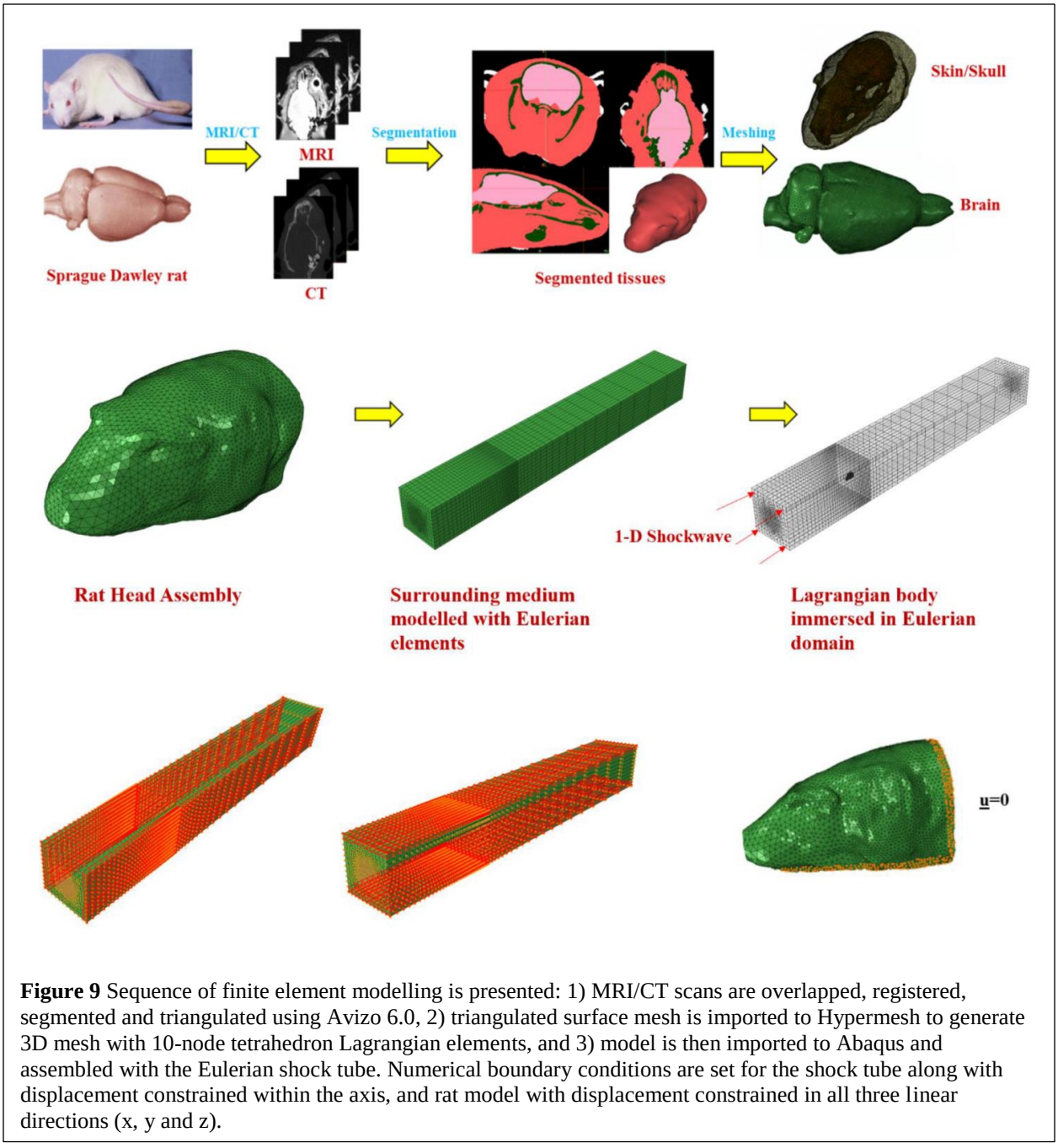


Figure 9 Sequence of finite element modelling is presented: 1) MRI/CT scans are overlapped, registered, segmented and triangulated using Avizo 6.0, 2) triangulated surface mesh is imported to Hypermesh to generate 3D mesh with 10-node tetrahedron Lagrangian elements, and 3) model is then imported to Abaqus and assembled with the Eulerian shock tube. Numerical boundary conditions are set for the shock tube along with displacement constrained within the axis, and rat model with displacement constrained in all three linear directions (x, y and z).

Except for the face on which the pressure is acting, velocity perpendicular to all other remaining faces of the shock tube is kept at zero, to avoid leaking of air from the shock tube. This configuration also maintains a planar shock front travelling in the longitudinal direction with no lateral flow. The interface between all components (skin, skull and brain) of the rat head was modelled as tied (i.e. no tangential and sliding) contact. The nodes on the bottom and rear faces of the rat head is constrained in all degrees of freedom.

An enhanced immersed boundary method is used to provide the coupling between the Eulerian and the Lagrangian domains. Here, the Lagrangian region resides fully or partially within the Eulerian region and provides *no-flow* boundary conditions to the fluid in the direction normal to the local surface. Further, the Eulerian region provides the pressure boundary conditions to the Lagrangian region. Thus, a combination of fixed Eulerian mesh and solid-fluid interface modeling through the enhanced immersed boundary method allows for the concurrent simulations of the formation and propagation of a primary blast wave in a fluid medium and accounts for the effects of both fluid-structure interaction and structural deformations once the blast wave encounters a solid. The interactions (contact conditions) between Eulerian (containing air and a propagating blast wave) and Lagrangian regions are defined using 'general contact' feature (card) in Abaqus. In general, contact constraints are enforced through the penalty method with finite sliding contact formulation. Various contact property models are available in general contact. In the present work, frictionless tangential sliding with hard contact is used as the contact property model.

Solution Scheme

The finite element model is solved using the nonlinear transient dynamic procedure with the Euler-Lagrangian coupling method (Abaqus 6.13). In this procedure, the governing partial differential equations for the conservation of momentum, mass and energy along with the material constitutive equations and the equations defining the initial and boundary conditions are solved simultaneously.
Conservation of mass (continuity equation):

$$\rho \frac{\partial v_i}{\partial x_i} + \frac{\partial \rho}{\partial t} + v \cdot \nabla \rho = 0 \qquad (1.5)$$

Conservation of momentum (equation of motion):

$$\frac{\partial \sigma_{ij}}{\partial x_j} + \rho b_i = \rho a_i \qquad (1.6)$$

Conservation of energy (energy equation):

$$\rho \frac{\partial e}{\partial t} + v \cdot \nabla e = \sigma_{ij} \frac{\partial v_i}{\partial x_j} - \frac{\partial q_i}{\partial x_i} + \rho q_S \qquad (1.7)$$

where, ρ is density, x, v and a are displacement, velocity and acceleration of a particle respectively, σ is Cauchy stress, b is body force, e is internal energy per unit mass, q is heat flow per unit area and q_S is rate of heat input per unit mass by external sources.

In Eulerian-Lagrangian method, the whole model is solved (i.e. both Eulerian and Lagrangian domains) with the same Lagrangian equations. The notion of a material (solid or fluid) is introduced when specific constitutive assumptions are made. The choice of a constitutive law for a solid or a fluid reduces the equation of motion appropriately (e.g., compressible Navier-Stokes equation, Euler equations etc.). For the Eulerian domain in the model the results are simply mapped back to the original mesh with extensions to allow multiple materials and to support the Eulerian transport phase for Eulerian elements. Eulerian framework allows for the modeling of highly dynamic events (e.g. shock) which would otherwise induce heavy mesh distortion. In Abaqus, the Eulerian time increment algorithm is based on an operator split of the governing equations, resulting in a traditional Lagrangian phase followed by an Eulerian, or transport phase. This formulation is known as "Lagrange-plus-remap." During the Lagrangian phase of the

time increment nodes are assumed to be temporarily fixed within the material, and elements deform with the material.

Model validation

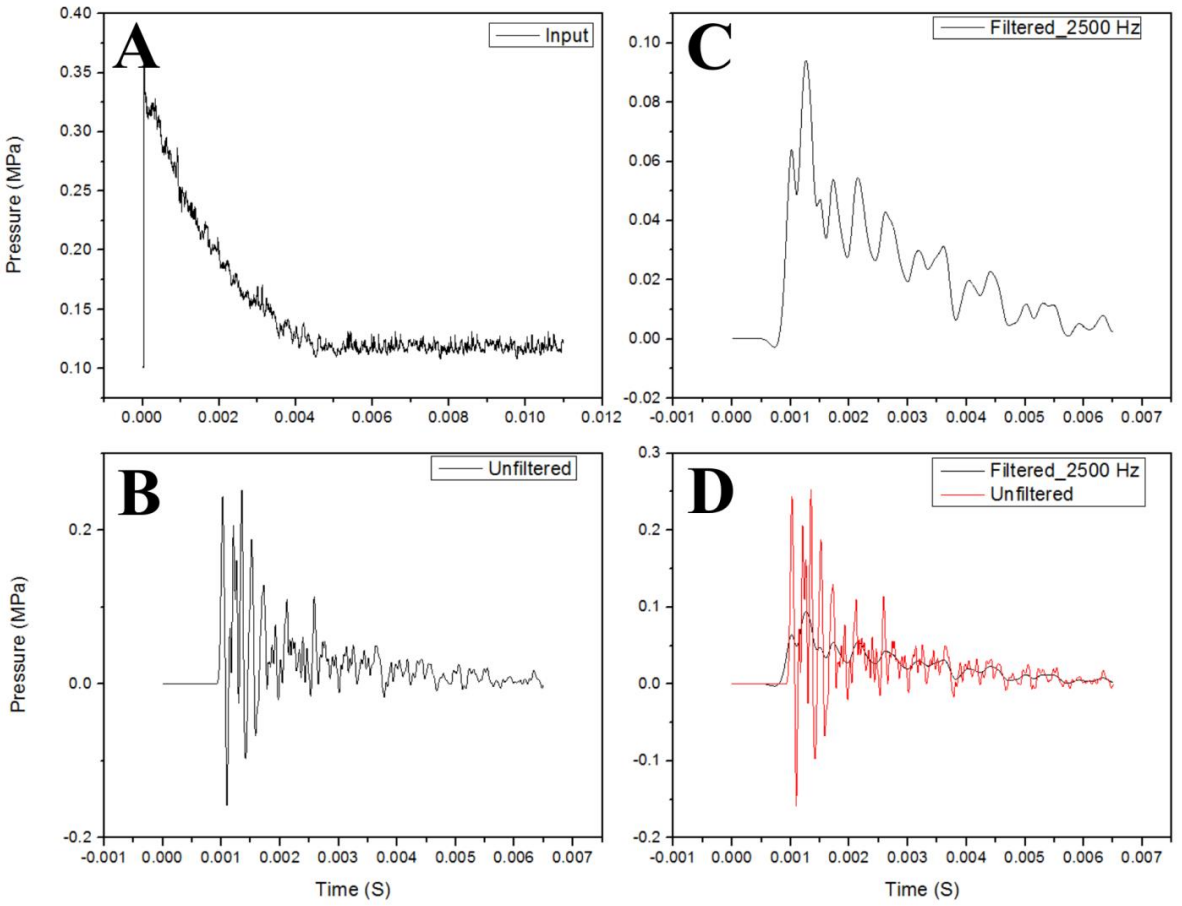


Figure 10 A. Input value (incident pressure), B. Unfiltered result at the hippocampus, C. Filtered using Butterworth filter at 2.5 kHz, and D. Comparison of filtered and unfiltered values.

Results of simulations will require further refinement, since excessive oscillations were observed within the pressure profiles obtained from the hippocampus region (fig. 10). The existence of the oscillations may be due to variation between the geometries of the Lagrangian vs the Eulerian domain, leading to inaccurate transfer of the blast wave from the air into the rat head. In order to eliminate the oscillations, the Butterworth filter was applied, which led to the peak pressure falling by half. Variation of mesh size (5 mm to 1 mm), made the oscillations higher. Also, literature was used to obtain alternate material properties for the skin, skull and brain, which was used for simulations. These modifications had no effect on the results of repeated simulations.

Wave transmission pathways

Numerical model allowed us to identify loading pathways in the rat brain during the blast exposure. As the blast wave impinges the rat, the wave first interacts with the snout and

undergoes diffraction, where it bends and converges towards the eye socket (pathway 1) and on top of the skull (pathway 2). The surface pressure loading along pathways 1 and 2 are transmitted to the rat brain, which start converging towards each other in the region of bregma, lambda and midline sutures. The loading through the snout (pathway 3) does not reach the brain before the transmitted pressure wave from pathways 1 and 2 completely load the brain.

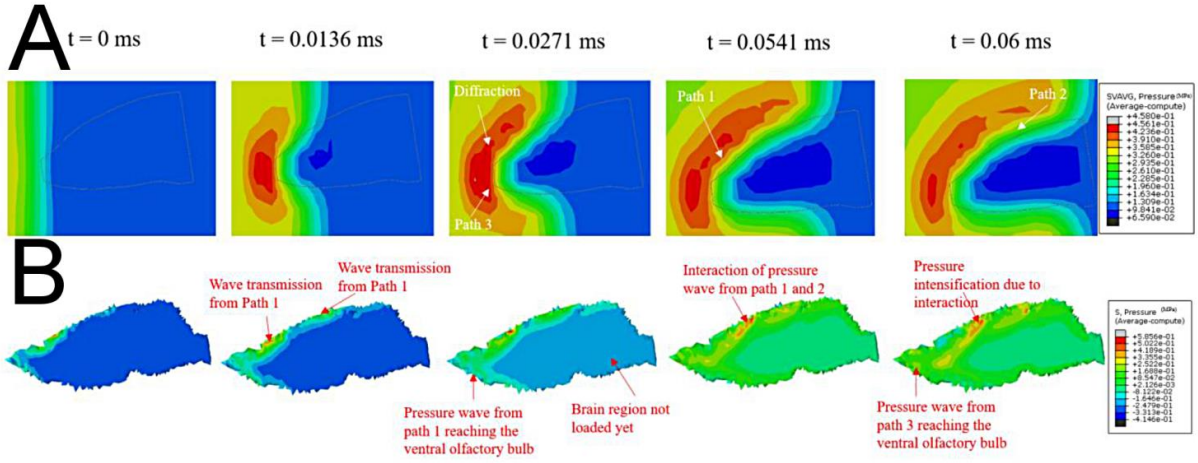


Figure 11: Differential loading of the brain caused by geometry of the rat's head and animal orientation with respect to incident shock wave: A. Pressure contour plots outside the rat head, B. Corresponding pressures inside the brain.

References

1. Skotak, M. *et al.* Rat injury model under controlled field-relevant primary blast conditions: acute response to a wide range of peak overpressures. *Journal of neurotrauma* **30**, 1147-1160 (2013).
2. Chandra, N., Skotak, M., Wang, F., Ganpule, S. & Haorah, J. in J. Neurotrauma, Vol. 30 A80-A80 (2013).
3. Yelveton, J.T. Pathology scoring system for blast injuries. *J Trauma* **40**, S111-115 (1996).
4. Abdul-Muneer, P.M. *et al.* Induction of oxidative and nitrosative damage leads to cerebrovascular inflammation in an animal model of mild traumatic brain injury induced by primary blast. *Free Radic Biol Med* **60**, 282-291 (2013).

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

Tasks in the **Specific Aim 2 (FY2)** include determination of pathologies associated with primary blast exposure corresponding to mild-to-moderate TBI. We are going to use the survival probability dose curve developed in FY1, to identify the blast parameters associated with 50-75-100% survival for detailed pathological and proteomic analysis.

We have initiated extensive biochemical characterization of various brain regions extracted from rats exposed to blast overpressure in the 130-180 kPa range and sacrificed 24 hours post injury (per experimental design included in the proposal). Goals indicated in the Statement of Work for FY2 will be met by utilizing qualitative Western blot analysis of these samples. This analysis will be supplemented with immunohistochemical data of coronal brain sections stained with various immunofluorescent probes to identify major pathological changes. Probing will be performed using approximately 50 sections per brain with signal quantification performed in the entire imaged area. For this task Aperio Versa 200 slide scanner. Additional information regarding the extent of bTBI will be obtained from proteomics analysis: the first series of samples is currently under analysis at facilities in the Cancer Center at Rutgers NJMS.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS

The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals.

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS

List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications:

1. M. Kuriakose, M. Skotak, A. Misistia, S. Kahali, A. Sundaramurthy, N. Chandra, Tailoring the Blast Exposure Conditions in the Shock Tube for Generating Pure, Primary Shock Waves: The End Plate Facilitates Elimination of Secondary Loading of the Specimen, PloS one 11(9) (2016) e0161597.
2. V. Mishra, M. Skotak, H. Schuetz, A. Heller, J. Haorah, N. Chandra, Primary blast causes mild, moderate, severe and lethal TBI with increasing blast overpressures: Experimental rat injury model, Scientific Reports 6 (2016) 26992.

  Books or other non-periodical, one-time publications.

Nothing to report.

  Other publications, conference papers, and presentations.

Conference presentations:
1. D. Younger, M.A.M. Peringady, D. Halder, N. Prasad, N. Chandra, Pathophysiological Changes Due To Blast Induced Neurotrauma Is Effected By Animal Orientation, National Neurotrauma Society Meeting, Lexington, KY, June 26-29, 2016 (J. Neurotrauma, 2016, pp. A87-A87).
2. B. Swenson, C. Miao, N. Chandra, B. Pfister, The Spatial And Temporal Deformation Pattern Of The Brain From Blunt Trauma, National Neurotrauma Society Meeting, Lexington, KY, June 26-29, 2016 (J. Neurotrauma, 2016, pp. A22-A22)
3. S. Kahali, M. Kuriakose, M. Skotak, E. Alay, A. Misistia, N. Chandra, Artifact-Free Loading Conditions In Compressed Gas Driven Shock Tube With End Plate: Focus In Primary Blast Injury Animal Models, National Neurotrauma Society Meeting, Lexington, KY, June 26-29, 2016 (J. Neurotrauma, 2016, pp. A93-A93) *
4. G. Ordek, M. Skotak, N. Chandra, M. Sahin, Electrophysiological Assessment Of Blast-Induced Injury In The Rat Cerebellum, National Neurotrauma Society Meeting, Lexington, KY, June 26-29, 2016 (J. Neurotrauma, 2016, pp. A40-A41)

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Full set of experimental data used for manuscript preparation is provided as a part of publication in Plos ONE: doi:10.1371/journal.pone.0161597.s009

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**

Theses:
1. A. Misistia, „Rodent Testing Device Surrogate for Shockwave Blast Testing", 2015.
2. S. Kahali, "Effect of endplate on the blast wave profile in a compressed gas shock tube", 2015

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name: Namas Chandra
Project Role: PI/PD
Researcher Identifier:
Nearest person month worked: 0.5
Contribution to Project: Design of the research, data interpretation

Name: Maciej Skotak
Project Role: Research Scientist
Researcher Identifier: 0000-0003-2584-7294
Nearest person month worked: 6
Contribution to Project: Exposure of animals, sensor implantation, supervision of students & staff, data analysis (pressure quantification), report preparation

Name: RamaRao Venkata Kakulavarapu
Project Role: Research Scientist
Researcher Identifier:
Nearest person month worked: 2
Contribution to Project: Overseeing western blot and histological analysis, supervision of students and staff

Name: Eren Alay
Project Role: Laboratory technician (instrumentation)
Researcher Identifier:
Nearest person month worked: 4
Contribution to Project: Assistance with exposure of animals, shock wave experimentation and sensor implantation, sensor calibration and instrumentation maintenance

Name: Matthew Kuriakose
Project Role: Graduate Student
Researcher Identifier:
Nearest person month worked: 4
Contribution to Project: Assistance with shock wave experimentation, exposure of animals, biochemistry work, data analysis, manuscript preparation

Name: Anthony Misistia
Project Role: Graduate Student
Researcher Identifier:
Nearest person month worked: 4
Contribution to Project: Assistance with exposure of animals and sensor implantation, RTD development, CAD development, data analysis

Name: Stephanie Iring
Project Role: Laboratory technician (biochemistry)
Researcher Identifier:
Nearest person month worked: 2
Contribution to Project: Western blot, staining of brain sections

Name: Debanjan Haldar
Project Role: Graduate student
Researcher Identifier:
Nearest person month worked: 2
Contribution to Project: Western blot, sectioning of brain sections, data quantification

Name: Daniel Younger
Project Role: Graduate student
Researcher Identifier:
Nearest person month worked: 1
Contribution to Project: Western blot, sectioning of brain sections, data quantification

Name: Sudeepto Kahali
Project Role: Graduate student
Researcher Identifier:
Nearest person month worked: 4
Contribution to Project: Numerical simulations

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

7. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

8. APPENDICES

a. Reprint of the paper published in Scientific Reports

www.nature.com/scientificreports

OPEN

Received: 01 December 2015
Accepted: 27 April 2016
Published: 07 June 2016

Primary blast causes mild, moderate, severe and lethal TBI with increasing blast overpressures: Experimental rat injury model

Vikas Mishra[1,*], Maciej Skotak[1,*], Heather Schuetz[2], Abi Heller[2], James Haorah[1] & Namas Chandra[1]

Injury severity in blast induced Traumatic Brain Injury (bTBI) increases with blast overpressure (BOP) and impulse in dose-dependent manner. Pure primary blast waves were simulated in compressed gas shock-tubes in discrete increments. Present work demonstrates 24 hour survival of rats in 0–450 kPa (0–800 Pa-s impulse) range at 10 discrete levels (60, 100, 130, 160, 190, 230, 250, 290, 350 and 420 kPa) and determines the mortality rate as a non-linear function of BOP. Using logistic regression model, predicted mortality rate (PMR) function was calculated, and used to establish TBI severities. We determined a BOP of 145 kPa as upper mild TBI threshold (5% PMR). Also we determined 146–220 kPa and 221–290 kPa levels as moderate and severe TBI based on 35%, and 70% PMR, respectively, while BOP above 290 kPa is lethal. Since there are no standards for animal bTBI injury severity, these thresholds need further refinements using histopathology, immunohistochemistry and behavior. Further, we specifically investigated mild TBI range (0–145 kPa) using physiological (heart rate), pathological (lung injury), immuno-histochemical (oxidative/nitrosative and blood-brain barrier markers) as well as blood borne biomarkers. With these additional data, we conclude that mild bTBI occurs in rats when the BOP is in the range of 85–145 kPa.

Exposure to blasts is one of the leading causes of trauma experienced by military personnel as a result of widespread use of high explosives. Our unique animal models of primary blast waves generated in compressed gas shock tubes in discrete increments shows that 24-hour survival of animals depends on the magnitude of blast overpressure (BOP)[1,2]. Blast-induced traumatic brain injuries (bTBI) are classified as primary, secondary, tertiary, and quaternary[3–5], based on the type of biomechanical loading. An incident pressure of a shockwave on the body within the time duration of few-to-ten milliseconds causes primary blast injury, while secondary blast injuries are caused by impact of high-velocity fragmentation and debris. Tertiary blast injuries result when the body is violently accelerated and is forced to impact other objects. Quaternary blast injuries are caused by exposure to heat and toxic gases released resulting from explosive detonation[6]. The present study is focused on primary bTBI that was recognized as a separate neuropathological condition during the World War I and dubbed as 'shell shock'[7]. Soldiers afflicted with shell shock in the battle field sustained plethora of neurological deficits (or even death) without any visible injuries long after the shelling had ended[8].

The emergence of bTBI among active military personnel in recent Iraq and Afghanistan war gained considerable attention and remains an important public health problem[3,9,10]. In the past decade, the Department of Defense reported more than 200,000 head injuries due to combat-related incidents and in non-deployed environment[11]. The severity of brain injury is clinically classified as mild[12–14], moderate[15–18], severe[3,19–23], and vegetative state TBI[3] as per 15-point Glascow Coma Scale (GCS) in humans (including blast TBI cases[24–26]). Over 150,000 of these head injured military personnel were diagnosed with mild Traumatic Brain Injury (mTBI) and Post-Traumatic Stress Disorder (PTSD) exhibiting a wide range of neurological and psychological symptoms[4,5]. Blast mTBI is

[1]Center for Injury Biomechanics, Materials and Medicine (CIBM3), Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ 07102-1982, USA. [2]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, 68198, NE, USA. *These authors contributed equally to this work. Correspondence and requests for materials should be addressed to J.H. (email: james.haorah@njit.edu) or N.C. (email: namas.chandra@njit.edu)

b. Reprint of the paper accepted for publication in Plos ONE

PLOS | ONE

RESEARCH ARTICLE

Tailoring the Blast Exposure Conditions in the Shock Tube for Generating Pure, Primary Shock Waves: The End Plate Facilitates Elimination of Secondary Loading of the Specimen

Matthew Kuriakose[☯], Maciej Skotak[☯], Anthony Misistia, Sudeepto Kahali, Aravind Sundaramurthy, Namas Chandra*

Center for Injury Biomechanics, Materials and Medicine (CIBM[3]), Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ, 07102–1982, United States of America

☯ These authors contributed equally to this work.
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OPEN ACCESS

Citation: Kuriakose M, Skotak M, Misistia A, Kahali S, Sundaramurthy A, Chandra N (2016) Tailoring the Blast Exposure Conditions in the Shock Tube for Generating Pure, Primary Shock Waves: The End Plate Facilitates Elimination of Secondary Loading of the Specimen. PLoS ONE 11(9): e0161597. doi:10.1371/journal.pone.0161597

Editor: Firas H Kobeissy, University of Florida, UNITED STATES

Received: May 17, 2016

Accepted: August 8, 2016

Published: September 7, 2016

Data Availability Statement: Data are provided within the Supporting Information.

Funding: This work was supported by grant no. 14059001 entitled "Primary Blast Injury Criteria for Animal/Human TBI Models using Field Validated Shock Tubes" received from the U.S. Army Medical Research and Materiel Command.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

The end plate mounted at the mouth of the shock tube is a versatile and effective implement to control and mitigate the end effects. We have performed a series of measurements of incident shock wave velocities and overpressures followed by quantification of impulse values (integral of pressure in time domain) for four different end plate configurations (0.625, 2, 4 inches, and an open end). Shock wave characteristics were monitored by high response rate pressure sensors allocated in six positions along the length of 6 meters long 229 mm square cross section shock tube. Tests were performed at three shock wave intensities, which was controlled by varying the Mylar membrane thickness (0.02, 0.04 and 0.06 inch). The end reflector plate installed at the exit of the shock tube allows precise control over the intensity of reflected waves penetrating into the shock tube. At the optimized distance of the tube to end plate gap the secondary waves were entirely eliminated from the test section, which was confirmed by pressure sensor at T4 location. This is pronounced finding for implementation of pure primary blast wave animal model. These data also suggest only deep in the shock tube experimental conditions allow exposure to a single shock wave free of artifacts. Our results provide detailed insight into spatiotemporal dynamics of shock waves with Friedlander waveform generated using helium as a driver gas and propagating in the air inside medium sized tube. Diffusion of driver gas (helium) inside the shock tube was responsible for velocity increase of reflected shock waves. Numerical simulations combined with experimental data suggest the shock wave attenuation mechanism is simply the expansion of the internal pressure. In the absence of any other postulated shock wave decay mechanisms, which were not implemented in the model the agreement between theory and experimental data is excellent.

c. Quad chart

Primary Blast Injury Criteria for Animal/Human TBI Models using Field Validated Shock Tubes
14059001
W81XWH-15-1-0303

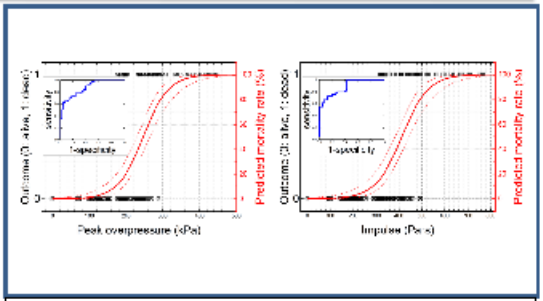
PI: Namas Chandra **Org:** New Jersey Institute of Technology **Award Amount:** 3,330,279

Study/Product Aim(s)

- Develop two master dose-response curves correlating mortality rate with blast overpressure (BOP, 0-450 kPa) and corresponding impulse in 10 wk old Sprague Dawley rats
- Identify and characterize the mild blast TBI thresholds using developed model and biochemistry: characterize oxidative stress, BBB damage and leakiness
- Evaluate pathophysiology of rats in wide range of BOPs (0-450 kPa)
- Develop numerical model of rat brain exposed to a single blast wave

Approach

Rats were exposed to a single blast wave at NJIT in the modular, multi-size shock tube capable of reproducing complex shock wave signatures. We evaluated survival, cardiac and pulmonary pathology following exposure at 60, 100, 130, 160, 190, 230, 250, 290, 350 and 420 kPa peak overpressure, and biochemical assays in 60-130 kPa range.



Accomplishment: Developed two master dose-response curves correlating mortality rates with blast overpressure (BOP) and impulse. These curves set the standard for further testing and the development of the blast TBI injury scale.

Timeline and Cost

Activities / CY	16	17	18	19
Master dose response curves				
Assess mild-moderate bTBI				
Effect of impulse on master curves				
Establish HIC for bTBI (x-species)				
Estimated Budget ($K)	**$1,064**	**$728**	**$773**	**$764**

Updated: September 14th, 2016

Goals/Milestones (3 years only)

CY16 Goal: Develop Master Dose Response Curves for 10 w/o SD rats

- ☑ Evaluate Mortality & Biomechanical Loading in Wide Range of BOP
- ☑ Determine Biomechanical Loading of Rat Brain in Simulated Blast
- ❑ Numerical Simulation of Brain Injury

CY17 Goal: Assess Pathologies of bTBI 24 hours After Exposure

- ☐ Evaluate the Lung Injuries Caused by Blast Exposure
- ☐ Assess Oxidative/Nitrosative Stress, BBB Damage and Inflammation
- ☐ Evaluate Alterations in Brain Proteome After Primary Blast Exposure

CY18 Goal: Effect of Blast Impulse on Master Dose-Response Curve

- ☐ Establish Master Impulse Dose-Response Curve at Three BOPs
- ☐ Protein Expression Due to Changes in Blast Impulse
- ☐ Effect of Changes in Impulse on Loading in the Rat Brain

Comments/Challenges/Issues/Concerns

- Timeline change: N/A.
- Spending change: N/A.

Budget Expenditure to Date

Projected Expenditure: 100%
Actual Expenditure: 75%