J Med Syst (2011) 35:765–770
DOI 10.1007/s10916-010-9464-5

ORIGINAL PAPER

Modeling Blast Induced Neurotrauma in Isolated Spinal Cord White Matter

Sean Connell · Hui Ouyang · Riyi Shi

Received: 19 October 2009 / Accepted: 18 November 2009 / Published online: 30 March 2010

Abstract Blast-induced neurotrauma (BINT) is a common injury associated with the present military conflicts. Exposure to the shock-wave produced from exploding ordnances leads to significant neurological deficits throughout the brain and spinal cord. Prevention and treatment of this injury requires an appropriate understanding of the mechanisms governing the neurological response. Here, we present a novel ex-vivo BINT model where an isolated section of guinea pig spinal cord white matter is exposed to the shock-wave produced from a small scale explosive event. Additionally, we define the relationship between shock-wave impact, tissue deformation and resulting anatomical and functional deficits associated with BINT. Our findings suggest an inverse relationship between the magnitude of the shock-wave overpressure and the degree of functional deficits using a double sucrose gap recording chamber. Similar correlations are drawn between overpressure and degree of anatomical damage of neuronal processes using a dye-exclusion assay. The following approach is expected to significantly contribute to the detection, mitigation and eventual treatment of BINT.

Keywords Neurotrauma · Blast injury · Spinal cord

H. Ouyang · R. Shi (✉)
Department of Basic Medical Sciences, Purdue University,
West Lafayette, IN, USA
e-mail: riyi@purdue.edu

S. Connell · H. Ouyang · R. Shi
Weldon School of Biomedical Engineering, Purdue University,
West Lafayette, IN, USA

Introduction

Blast-induced neurotrauma (BINT) is the signature injury modality associated with the current war efforts and increasing levels of terrorist activity [1, 4]. Exposure to the shock-wave generated by explosive devices results in both traumatic brain and spinal cord injury and is responsible for many of the war related pathologies [6].

Specific sequelae leading to these pathological events, however, are unknown. Furthermore, the magnitude and injury thresholds dictating anatomical and functional loss after BINT are not well-established. Understanding the governing mechanisms requires investigation of the critical link between the impact of an explosive shock-wave and the ensuing damage imparted on neuronal tissue.

Therefore, developing a model of BINT for the accurate reproduction of the primary injury is paramount in elucidating the insult mechanisms. Several experimental models have been designed to study this phenomenon, but are inadequate. For instance, previously designed global *in vivo* blast models analyzed whole body responses, but failed to annotate the physical injury at the tissue level. Global models are also susceptible to confounding factors, including systemic and respiratory reactions that alter the neuronal response [2, 5]. Conversely, *in vitro* models isolate cell systems from the extracellular matrix, which modifies their intrinsic cellular characteristics and significantly affects their connectivity and structural surroundings [7, 8]. Lack of an extracellular matrix or the support of surrounding incompressible fluids alters the response of neuronal cells to mechanical trauma, yielding a biologically irrelevant response to BINT. Therefore, a substantial need exists to improve the methodologies used to investigate the cellular and functional responses within a controlled environment at the tissue level.

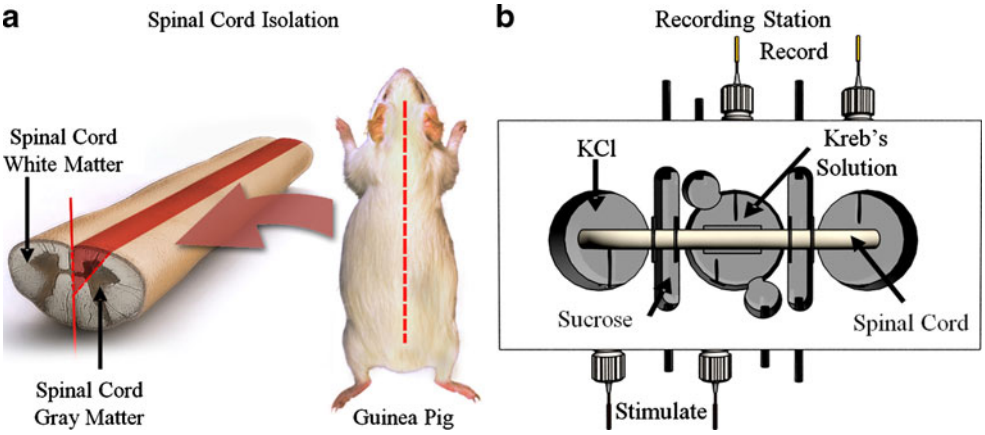

Fig. 1 **a** Spinal cord extraction and isolation of ventral white matter **b** Double sucrose gap recording chamber (*top view*) and isolated section of guinea pig spinal cord white matter depicting stimulus application and recording of evoked CAPs

Here, we address the aforementioned need using a novel *ex vivo* BINT model. The apparatus exposes extracted sections of guinea pig spinal cord white matter to a well-controlled, small-scaled explosive event. The well-understood anatomical layout of the spinal cord and known geometry of the samples allows for precise mechanical, structural, and functional analysis. Additionally, spinal tissue provides a physiologically pertinent environment (i.e. anisotropic tissue with axonal processes, connective tissue and interstitial fluid) with an intact ECM for the precise reproduction of neuronal responses to blast injury. This approach will demonstrate a clear relationship concerning the shock-wave impact, tissue deformation, and resulting anatomical and functional deficits associated with blast neurotrauma.

Materials and methods

Isolation of spinal cord white matter

The experimental protocol used for this study was approved by the Purdue University Animal Care and Use Committee. Twelve guinea pigs, weighing between 250 g and 350 g were anesthetized (ketamine 60 mg/kg and xylazine 10 mg/kg) prior to perfusion with cold oxygenated Kreb's solution (124 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 2 mM $CaCl_2$, 20 mM dextrose, 26 mM $NaHCO_3$ and 10 mM sodium ascorbate). The vertebral column was removed and the spinal cord ventral white matter was carefully excised by cutting through the pedicles longitudinally along the column similar to previously described techniques and shown diagrammatically in Fig. 1a [9, 10]. Prior to testing, the ventral white matter was allowed to recover and equilibrate in continuously oxygenated Kreb's solution for at least 1 h.

Blast induced injury

The primary blast injury was created using a novel blast generator. One-inch sections of shock tubing (NONEL Lead Line with an explosive lining of 0.1 grains/foot composed of tetranitramine (HMX) and aluminum) housed in a hollow aluminum blast nozzle approximately 6 inches in length was detonated with a remote initiator (Wizard Shock Tube Initiator plasma discharge device). Excised sections of spinal cord white matter were secured in the double sucrose gap chamber directly beneath the blast nozzle for continuous monitoring of electrophysiological function, as shown in Fig. 1b and Fig. 2.

The BINT model will address the direct effects of the shock-wave impacting neuronal tissue by creating three

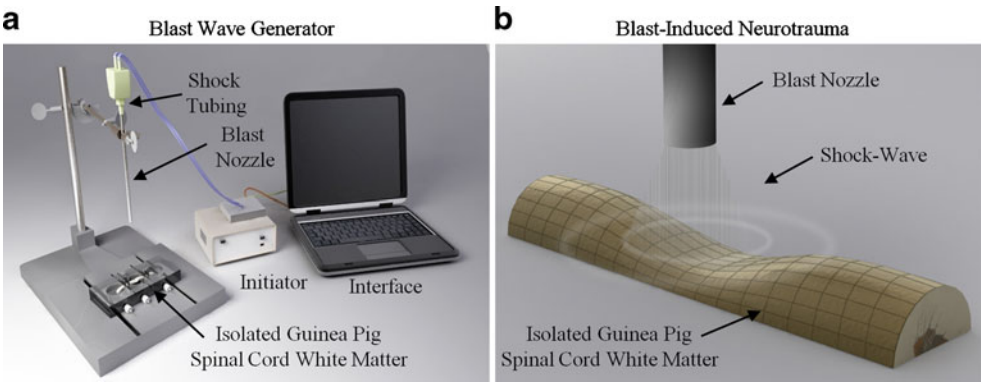

Fig. 2 **a** BINT model consisting of the computer interface measuring electrophysiological function, initiator for detonation and blast chamber containing the recording station and shock tubing housed within the blast nozzle. **b** Detailed view of ensuing deformation from the supersonic shock-wave impacting the excised section of spinal cord white matter

distinct injury levels. According to Hopkinson's Rule, overpressure (i.e. blast intensity) decreases with increasing distance from the epicenter, as demonstrated in Fig. 3. Blast intensity levels were created by calibrating the distance between the spinal cord section and the blast nozzle. Mild blast injury occurred at a distance of 1.75 cm, followed by Moderate exposure at 1.5 cm and finally Severe blast injury at 1.25 cm. Overpressure values for each injury level were determined independently using a dynamic pressure transducer (DPX101 by Omega) mounted normal to the propagating pressure wave.

Electrophysiological recording

Electrophysiological function of excised spinal cords was continuously monitored before and after exposure to a blast injury using a double sucrose gap-recording chamber. (Fig. 1b) Ventral white matter strips were placed across the chamber with the middle of the cord housed in the central compartment under constant perfusion of oxygenated Kreb's solution maintained at 37°C, while either end of the cord rested in the side compartments containing isotonic KCl (120 mM). Both gaps separating the side compartments from the central portion were perfused with sucrose (320 mM) to avoid ion exchange. One end of the cord was stimulated with a 0.3 V constant voltage pulse every 3 s and the corresponding CAPs were recorded from the distal end using Ag-AgCl electrodes. A detailed description of the construction and dimensions of the chamber are reported in previous studies [11].

Membrane integrity and HRP analysis

The HRP exclusion assay was performed as previously described to quantify the degree of membrane damage [9, 10]. Immediately following exposure to blast injury, the

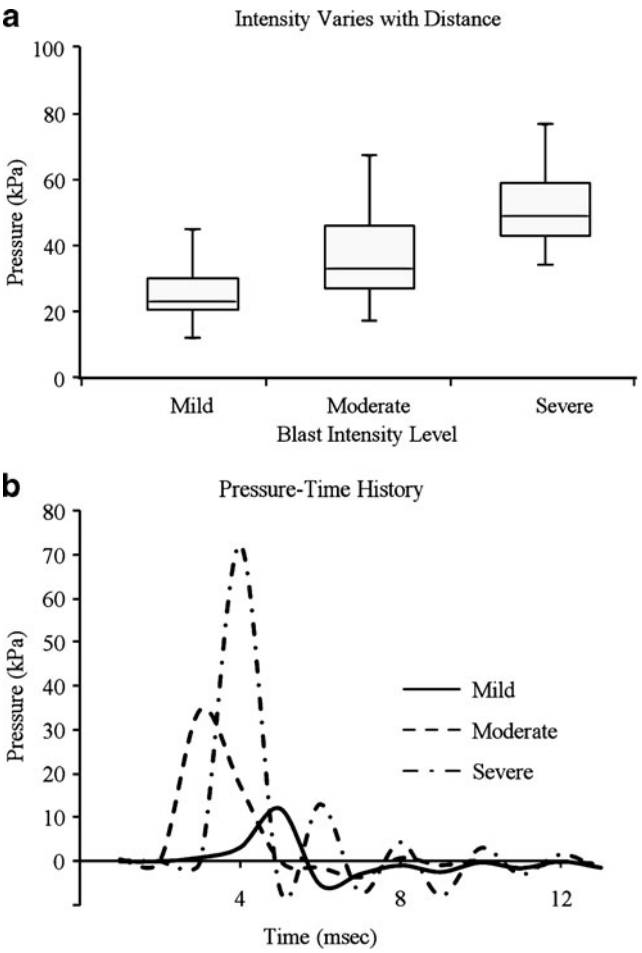

Fig. 4 **a** Peak pressure outputs for Mild, Moderate and Severe levels of blast injury ($p<.05$), $n=8$ **b** Representative pressure-time history for the various shock-wave intensity levels depicting the characteristic Friedlander waveform expected in open-air explosions

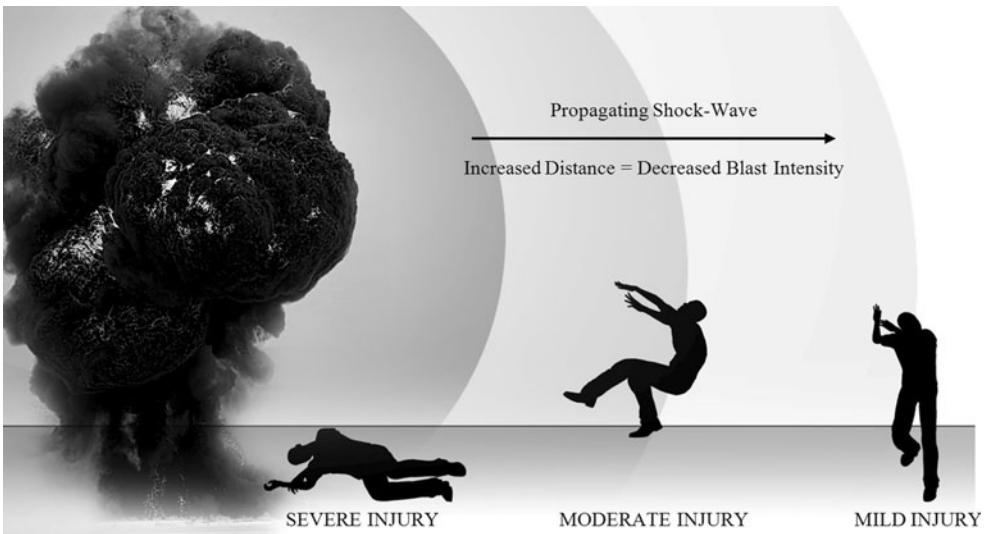

Fig. 3 Blast intensity decreases with increasing distance from the epicenter of the shock-wave. Exposure within the immediate vicinity results in severe injury while exposure at greater distances results in either moderate or mild injury. Correlation between distance and blast intensity was applied to produce three distinct levels of blast injury to analyze the effects of shock-wave intensity on neurotrauma

ventral white matter strips were transferred into an oxygenated Kreb's solution containing 0.015% HRP for one-hour before being placed in a 2.5% glutaraldehyde solution in phosphate buffer for two-hours. Treated spinal cords were then cut into 30 μm transverse sections at the center of the injury site using a vibratome. Sections were processed with diaminobenzidine (DMB) to visualize the degree of HRP uptake in damaged axons. Digital images of HRP-stained sections were used to quantify the total number of stained axons and results are reported as a mean density (axons/mm^2).

Statistical analysis

Statistical significance was determined using one-way ANOVA. Subsequent comparisons were performed using a Tukey test with significance level of $P<0.05$. Values are reported as means±standard error.

Results

Blast induced injury

Peak pressure was calibrated as a function of distance from the ventral white matter segment to produce three distinct blast injury levels. Resulting peak pressure values are shown in Fig. 4a. The three significantly different levels were designated as Mild (1.75 cm at 25.25±3.69 kPa), followed by Moderate exposure (1.5 cm at 38.21±6.26 kPa) and finally Severe exposure (1.25 cm at 52.4±5.33 kPa).

Representative pressure-time histories of shock-waves from each level are shown in Fig. 4b. Plots illustrate the varying magnitudes of the shock-wave and the sharp increase in overpressure immediately following detonation. Peak overpressure is followed by a negative underpressure wave and eventual return to ambient pressure. Traces are representative of the typical Friedlander waveform for open air detonations. Minimal fluctuations in the sinusoidal pressure wave following the initial detonation of the shock tubing is attributed to the equilibration of the force plate on the pressure transducer.

Electrophysiological recording

Loss of neurological function was correlated with electrophysiological deficits by continuously monitoring CAP amplitudes using the double sucrose gap-recording chamber. Percent recovery in CAP amplitudes for each pressure level was determined by comparing pre-and post-blast amplitudes, as reported in Fig. 5a. Increasing pressure levels resulted in a significant decrease in CAP recovery. Percent recovery ranged from 99±10% for Mild exposure, 61.9±7% for the Moderate injury, to 41.1±4.3% recovery for Severe exposure. Application of the increasing shock-wave intensities reveal and inverse correlation with respect to CAP amplitudes. Subsequent plots in Fig. 5b and 5c demonstrate the reduction in amplitude following exposure to a Moderate level blast injury. No significant change in latency was detected (values not reported).

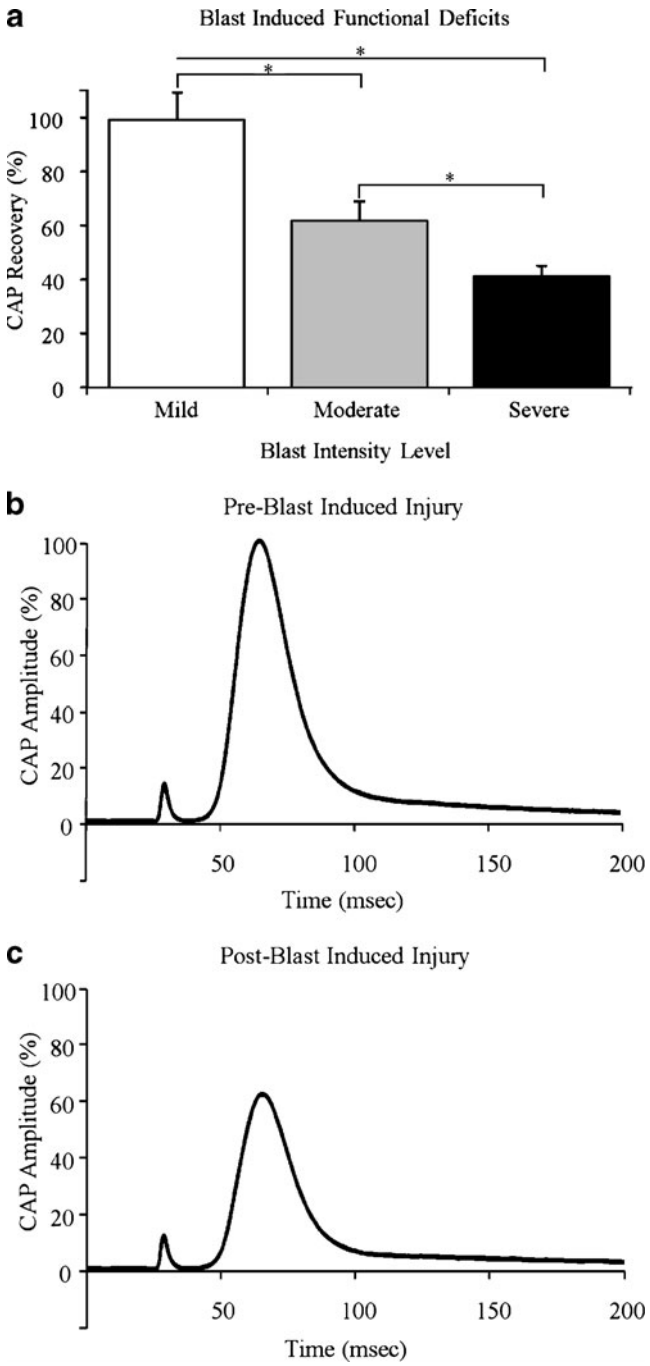

Fig. 5 **a** Functional recovery following exposure to varying degrees of blast injury. ($p<.05$), $n=3$ **b–c** Representative CAPs depicting significant reduction in amplitude of evoked CAPs after sustaining a Moderate blast injury

Membrane integrity and HRP analysis

Anatomical damage was quantified using an HRP-exclusion assay to visualize damaged axons in the spinal cord sections exposed to each level of blast injury. A significant difference between treatment groups was found in comparison to the uninjured control. Exposure to a Severe-level blast injury damaged 2916.45±646.39 axons/mm^2, followed by Moderate exposure at 966.38±356 axons/mm^2, Mild exposure at 335.27±77.64 axons/mm^2 and finally the uninjured control at±1.7 axons/mm^2, as reported in Fig. 6a. Similar to CAP recovery, anatomical damage was found to be inversely related to the degree of blast injury. Photomicrographs for injured ventral white matter sections are depicted in Fig. 6b for visual comparison of injured axons to unstained healthy axons for each treatment group and the uninjured control section.

Discussion

The prevalence of BINT is increasing due to modern war efforts and continuing terrorist activity around the world. As a result, significant research efforts are being directed to the study of this unique injury modality. Basic understanding of the injury mechanisms and pathological response of the CNS are crucial for future treatment and ultimately the prevention of traumatic blast-induced neurotrauma.

The current study expands on this research demand by creating a novel BINT model. As demonstrated, the production of significantly different blast injury levels (i.e. Mild, Moderate and Severe) demonstrates an inverse relationship between the magnitude of the shock-wave and both anatomical and functional deficits.

Based on this evidence and application of the BINT model we postulate the mechanisms responsible for the damage is the high strain rates produced by the impacting shock-wave. Shock-waves, like other pressure disturbances, propagate through space by transferring energy to adjacent particles within the medium. The force created by the collision causes the surrounding material to be deformed, thus transmitting energy and waveform propagation. Similar scenarios might occur when the shock-wave impacts bodily tissue. For example, recent studies demonstrate a substantial portion of the shock-wave and associated energy is absorbed upon impact [3]. Absorption of the shock-wave indicates the possible transmission of force and rapid deformation of neuronal tissue under high strain rates. Subsequent deformation in response to compressive and tensile forces could cause permanent deficits by disrupting

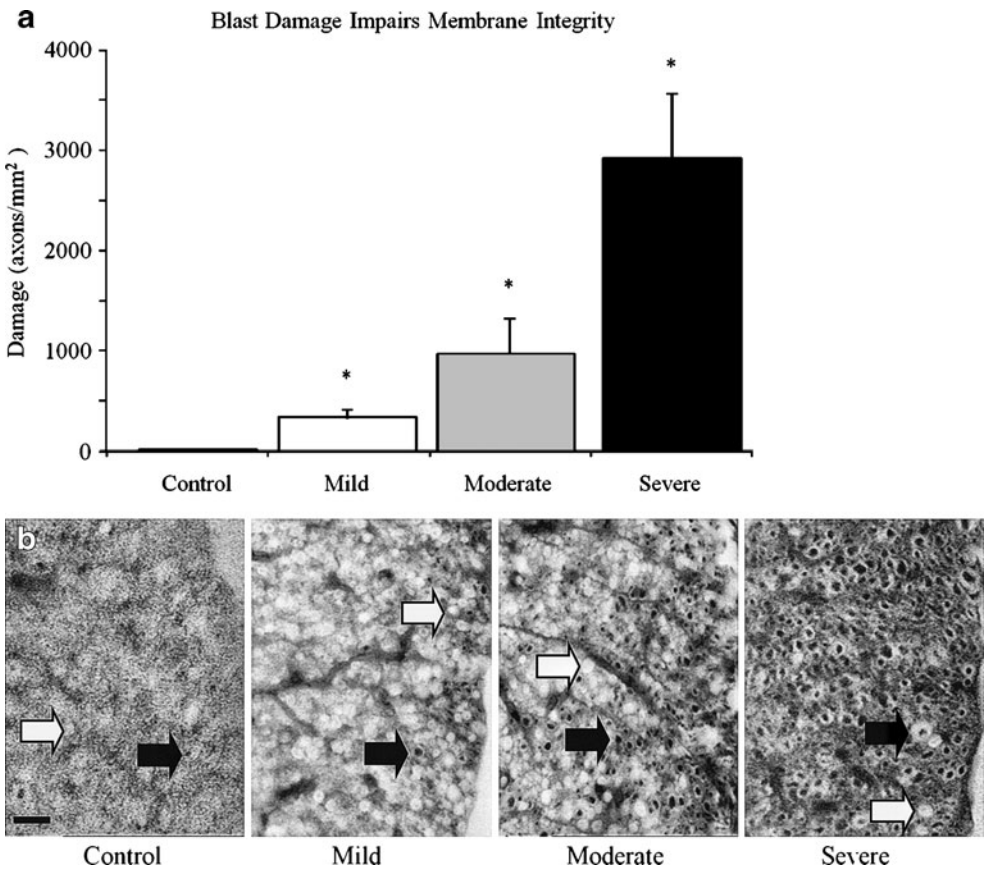

Fig. 6 **a** HRP-exclusion assay for testing membrane integrity of axons for three levels of blast injury based on HRP uptake. '*' Represents significance from control, ($p<.05$), $n=3–5$ **b** Representative photomicrographs of HRP stained sections. Black arrows indicate damaged axons and HRP uptake. White arrows indicate undamaged axons. Scale bar represents 10 μm

the structural integrity of axons and inhibiting the propagation of neuronal signals.

Application of this novel *ex vivo* BINT apparatus will satisfy a major deficiency in existing blast models by creating a precise, reproducible blast injury event in an isolated and simplified environment. This *ex vivo* model will allow for rapid characterization of the injury by mitigating any confounding factors associated with *in vivo* models, a significant advantage when compared to previously established blast models. Thus, allowing for a basic understanding of the key components of blast injury. Currently, no blast injury models exist that can reproduce the injury in such a well-controlled and maintained fashion.

Furthermore, this model will allow for the implementation and testing of various treatment options. Future expansion of this model will include the global response of a blast injury *in vivo* in order to correlate immediate tissue deformation and functional loss to the global onset of symptoms associated with traumatic blast-induced neurotrauma. Future studies will also include a mechanistic approach for the prevention of blast injury in CNS by allowing for the rapid characterization of protective gear, armor or application of future preventative therapies.

Conclusion

BINT is a debilitating injury affecting the livelihood and well-being of afflicted victims. Despite previous efforts, the mechanisms and etiology of the pathological response associated with BINT remain unidentified. Basic understanding of the injury mechanisms and pathological response are crucial for developing future treatments and ultimately preventing blast injury-induced neurotrauma.

The current investigation provides the fundamental tools to analyze damage associated with BINT. Application of the novel model is expected to contribute significantly to the detection, and prediction of blast trauma in the CNS. More importantly, such knowledge will facilitate the preservation of sensory, motor, and cognitive function of the victims through effective medical intervention and increase the post-injury quality of life.

Ackowledgement The authors thank Michel Schweinsberg for the graphic illustrations and Mike Koppes for providing the explosives used throughout the study. The funding for this study was partially provided by the State of Indiana and the Indiana Spinal Cord and Brain Injury Research Fund.

References

1. Argyros, G. J., Management of primary blast injury. *Toxicology* 121:105–115, 1997.
2. Cernak, I., Wang, Z., Jiang, J., et al., Ultrastructural and functional characteristics of blast injury-induced neurotrauma. *J Trauma* 50:695–706, 2001.
3. Chavko, M., Koller, W. A., Prusaczyk, W. K., et al., Measurement of blast wave by a miniature fiber optic pressure transducer in the rat brain. *J Neurosci Methods* 159:277–281, 2007.
4. Jones, E., Fear, N. T., and Wessely, S., Shell shock and mild traumatic brain injury: a historical review. *Am J Psychiatry* 164:1641–1645, 2007.
5. Kaur, C., Singh, J., Lim, M. K., et al., The response of neurons and microglia to blast injury in the rat brain. *Neuropathol Appl Neurobiol* 21:369–377, 1995.
6. Mayorga, M. A., The pathology of primary blast overpressure injury. *Toxicology* 121:17–28, 1997.
7. Murphy, E. J., and Horrocks, L. A., A Model for Compression Trauma—Pressure-Induced Injury in Cell-Cultures. *Journal of Neurotrauma* 10:431–444, 1993.
8. Shepard, S. R., Ghajar, J. B., Giannuzzi, R., et al., Fluid percussion barotrauma chamber: a new in vitro model for traumatic brain injury. *J Surg Res* 51:417–424, 1991.
9. Shi, R., The dynamics of axolemmal disruption in guinea pig spinal cord following compression. *J Neurocytol* 33:203–11, 2004.
10. Shi, R., Asano, T., Vining, N. C., et al., Control of membrane sealing in injured mammalian spinal cord axons. *J Neurophysiol* 84:1763–1769, 2000.
11. Shi, R., and Blight, A. R., Compression injury of mammalian spinal cord in vitro and the dynamics of action potential conduction failure. *J Neurophysiol* 76:1572–1580, 1996.