

Mild Neurotrauma Indicates a Range-Specific Pressure Response to Low Level Shock Wave Exposure

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Abstract—Identifying the level of overpressure required to create physiological deficits is vital to advance prevention, diagnostic, and treatment strategies for individuals exposed to blasts. In this study, a rodent model of primary blast neurotrauma was employed to determine the pressure at which acute neurological alterations occurred. Rats were exposed to a single low intensity shock wave at a pressure of 0, 97, 117, or 153 kPa. Following exposure, rats were assessed for acute cognitive alterations using the Morris water maze and motor dysfunction using the horizontal ladder test. Subsequently, histological analyses of three brain regions (primary motor cortex, the hippocampal dentate gyrus region, and the posteromedial cortical amygdala) were conducted. Histological parameters included measuring the levels of glial fibrillary acidic protein (GFAP) to identify astrocyte activation, cleaved caspase-3 for early apoptosis identification and Fluoro-Jade B (FJB) which labels degenerating neurons within the brain tissue. The results demonstrated that an exposure to a single 117 kPa shock wave revealed a significant change in overall neurological deficits when compared to controls and the other pressures. The animals showed significant alterations in water maze parameters and a histological increase in the number of GFAP, caspase-3, and FJB-positive cells. It is suggested that when exposed to a low level shock wave, there may be a biomechanical response elicited by a specific pressure range which can cause low level neurological deficits within the rat. These data indicate that neurotrauma induced from a shock wave may lead to cognitive deficits in short-term learning and memory of rats. Additional histological evidence supports significant and diffuse glial activation and cellular damage. Further investigation into the biomechanical aspects of shock wave exposure is required to elucidate this pressure range-specific phenomenon.

Keywords—Blast, Neurotrauma, Injury mechanism, Brain.

INTRODUCTION

Blast-related injury is currently a complex problem for the present military and civilian populations. A blast event consists of an explosion which produces an associated pressure or primary blast wave that rapidly expands as it approaches an individual. The primary concern is the effect on the human body which varies depending on the individual's orientation and location from the epicenter of the explosion. The Joint Theater Trauma Registry reported that soldiers participating in Operation Iraqi Freedom and Operation Enduring Freedom (OIF/OEF) from October 2001 through January 2005 experienced a greater proportion of head and neck injuries as compared to other previous conflicts. Improvised explosive devices (IEDs) are reported to account for 78% of injuries, the highest proportion found for any large scale conflict.⁴⁴ In addition, Hoge *et al.* surveyed over 2500 U.S. Army Infantry soldiers and found that 43.9% of soldiers reported loss of consciousness, memory problems (24.6%), concentration problems (31.4%), and irritability (56.8%).²⁵ There is debate to the extent that blast-exposed soldiers are categorically different than patients suffering from traditional blunt trauma injuries.⁴ A victim exposed to a primary blast wave may appear normal at first but can rapidly demonstrate neurological deficits for a period of time following the blast event.¹⁰ These studies indicate that there are neurological alterations sustained by individuals exposed to blast environments. However, the effect that primary blast wave has on the central nervous system (CNS) is less understood

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as compared to other blast-related pathologies such as lung injury.^{4,49} This lack of understanding of the exact mechanism for blast neurotrauma has caused much debate in the scientific community. There is a strong thrust to provide evidence on the effects of blast neurotrauma. Clinically, identifying blast neurotrauma victims who demonstrate no outward signs of injury will lead to more effective treatments for these individuals.

There are several hypotheses proposed in the literature regarding the mechanism of how pressure waves from blast can injure the brain. Ongoing hypotheses include acceleration of the head and blast wave passage either directly through the cranium or indirectly through a pulmonary or vascular mechanism.^{5,8,11,13,14} While computational models have suggested that skull flexure may contribute to blast neurotrauma,^{41,58} a recent report by Bolander *et al.* provided experimental data which demonstrated that shock wave exposure causes a complex multimodal biomechanical response in rats which may play a significant role in blast energy transmission to the brain.⁶ They established that the intracranial pressure developed in the brain during shock exposure correlated with skull surface strain. Importantly, those authors note that the dependency of the transmitted stress will be unique to the skull dynamics of the species. Further understanding of the level of overpressure required for developing neurological changes in animals, or a threshold for mild blast neurotrauma, is vital to the development of mitigating systems to protect against shock wave exposure. Therefore, the purpose of this study was to test a series of shock wave intensities to identify a pressure level that causes acute neurological deficits in the rodent model.

METHODS

Animals and Testing Parameters

Male Sprague–Dawley rats (Harlan Labs, San Diego, CA), approximately 250 g, were used in this study. The rats were allowed to acclimate for a period of 5 days before testing. They were handled by researchers to help diminish fear, and were given food and water *ad lib* while being cycled on a 12-h light/dark schedule. A testing regimen of 16 groups, each containing five rats was designed. These groups each consisted of a different combination of exposure pressure and time to post-exposure neurocognitive testing (Table 1). Since all animals were evaluated within 72 h following injury, the assessments were optimized for the detection of acute neurotrauma. Approval of all the experiments was obtained from the Wayne State University Institutional Animal Care and Use Committee before testing.

TABLE 1. Test matrix indicating exposure pressures and time in which the rats were cognitively tested following exposure. Each group contained 5 animals.

	0 kPa	97 kPa	117 kPa	153 kPa
3 h	5	5	5	5
6 h	5	5	5	5
48 h	5	5	5	5
72 h	5	5	5	5

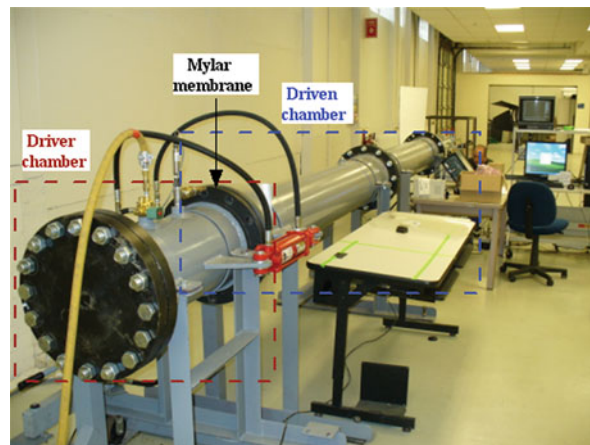


FIGURE 1. Shock wave generator located at Wayne State University.

Shock Wave Exposure

A custom-built 0.305-m diameter shock tube (ORA Inc., Fredericksburg, VA) located at the Wayne State University Bioengineering Center was used for producing the shock front and dynamic overpressure as previously described by Leonardi *et al.*³⁴ (Fig. 1). In brief, compressed helium and Mylar sheets of varying thicknesses (GE Richards Graphics Supplies, Inc., Landisville, PA) were employed to obtain different peak pressures. Pressure sensors (PCB model number 102A06 (max capacity 500 psi), Piezotronics Inc., New York, NY) were systematically placed in the driver sections to collect the incident (side-on) pressure within the tube. An additional pressure sensor (PCB model number 137A22 (max capacity 500 psi), Piezotronics Inc., New York, NY) was placed in the platform holding the rat to most accurately determine the level of incident pressure the rat was exposed to. The sensor distance was 0.0508 m in front of the rat's head. Pressure data were collected at 250 kHz using a DASH HF-HS data acquisition system (Astro-med Inc, WestWarwick, RI). Shock wave profiles were verified through the DASH HF-HS to confirm incident pressures within the shock tube (Fig. 2). Animals were exposed to a single shock wave with an intensity of 0, 97, 117, or 153 kPa.

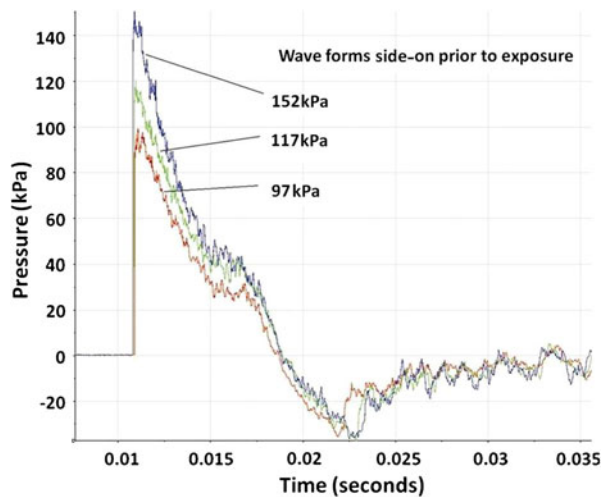


FIGURE 2. Examples of the pressure profiles for the incident shock wave intensities reported from the pressure sensor just below the animal.

Animal Testing Procedure

The rats were anesthetized with 3% isoflurane mixed with 100% oxygen for 4 min, after which they were weighed and placed on a nose cone with the same percent of anesthesia for an additional period of 2 min. During this time, the rat was placed into a custom harness and moved approximately 1.09 m within the tube so that the rat's head would face the shock wave. The rat was restrained in a custom harness located on a sled. The purpose of the sled was so that the rat would not be translated by the dynamic pressure of the shock wave and of the overall gas dynamics that occur within the shock tube, but to reduce the intensity of the loading that the rat was exposed to. Thus, the sled mitigates the dynamic pressure effects. The end of the tube was made of clear Lexan so that high speed video could be taken during the exposure. After a single shock wave exposure, the rat was removed and the time to awaken from anesthesia was recorded. Rats underwent both motor assessment and cognitive testing at the predetermined time point of either 3, 6, 48, or 72 h following exposure.

Sham Exposures

Sham rats were treated in a similar manner as the other animals except that they were not exposed to the shock wave. The shams were then subjected to the same battery of motor and cognitive tests that were undertaken by the exposed rats.

Motor Coordination Testing

The horizontal ladder test was used for assessing the motor capabilities of the rat to ambulate over a given

distance.³⁹ For this test, a horizontal ladder (0.12 m wide by 1.27 m long) was mounted between two platforms with a height of 0.762 m above the ground. The rods on which the rat stepped on had a diameter of 0.00645 m. The distance between the rods was random with the distances not being more than 0.0125 m apart. The rat was placed on the end of one side of the ladder. The rat would then travel to the other end as an escape from being so high off the ground. Following the trial, the rat would then rest for 3 min. A total of three trials were applied for each rat. Performance of the rats was recorded during the motor coordination test. Distance traveled and the number of total slips were reported by a manual count when reviewing the data.

Neurocognitive Testing

The Morris water maze (MWM) test was selected for this study because of its ability to assess spatial learning and memory in the rat. Research groups have utilized this test because it is sensitive to cognitive damage associated with mild brain injury.^{9,38,52,54} Furthermore, a 1-day version of this test has been reported to demonstrate deficits in spatial learning and memory in both mice and rats.^{2,18,19}

A fiberglass pool (1.83 m in diameter) was filled with water, approximately 23 °C, made opaque with black acrylic, non-toxic paint. A platform 0.11 m in diameter was placed in the middle of one of the four designated quadrants, 0.02 m below the water. Data acquisition was performed using Ethovision XT (Noldus Information Technology Inc., Leesburg, VA), a video tracking and analysis software. The trial time and total distance traveled were calculated using this software.

Following shock wave exposure and at the designated time point (Table 1), the rat was placed in one of the three quadrants not containing the platform facing the wall. The order of positions in which the rat was placed in the water, facing the wall, was predetermined to allow for consistent starting points for each rat. After locating the platform, the rat was removed from the pool following an association period of 10 s. Rats that did not find the platform after the maximum trial time of 90 s were placed on the platform for the association period before being returned to their cages. Rats remained in their cages for 5 min before the start of the next trial. Each rat was subjected to four trials.

Glial Fibrillary Acidic Protein and Caspase-3 Immunostaining and Analysis

Immediately after MWM testing, all animals were euthanized by overdose with sodium barbital (200 mg/kg i.p.) and transcardially perfused with saline (0.9%

sodium chloride) followed by fixative solution containing 4% formaldehyde. Brains were removed and stored in a fixative solution containing 15% sucrose. After 48 h, the brains were placed in OCT embedding medium and allowed to freeze on dry ice. The samples were then cut into 40- μ m sections using a microtome. In order to assess neuropathology, we utilized two standard neurohistological parameters. Since reactive astrocytosis occurs prominently in response to most forms of CNS injury or disease⁵⁶ and has been reported to be increased in the hippocampus of blast-exposed animals,^{3,51,57} the level of glial fibrillary acidic protein (GFAP) was determined within the brain tissue sections. Apoptotic cell death is a standard histological assessment in traumatic brain injury (TBI) and was measured by quantifying cleaved caspase-3 (Casp-3), an indicator of early-stage apoptosis.^{45,60} The range of sections analyzed were bregma -2.04 mm to bregma -4.08 mm, and the results are reported as an average of all the sections per group. In order to gauge how diffuse the histological response was, three areas of the brain section were assessed: the primary motor cortex (PMC), the hippocampal dentate gyrus region (DG), and the posteromedial cortical amygdala (PCA). These regions were selected as they play an important role in cognitive and behavioral deficits observed in TBI patients. The PMC is known to play a supporting role when cellular injury occurs to the hippocampus. Any damage to the PMC and the hippocampus (especially the DG) could lead to irreversible damage and cognitive impairment.¹⁶ The DG region of the hippocampus was chosen as it is known to contain stem cells that play a supporting role in injury repair *via* generation of new neurons or astrocytes upon demand.³⁵ The amygdala was examined because it is considered as the fear and anxiety center of the brain. Furthermore, the amygdala has a prominent role on innervating other brain regions such as basal ganglia (mainly hippocampus and nucleus accumbens), motor cortex *via* thalamus, and cerebellum.⁴⁶

In brief, tissue sections were first washed in phosphate saline buffer (PBS) and incubated in 5% gelatin-blocking buffer. Sections were then incubated with a primary antibody (anti-cleaved caspase-3 (Cell Signaling, 1:100) or anti-GFAP (Invitrogen, 1:50)) overnight at 4 °C. Following a PBS wash, the samples were incubated for 1 h with secondary anti-rabbit IgG antibodies (Vector Laboratories, Burlingame, CA). After a PBS wash, samples were placed for 1 h in avidin biotin conjugate (Vector Laboratories), washed with PBS, then incubated in DAB peroxidase substrate (Vector Laboratories) for 5 min. The samples were cleared in xylene, air dried, and coverslipped with Permount (Fisher Inc., Fair Lawn, NJ). Sections were examined at 200 \times on a Zeiss AxioVision microscope,

and analysis was conducted with 20 \times magnification. The number of GFAP+ and Casp-3+ cells within the regions of interest were counted per mm². The Casp-3 and GFAP stainings were scored independently to determine the correlation between individual staining intensity and pressure magnitude.

Fluoro-Jade B (FJB) Staining and Analysis

Hippocampal sections were stained with Fluoro-Jade B (FJB) as described by Schmued *et al.*⁵⁵ to identify degenerating neurons. In brief, tissue sections were incubated in the solution of 1% alkaline (NaOH) in 80% ethanol, and then hydrated in 70% ethanol and distilled water. The sections were then incubated in a solution of 0.006% potassium permanganate, rinsed in distilled water, and incubated in a 0.0004% solution of FJB (Histo-chem Inc., Jefferson, AR). Sections were then rinsed in distilled water, air-dried, and placed on slide warmer until they become fully dry. The dry slides were cleared in xylene and mounted with DPX (Sigma-Aldrich Co. Ltd, St. Louis, MO). Hippocampal sections were examined at 200 \times on a Zeiss AxioVision microscope, and analysis was conducted at 20 \times . The number of FJB+ neurons within the DG region of the hippocampus was counted per mm². FJB+ neurons were manually counted based on the morphology, size, fluorescent intensity, and location of the staining. Neurons with less intensity of the staining were not counted.

Statistical Analysis

Analysis of total slips was completed using a two-way analysis of variance (ANOVA) comparing the combined effect of time point and distance. Distance, latency, and velocity required for the rat to reach its target during the MWM were measured. The MWM data were averaged for trials 2–4. It was expected that uninjured rats would be able to find the target more efficiently and would therefore have lower average values after the initial introduction phase (trial 1). Comparison between the means at each time point was achieved by ANOVA, and *post-hoc* comparisons of the exposure pressures to the shams at a specific time point were achieved by Dunnett's test. In addition, tests for equal variances were achieved before statistical analysis to determine if ANOVA would be appropriate given small sample sizes. Analysis of the MWM was carried out using SAS JMP (SAS Institute, Cary, NC). Significance was determined at ($p < 0.05$) and was reported as mean \pm standard error of the mean (SEM). Histological statistical analysis was calculated with a two way ANOVA followed by a *post-hoc* LSD test with significance achieved with ($p < 0.05$). Data were reported as percent of sham \pm error percent.

RESULTS

Horizontal Motor Coordination Test

The results for the horizontal ladder test were not significant in determining performance differences for rats exposed to different pressure intensities, including shams. The number of slips between groups was not great enough to indicate large enough differences to be significant. The p -value of the ANOVA was 0.8255.

Morris Water Maze

Latency or the time required for the animals to find the goal platform following exposure to 117 and 153 kPa was significantly increased ($p < 0.05$) over shams. The largest effect was found 48 h after exposure (Fig. 3). This indicated that the rats exposed to a 117 kPa shock wave swam the greatest distances within the pool without achieving success, which indicated neurological dysfunction. In addition, these same animals were found to have an increased swim speed as compared to all other groups ($p < 0.05$) (Fig. 4). This association was only found at the 48-h time point.

Histological Assessment

Astrocyte Reactivity Measured by GFAP Elevation

In order to validate astrocytic changes within the brain tissue, three regions of the tissue sections were examined for GFAP. It was found that astrocyte reactivity was elevated at specific blast magnitudes and specific time points. Overall, animals exposed to an overpressure of 117 kPa had an increase of GFAP expression throughout the time evaluated. Interestingly, the level of reactivity varied between the regions, with the PMC having the highest level of activation 3 h

following exposure (Fig. 5). While GFAP expression remained significantly higher at six and 72 h following exposure in the PMC ($p < 0.05$), the level was reduced as compared to the early time point. Expression in the PCA followed the same temporal expression pattern as in the PMC, while the DG only demonstrated an early response to the shock wave exposure. Interestingly, there was a decrease in GFAP levels at 48 h in all regions which may be linked with the increase of apoptotic cells or neuronal degeneration found at 48 h.

Early-stage Apoptosis Measured by Cleaved Caspase-3 (Casp-3)

A diffusely elevated Casp-3 expression was found as compared to sham. Specifically, animals exposed to a 117-kPa shock wave were noted to have a high level of cell death in all three areas of the brain examined ($p < 0.05$) (Fig. 6). Peak Casp-3 expression was found at 3 h following exposure in all regions, with the PCA reporting the greatest level. Furthermore, PCA was found to have significantly higher levels of Casp-3+ cells at all time points observed demonstrating a persistent level of apoptosis up to 72 h following exposure. In addition, it was found that the animals exposed to 153 kPa had a delayed expression of Casp-3 in the PMC and the PCA, which became significant as compared to sham at 72 h ($p < 0.05$).

Neurodegeneration Evaluated by Fluoro-Jade B (FJB)

Neuronal degeneration was evaluated in the DG to link cognitive deficits to neuronal injury. Figures 7 and 8 depict the number of neurons degenerating per mm^2 which was significantly increased after blast exposure when compared to sham. All three pressure groups were significantly higher than sham at 3, 48, and 72 h following blast. The most significant elevation was found in the 117-kPa group at 48 and 72 h ($p < 0.01$).

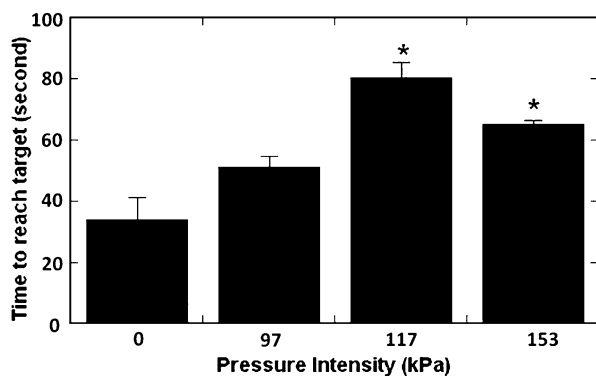


FIGURE 3. Animals were tested using a one-day Morris water maze. Animals exposed to either a 117 or 153 kPa shock wave took significantly longer to complete the task 48 h following exposure as compared to the sham group (* $p < .05$).

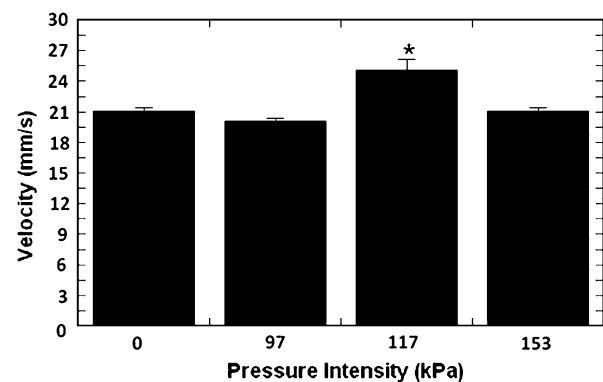


FIGURE 4. Velocity (mm/s) of the animal's swim was measured during the Morris Water Maze assessment. Animals exposed to a 117 kPa shock wave swam significantly faster than other groups 48 h following exposure (* $p < .05$).

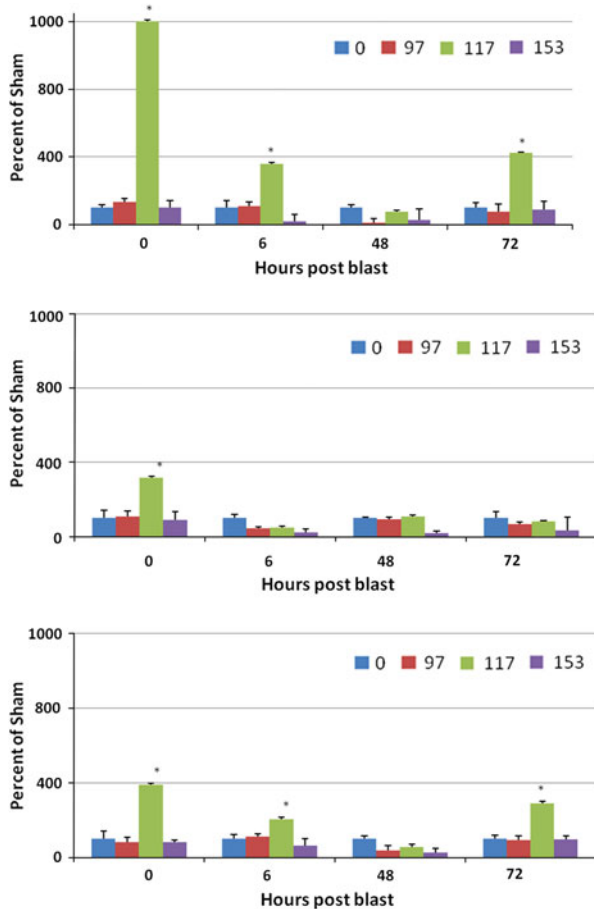


FIGURE 5. Astrocyte reactivity measured by GFAP expression was significantly higher in the cortex (top), DG region of the hippocampus (middle), and amygdala (lower) of the 117 kPa group as compared to sham ($*p < 0.05$).

The two-way ANOVA indicated that pressure ($p < 0.001$), and combined pressure and time point ($p < 0.001$), resulted in significant differences where time point ($p = 0.8689$) did not.

DISCUSSION

A thrust for advanced research efforts to understand the injury mechanisms and subsequent pathophysiology of blast neurotrauma are underway. The current study was conducted to determine an overpressure injury threshold for mild blast neurotrauma based on neurocognitive and histological changes in the rodent model. Based on the combination of cognitive and histological data, it was demonstrated that learning and memory impairment after shock wave exposure may be associated with cellular injury and death in key brain regions that initiate and support cognitive functions. Kamnaksh *et al.*²⁸ found similar levels of elevated GFAP and apoptosis within the amygdala, hippocampus, and prefrontal cortex of rats exposed to

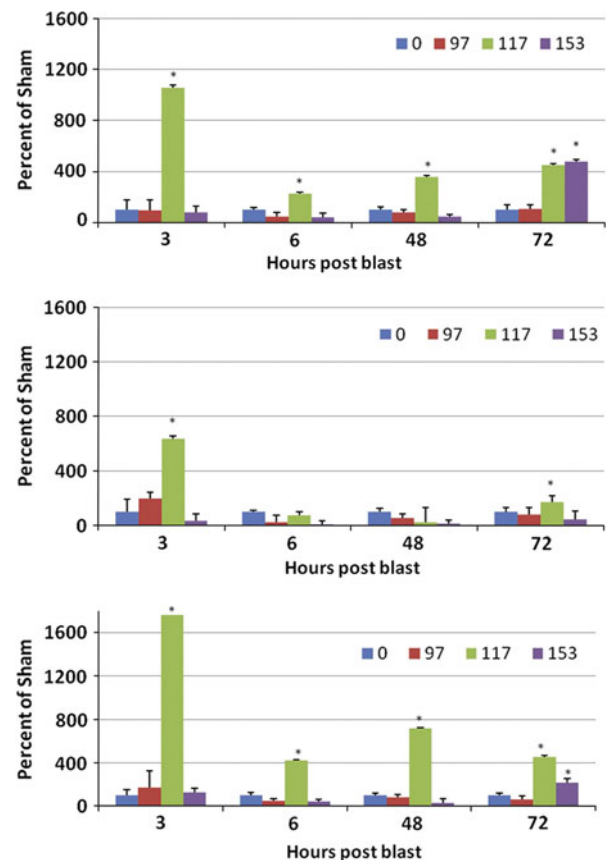


FIGURE 6. There was a significant increase in the number of apoptotic cells measured by cleaved caspase-3 positive (casp+) cells in the cortex (top), DG region of the hippocampus (middle), and amygdala (lower) of the 117 kPa group over sham ($*p < 0.05$).

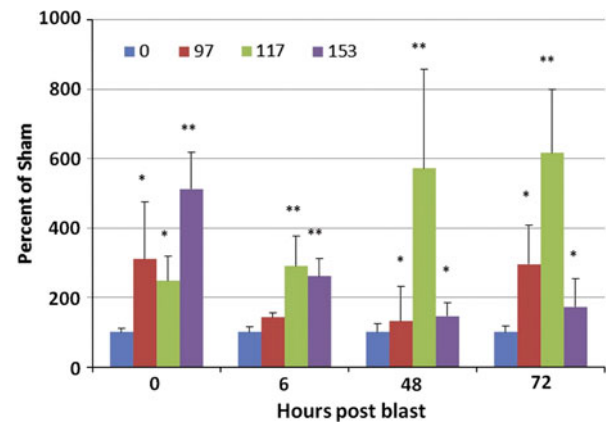


FIGURE 7. The number of degenerating neurons within the DG was measured by Fluor-Jade B (FJB) positive cells. All animals exposed to a shock wave demonstrated a significantly higher number of FJB positive cells as compared to sham animals ($*p \leq 0.02$, $**p \leq 0.005$).

mild blast overpressure. While the motor cortex has a fundamental function of controlling movements, evidence is now emerging that cortical areas play a

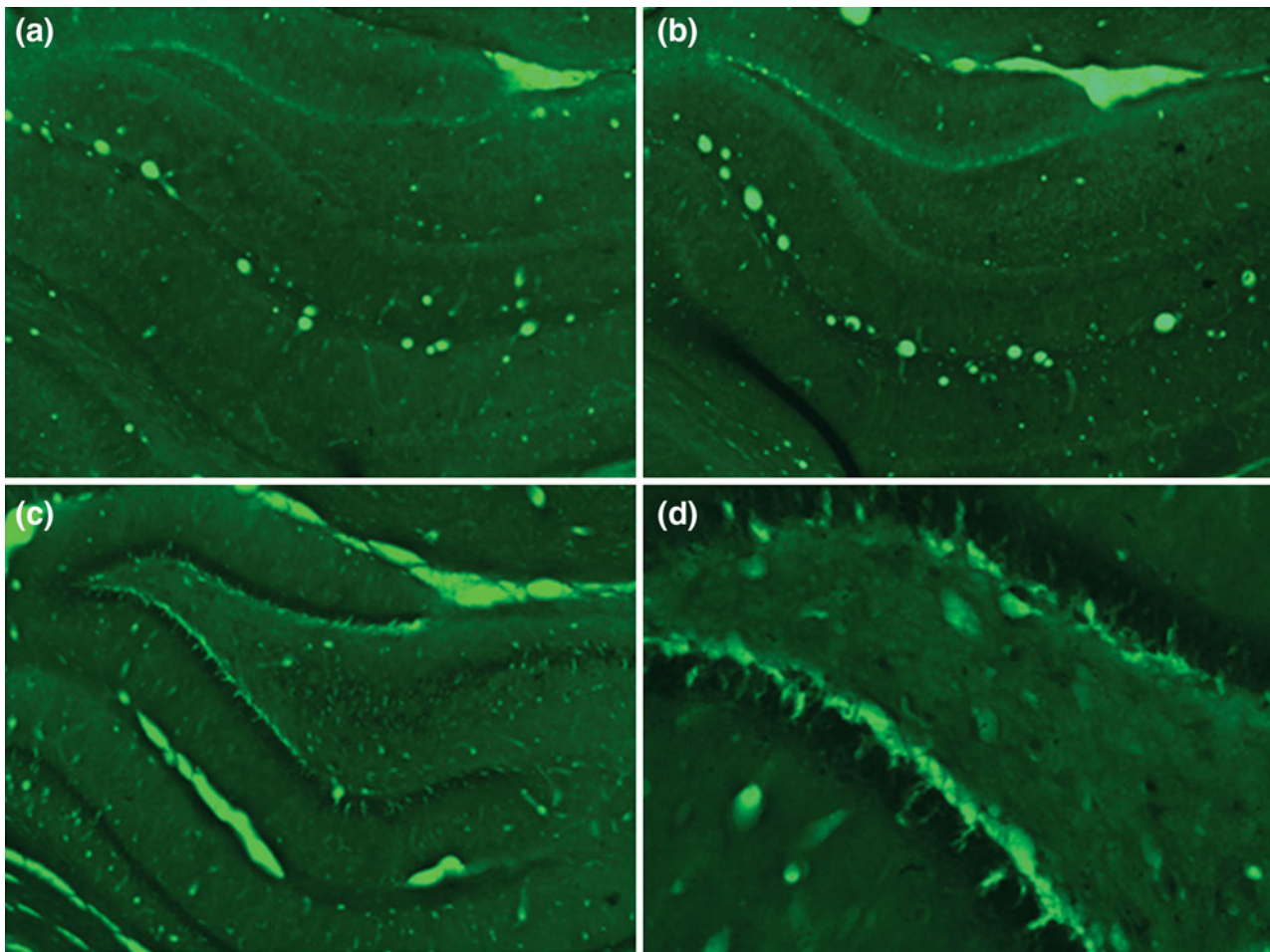


FIGURE 8. FJB positive cells are observed in the DG following exposure to blast. In control animals, there is minimal FJB positive cells (a, 200 \times), as compared to animals at 6 (b, 200 \times) and 48 h (c, 200 \times) post blast exposure. Specific staining is highlighted in the subgranular zone and inner layer of the DG at 48-h post exposure (d, 400 \times).

supporting role in learning and cognition.^{20,53} Furthermore, learning and memory networks link the PMC, DG, and PCA regions. Injury to the motor cortex using the controlled cortical impact and fluid percussion models has demonstrated significant cognitive and histological deficits in animals.^{31,50} The DG region of the hippocampus is where neurogenesis is known to occur throughout adulthood and is associated with cognition and behavior.^{22,29} Neurogenesis helps in the development of the working, spatial, and learning memories. There are many factors that can influence the regulation of the neurogenesis process, including brain trauma, stress, and aging.^{17,23,32} Degeneration of these neural progenitor cells could be a contributing factor of the cognitive issues associated with blast-induced neurotrauma. We found degenerating neurons mainly in the subgranular zone and inner layer of the DG. This could contribute to the memory impairment associated with short-term memory.^{33,36,40} Similar results have been reported within the various rodent models of impact-related TBI in

which the process of neurogenesis was significantly down regulated after injury.^{1,21,24,26,27,42,43,59} It has been shown that, using the cortical contusion model of TBI, the DG region had the highest number of degenerating neurons as compared to the CA1 and CA3 regions of the hippocampus.¹ Using the Marmarou model of TBI, others have demonstrated an association between neuronal degeneration in the hippocampus with cognitive deficits using the Morris Water Maze within 72 h following injury.^{1,15,26} Moreover, animals which have a specific lesion in the DG alone demonstrate a strong correlation between DG cell density and poor performance in the working memory task.²⁴

Studies investigating the link between the hippocampus and the amygdala to cognitive development and repair are well documented.^{47,48} While TBI studies have shown there is significant down regulation of neurogenesis in the DG, anxiety-related studies and cellular stress on the amygdala have also been shown to cause a decrease of neurogenesis.³⁰ Neurogenesis is

believed to occur as a compensatory mechanism for injury repair in the hippocampus and surrounding regions. Direct and indirect neuronal signaling from amygdala and motor cortex circuits to DG could not only effectively increase cellular stress locally but could also lead to decreased neurogenesis. Furthermore, the role of motor cortex in coordination and cognitive outcome is linked with amygdala and DG *via* neuropeptides signaling.^{7,62} These regulatory effects interlinked between these regions profoundly affect cognitive outcome. In this study, it is evident that there is diffuse cellular injury in motor cortex, DG, and amygdala. Clinical and experimental studies commonly describe histological changes in multiple regions of the brain after closed head injuries.^{12,61} The extent of cognitive deficit was able to be estimated from the rats' performance on the MWM. The results of this study demonstrated that animals exposed to a 117-kPa shock wave, notably at the 48-h time point, performed worse overall as compared with other pressure groups and significantly worse than sham animals. Collectively, the histological and cognitive findings suggest that cell death in cognition pathways of the brain may lead to functional deficit after blast neurotrauma.

A unique finding with our dataset was that the mid-range pressure exposure demonstrated to be the most injurious, which is unusual when investigating the concept of injury thresholds. The data indicated that a pressure intensity of 117 kPa produced the overall greatest neurological changes as compared to sham animals. Furthermore, it was noted that this pressure level was more injurious as compared with other pressure exposure intensities. This range-specific susceptibility to mild blast injury is likely the result of a biomechanical response of the skull to the shock wave parameters (i.e., peak magnitude, positive impulse, rate of pressure change, and pressure differentials). Since the exact mechanism of cellular injury from blast is unknown, these parameters of the blast energy need to be evaluated. Bolander *et al.* studied the biomechanical response of the rodent skull during exposure to various shock waves.⁶ The results indicated that a combination of biomechanical interactions developed depending on skull maturity and shock wave intensity. It is possible that given the highly nonlinear viscoelastic nature of the skull–brain interaction with the blast wave, a specific range of loading may be synchronized to generate a more damaging profile of energy transmission. Since the skull–brain interactions are likely to play a key role in the blast energy transmission to the brain cells, it is important to note that the window of vulnerability will most likely be different for other animals and humans. It is expected that different loading conditions will be required to identify a similar range-specific sensitivity to blast. In this study, deficits in both cognitive

performance and histological outcome demonstrated a range-specific injury threshold for rodents exposed to low-level shock waves. Further investigation of the high-rate mechanical properties of the skull and brain tissue will likely elucidate how shock wave energy propagates, helping to isolate the injury mechanism of blast neurotrauma.

CONCLUSION

While reports of blast neurotrauma are increasing, the injury mechanism of brain trauma from blast exposure is unknown, yet highly speculated. With impact-related TBI, there is a large amount of data which indicated that a greater magnitude of insult would be associated with greater neuropathology. However, a threshold for blast neurotrauma and response to increasing intensity are still unknown. Within low-to-moderate shock wave intensities, our data suggest a specific range of shock wave intensity triggered an exaggerated neuropathological response resulting in cognitive and histological deficits. Interestingly, neuronal stem cells within the DG are in various development stages and may be more sensitive to biomechanical and physiological stresses.^{37,63}

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CONFLICT OF INTEREST

No conflicting financial interests exist.

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