Impact of Single Blast Exposure on Neuronal Damage and Protein Levels in the Rat Brain at Varying Pressures

Puru Bansal\(^1\) Dhananjaya I. Bhat\(^2\) Chirag Jain\(^3\) Sarada Subramaniam\(^4\) Anita Mahadevan\(^5\) Dhaval Shukla\(^3\) B. Indira Devi\(^6\)

\(^1\)Department of Neurosurgery, Bansal's Arogya Hospital, Thane, Maharashtra, India
\(^2\)Department of Neurosurgery, RV Aster, Bengaluru, Karnataka, India
\(^3\)Department of Neurosurgery, National Institute of Mental Health and Neuro-Sciences, Bengaluru, Karnataka, India
\(^4\)Department of Neurochemistry, National Institute of Mental Health and Neuro-Sciences, Bengaluru, Karnataka, India
\(^5\)Department of Neuropathology, National Institute of Mental Health and Neuro-Sciences, Bengaluru, Karnataka, India
\(^6\)National Institute for Health and Care Research, University of Cambridge, United Kingdom

Indian J Neurotrauma

Abstract

Introduction Brain injuries from blasts are increasingly common in both civilian and war settings. The impact of blast injuries at different pressure levels remains unclear, and their long-term effects are not well understood. This study investigates how varying blast pressures affect the rat brain over time.

Materials and Methods Forty adult Sprague-Dawley rats were randomized into four groups of 10. Three groups were exposed to blasts, while one served as a control and was only subjected to blast sounds. Each group received a single blast at different pressures, followed by neuropsychological tests. After 28 and 84 days, the rats were sacrificed to measure tau protein and acetylcholine esterase levels and to conduct histological examinations of brain tissues.

Keywords
- blast injury
- traumatic brain injury
- acetylcholinesterase
- rat model
- tau
- visuospatial memory

Results A single blast exposure did not significantly impact visuospatial memory or recall. Despite the lack of noticeable cognitive deficits, histopathological and biochemical analyses revealed reduced tau protein levels, indicating ongoing neuronal damage.

Conclusion While a single blast did not significantly impair visuospatial memory or recall in this rat model, there were decreases in tau protein and acetylcholine esterase levels, along with histological signs of neuronal damage.

ISSN 0973-0508.

© 2024. The Author(s).
This is an open access article published by Thieme under the terms of the Creative Commons Attribution License, permitting unrestricted use, distribution, and reproduction so long as the original work is properly cited.
Introduction

Blasts and blast waves are a common cause of traumatic brain injury in the military, both during training as well as during wartime. Civilians may also get exposed to blasts during terrorist attacks and domestic or industrial accidents.

The pathophysiology, outcomes, and management options for blast-induced neurotrauma (BINT) differs from the more commonly seen impact neurotrauma. The outcomes of blast injury depend on the severity of injury with moderate and severe injuries having fatal or highly morbid outcomes. On the other hand, mild blast injury has a more indolent course without any visible features on imaging, but with the risk of long-term cognitive sequelae. Multiple exposures produce a syndrome quite similar to chronic traumatic encephalopathy and usually occur in military personnel, while single exposure blasts are more common in the civilian population.

This study utilizes a rat model of BINT using a novel, cost-effective method to study the cognitive effects of mild blast injuries at different pressures. The apparatus used to generate the blast shockwave is a novel tube of Indian origin known as the modified Reddy's tube which has been validated by the investigators in an earlier study.

This study considers mild BINT as a unique entity with a distinct pathology. It has some features of diffuse axonal injury and is associated with electrophysiological changes, genetic changes, and systemic changes of autonomic, endocrine, and immunological dysfunction.

Using this model, we have studied the effects that mild BINT has on the retrograde spatial memory of a rat. We have also evaluated the hippocampus for change in tau protein content and acetylcholine esterase (AChE) activity, and the structural damage caused by the blast shockwaves as seen on light microscopy.

Materials and Methods

Forty 2-month-old male Sprague-Dawley rats weighing 200 to 250 g were procured from the Central Animal Research Facility, National Institute of Mental Health and Neuro-Sciences (NIMHANS), Bengaluru, Karnataka, India after ethical clearance from the Institutional Animal Ethics Committee. The rats were housed in 12 hours light and 12 hours dark cycles with access to standard rat chow and water ad libitum. They were randomly divided into 4 groups of 10 rats each. Then, the rats were placed in 8 cages, with 5 rats in each cage. After the division, they were labeled and marked with numbers 1 to 5. Following placement into the 8 cages, the entire cage of rats was exposed to the same pressure of blast shockwave. The pressure subjected to each cage was separately recorded on a key Excel sheet that was unavailable to the research team until the analysis was completed. On day 28 postblast exposure, half the rats were sacrificed. There were two rats randomly chosen by their marked numbers from each cage first followed by one rat from the cages labeled 1 to 4. The remaining rats were sacrificed on postblast exposure day 84. Samples collected were labeled by the cage and rat number. Statistical analysis of data generated was done using cage numbers. After the entire analysis was completed, the key and cage numbers were tallied to determine which rat belonged to which group and then the results were interpreted.

Barnes Maze Trials

All four groups were subjected to trials on a Barnes maze. The 40 rats underwent acquisition training with three trials per day of the Barnes maze for four consecutive days. In this protocol, the rat was placed in the center of the maze and covered with a black cylinder. The cylinder was suddenly removed, while at the same time, an overhanging lamp was switched on. The rats rushed away from the light source and explored the holes in the Barnes maze to escape. Each look into a wrong hole was counted as an error. The entry in the correct hole and consequently the chamber was considered as completion. The time taken from the removal of the covering cylinder to the completion was recorded along with the number of errors made by the rat. After acquisition training, on the fifth day, 30 of the rats were exposed to blast shockwaves from the modified Reddy's tube. The rats were allowed to recover and 2 days after the blast or sham exposure the rats were reevaluated with three trials of the Barnes maze task. They were again evaluated on day 10 and day 21 postblast exposure.

Blast Exposure

Three groups of 10 rats each were randomly divided and exposed to three different blast pressures on the 5th day after procurement. The exposure blast pressures were 81, 160, and 210 kPa as noted at 1 cm from opening of the driven section, measured using a piezoelectric sensor.

The rats were exposed in the prone position with the fore- and hind-limbs restrained using a cellophane sheet, such that only the head of the rat was exposed. The neck was restrained with the help of a rubber collar and metal prongs which held the collar in place. The snout of the rat was at 1 cm from the opening of the modified Reddy's tube. After exposure to the blast, the rat was freed and observed for any external injuries. All the rats were stunned for 5 to 15 minutes after exposure to the blast shockwave.

The remaining 10 rats were used as controls, and they underwent sham blasts. These rats were restrained in a similar manner on the 5th day after the commencement of acquisition and exposed to the sound of the blast without being exposed to the shockwave (► Figs. 1 and 2).

Sacrifice

The experimental animals (those exposed to blast waves) were equally and randomly divided into two groups which were sacrificed on day 28 and day 84 postblast exposure. The rats were sacrificed by manual cervical dislocation, following which the skull caps were dissected out and the brain was removed. The cerebral hemispheres were placed on an ice-cold surface and divided in the mid sagittal plane using a scalpel blade. One half of the brain was preserved in 10% neutral-buffered formalin. From the other hemisphere, the hippocampus was dissected out and placed in an Eppendorf tube and frozen at
-20°C. The remaining portion of the brain was preserved in another container at -20°C. The hippocampi were homogenized in 50 mmol tris-buffered saline, pH 7.4 containing 0.5% TritonX-100 with pestle and sonicated with 3 pulses of 10 seconds each, and centrifuged at 10,000 revolutions per minute for 15 minutes at 4°C. The supernatants were collected and subjected to tau enzyme-linked immunosorbent assay (ELISA) and acetylcholinesterase assay.

The other half of the brain was fixed in 10% neutral-buffered formalin for a minimum period of 4 weeks prior to processing. The tissue was then processed for paraffin embedding. The brainstem and cerebellum were disconnected at the level of crus cerebri and embedded in the mid-sagittal plane. The cerebral hemisphere was sliced at the level of the olfactory bulb, infundibulum, and mammillary bodies.

Five-micron thick serial sections were taken and stained with hematoxylin and eosin (H&E), Luxol fast blue (LFB) for myelin, and Cresyl violet fast stain for neurons.

Each case was evaluated for alterations in neurons (neuronal shrinkage and dark neurons on H&E, Cresyl violet stains: reflecting ischemic changes), perivascular inflammation, microglial nodules, and ventricular dilatation. All changes were semiquantitatively graded as mild, moderate, and severe.

**Rat Tau Protein ELISA**

Total tau in the hippocampal homogenate was estimated using commercially available rat tau quantitative ELISA kit (My Bio Source, United States). The assay sample (after being appropriately diluted) was incubated with the buffer and tau-horseradish peroxidase (HRP) conjugate in a precoated plate for 1 hour at 37°C. After the incubation the wells were decanted and washed five times. The wells were then incubated with a substrate for HRP enzyme. The product of the enzyme substrate complex was blue. The substrate used was 3,3′,5,5′ tetramethylbenzidine. A stop solution was added which arrests the reaction and turns the solution yellow. The intensity of the color was measured spectrophotometrically at 450 nm in a microplate reader and was inversely proportional to the tau concentration, as both the tau from the sample and the tau-HRP conjugate compete for binding sites on the anti-tau antibody immobilized in the well. A standard curve was plotted between the optical density and concentration of standards from which the concentration of tau was extrapolated.

**Acetylcholine esterase Assay**

The principle of this assay is based on the measurement of the activity of the enzyme AChE on the substrate acetylthiocholine iodide (ACTI). The enzyme cleaves the
substrate into thiocholine and acetate. The thiocholine reacts with a coloring agent (in this case dithiobisnitrobenzoate) to form a yellow-colored product. The esterase activity in the homogenized hippocampus of the rat was measured by following the earlier described procedure. The buffer used was 0.1 M phosphate buffer at a pH of 8.0. The standard value for different concentrations of AChE from electric eel (Sigma Aldrich, Bengaluru, Karnataka, India) in hydrolyzing ACTI (Sigma Aldrich) was recorded as optical density at 412 nm. The readings were then plotted on a graph. Amount of enzyme activity in the hippocampal homogenates was extrapolated from the standard curve.

**Statistical Analysis**
The completion time and errors were analyzed using a nonparametric analog to two-way repeated measures analysis of variance using a package called nparLD. It tested the effects of groups (sham, low blast pressure, medium blast pressure, and high blast pressure) and the effects of time (postinjury day 3, postinjury day 7, postinjury day 21) on the outcome variables (time to completion and errors). The Kruskal–Wallis test was used to compare the tau protein levels and acetylcholinesterase activity levels at various time points (►Fig. 3).

**Results**
All 40 rats underwent acquisition and recall using Barnes maze trials on day 7, day 14, and day 21. A rat each from the sham exposure group, low pressure blast group, and high pressure blast group were excluded due to fall from the Barnes maze. Three rats in the sham group were sacrificed on day 28 and three rats on day 84 postinjury. In the low blast pressure group, four rats were sacrificed on day 28 and five rats on day 84 postinjury. Seven rats were sacrificed on day 28 and three rats on day 84 postinjury in the medium blast pressure group. Six rats were sacrificed on day 28 postinjury and three rats on day 84 postinjury in the high blast pressure group. The rats and samples were labeled randomly after blast exposure and a key was generated to record the assignment. The results were calculated and interpreted while blinded to the key. The final analysis was then done after decoding from the key.
The results show that the completion time had an improvement in performance on postblast exposure day 3 with shorter times taken to complete the maze in the low and high blast pressure groups. Following this the subsequent time taken to complete the Barnes maze trial continued to decrease with an improvement in performance in the low blast pressure blast groups. The rats exposed to high pressure blast wave had a significantly better performance as compared to the other rats on postblast injury day 3. But after the initial improvement seen on the 3rd postexposure day (likely some error as there is no possible explanation for this phenomenon, it is likely due to increased familiarity with the maze after continued learning or increased vigilance after exposure to trauma), performance was static over time (performance declined from the very low value of postblast 3rd day test, but was still better than the preblast value), this likely indicates impaired retention over time.

The change in time taken to completion is statistically significant among the rats exposed to high blast pressures when compared with rats exposed to sham blasts or low blast pressures.

The errors made by the rats did not show any significant differences when comparing the blast-exposed groups with the control groups (Figs. 4–6) (Table 3).

The hippocampal homogenates of the blast-exposed rats show a transient reduction in the levels of total tau protein on postblast exposure day 28 compared to the controls. Following this, the total tau protein levels on postexposure day 84 were similar in all the groups. This could reflect a repair phenomenon leading to a rise in total tau levels by day 84. Alternatively, the transient reduction at day 28 postinjury could be due to a wash out of tau from the brain into the circulation5 (Table 4).

The acetylcholinesterase activity did not show any statistically significant change at the different blast pressure exposed groups and the control groups at both postexposure day 28 and post exposure day 84.

### Histopathology

The rats exposed to different blast pressures and sham blasts were sacrificed at two time points—4 and 12 weeks postinjury. Changes in gray and white matter in all neuroanatomical areas were assessed. The white matter showed a greater degree of involvement than the gray matter. The brain sections at 4 weeks postinjury showed evidence of variable edema and vacuolation in white fiber tracts predominantly involving the cerebellar white matter and corpus callosum with demyelination highlighted by LFB staining. In contrast, the brainstem white matter, internal capsule, anterior commissure, optic tract, and crus cerebri were spared. The rats sacrificed at 12 weeks postblast exposure showed evidence of variable edema and vacuolation in white fiber tracts predominantly involving the cerebellar white matter and corpus callosum with demyelination highlighted by LFB staining.
Fig. 4 Comparison of mean time to completion in different groups at various time points.

Fig. 5 Comparison of mean errors in different groups at various time points.
exposure demonstrated more prominent demyelination on LFB in cerebellar white matter and corpus callosum. In addition, there was focal gliosis seen in the lower portions of the corpus callosum in midline, at the point of insertion of the septum. Distortion of white matter fibrillar pattern with interspersed oligodendroglial cells was seen in the corpus callosum, external capsule, internal capsule, and crus cerebri. The control group also showed white matter vacuolation and edema suggesting that these changes are likely secondary to hypoxia as a terminal event. Gray matter changes were limited to the cingulate/frontal cortex and hippocampus with conspicuous sparing of caudate, putamen, thalamic nuclei, and brainstem nuclei. The hippocampus showed the most characteristic damage pattern that correlated well with the blast pressures. The dentate gyrus, CA1, and CA3 demonstrated dark neurons, with pyknotic nuclei, and intensely stained cytoplasm. This change was most noticeable in the basal layers and the angle of the dentate gyrus and prominent at 4 weeks postblast injury. The rats sacrificed at 12 weeks postblast injury showed recovery from injury, with normal neuronal cells. However, there was evidence of scarring and distortion of architecture. The basal ganglia, thalamus, and sensorimotor cortex showed minimal damage which did not seem to increase with increasing pressures. The sensorimotor and pyriform cortices showed mainly focal neuronal damage in middle and deeper layer foci of damage in the blast-exposed rats when compared with the control rats.

In the cerebellum, the Purkinje cells revealed varying degrees of hypoxic/ischemic change that did not correlate with the blast pressure exposure. The control animals showed similar changes, suggesting that these changes are likely secondary to hypoxic injury occurring as a terminal event (Figs. 4–8).

**Discussion**

In this study, a rodent model of blast injury was studied, and the cognitive, behavioral, biochemical, and pathological changes were evaluated following blast pressures using an indigenously developed modified Reddy’s tube which can deliver predetermined blast pressures in a controlled manner.

A small laboratory-based simulation for BINT is possible due to the availability of reliable and portable shockwave tubes. Although these tubes mimic the effects of primary blast wave well, their utility in replicating real-life situations of blast explosion-induced trauma is limited. However, for the study of the pathobiology and physics of the blast exposure, these studies are adequate, especially due to minute control that can be exerted over the physical attributes, the environment, and the nature of the subject as well.

The blast pressure was chosen based on previous pilot study done by one of the senior authors. The blast pressures chosen were the pressures at which blast/pressure-stratified white matter and gray matter injury was seen.4

**Table 3** Comparison of total tau protein levels (pg/100 mg tissue) in different groups at various time points

<table>
<thead>
<tr>
<th>Pressure group</th>
<th>Day 28</th>
<th>Day 84</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>2050 (1250–3400) [n = 3]</td>
<td>2150 (1400–2900) [n = 3]</td>
</tr>
<tr>
<td>Low pressure group (81 kPa BOP)</td>
<td>880 (550–1500) [n = 4]</td>
<td>2240 (1300–3450) [n = 5]</td>
</tr>
<tr>
<td>Medium pressure group (160 kPa BOP)</td>
<td>536.5 (420–700) [n = 7]</td>
<td>2133.3 (550–3300) [n = 3]</td>
</tr>
<tr>
<td>High pressure group (210 kPa BOP)</td>
<td>754.2 (830–1400) [n = 6]</td>
<td>2100 (1650–3000) [n = 3]</td>
</tr>
</tbody>
</table>

Abbreviation: BOP, blast overpressure.

**Table 4** Comparison of acetylcholinesterase activity (U/mg tissue) in different groups at various time points

<table>
<thead>
<tr>
<th>Pressure group</th>
<th>Day 28</th>
<th>Day 84</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>1.408 [n = 3]</td>
<td>1.526 [n = 3]</td>
</tr>
<tr>
<td>Low pressure group (81 kPa BOP)</td>
<td>1.372 [n = 4]</td>
<td>1.455 [n = 5]</td>
</tr>
<tr>
<td>Medium pressure group (160 kPa BOP)</td>
<td>1.754 [n = 7]</td>
<td>1.365 [n = 3]</td>
</tr>
<tr>
<td>High pressure group (210 kPa BOP)</td>
<td>1.892 [n = 6]</td>
<td>1.445 [n = 3]</td>
</tr>
</tbody>
</table>

Abbreviation: BOP, blast overpressure.
The rat is a commonly used laboratory model and a sturdy animal; with earlier studies establishing its analogous and homologous anatomical structures. Due to its small size, maintenance is easy, and the environment can be carefully controlled to avoid confounders. The parameters of the experiment can be easily varied with controlled conditions. For this study, Sprague-Dawley rats were chosen.

Male rats aged 2 months were used as this age group corresponds with young adults in humans, and most of the central nervous system development is complete at this age. The rats were exposed to the blast pressure at a distance of 1 cm from the driven end of the shockwave tube while the rat was in a prone position. The prone position of the rat lets the injury be limited to the brain and cranial structures due to the blast wave pressure decaying before it reaches the thorax. The effects of primary blast injury on the brain alone without the effects of systemic exposure can thus be studied.

The rats exposed to higher blast pressures showed a deterioration of completion time at later recalls which was significant when compared to animals exposed to low blast pressure.

Human exposure to blasts leads to the chest or the back bearing the main brunt of the blast shockwave.

The Barnes maze is a tool with diverse applications, and it is a physiological and sensitive test of visuospatial memory and learning. The results noted in the current study corroborate with those seen in other studies that show that there is no significant change in retrograde recall of previous path and visual cues, resulting in similar completion time across different blast pressures and control animals. The initial improvement with a faster response and completion of the Barnes maze test seen in rats exposed to 210 kPa shockwave blast overpressure could be a consequence of increased vigilance following blast exposure as other studies show normalization of retrograde memory function after the initial 24 hours.

The rats exposed to higher blast pressures showed a deterioration of completion time at later recalls which was significant when compared to animals exposed to low blast pressure.
Fig. 8 (A–D) White matter changes (Luxol fast blue stain). (A, B) Controls showing well preserved myelinated fiber tracts in whole mount section of brain at level of mammillary body with closeup view showing compact myelinated tracts (B). (IC, internal capsule; EC, external capsule; F, fornix; T, thalamus; H, hippocampus; Hy, hypothalamus). (C) Disarray of fiber tracts, with patchy demyelination and scarring at junction of internal and external capsule day 84 postinjury in rats exposed to medium blast pressure of 160 kPa. (D) Severe demyelination in IC 28 days postinjury in rats exposed to high blast pressure of 210 kPa. (magnification = scale bar).

Fig. 9 (A–D) Neuronal morphology as seen in rats exposed to 210 kPa blast overpressure (Nissl stain). (A) Well-preserved neurons in laminar arrangement in sensorimotor cortex. (B) Several dark, pyknotic neurons in superficial and middle layers of the cingulate cortex, day 28 postinjury. (C) Neurons in thalamic nuclei well preserved. (D) Neurons in caudate nuclei well preserved. (magnification = scale bar).
pressures and sham control animals which do indicate perhaps an impairment in anterograde learning as seen in multiple studies. It may also indicate ongoing secondary effects of the primary blast injury. This is supported by the demonstration of parenchymal injury mainly seen in the hippocampus. The dentate gyrus shows maximal effect followed by CA3 and CA1, while CA2 is spared. The hippocampal injury is maximal in acute phase but persistent at 4 weeks, in the high blast pressure-exposed rats; however, these did not have significant effects on the spatial memory as demonstrated by relatively similar times to completion among the different groups on recall day 21.

Apart from evidence of neuronal death, the white fibers show a disorganized pattern in multiple areas which may contribute to the cognitive dysfunction and delays in impulse transmission.
4 weeks. The current study indicates that the hippocampus acetylcholinesterase activity remains normal even at 12 weeks postblast. Acetylcholinesterase activity is seen to be decreased in several other areas, while the hippocampus was spared.6,8

The levels of total tau protein appear to transiently decrease in blast-exposed rats at day 28 postinjury when compared to control animals. The fall in total tau may indicate structural damage to cells with elution of tau protein into cerebrospinal fluid and venous system. The fall in total tau protein levels may also be due to decreased binding of the tau ELISA antibody due to posttranslational modification of the tau proteins because of the blast exposure.7 The delayed sacrifice at 84 days postblast injury shows normalization of levels, comparable between blast-exposed and control animals likely reflecting reparative response. These minor changes seen in the cellular and fiber architecture on histopathology without any major gross demyelination or severe neuronal injury may be a cause of the delayed cognitive deficits seen in humans. The drastic fall in tau protein levels may lead to ultrastructural reorganization at a later point of time which may be a cause of impaired function due to aberrant recovery over time.

There are few studies that assess the structural or biochemical status of the rat brain after the first month postinjury.9 The delayed sacrifice at 84 days postinjury reveals evidence of recovery with no persistent deficits.

**Summary**

A single blast exposure did not significantly affect the visuospatial memory and its recall. Although functionally, cognitive deficits were not prominent, there was histopathological and biochemical evidence of decreased tau protein levels that reflected ongoing neuronal damage (► Figs 7–11).

**Conflict of Interest**
None declared.

**References**

6. Ahlers ST, Vasserman-Stokes E, Shaughnessy MC, et al. Assessment of the effects of acute and repeated exposure to blast overpressure

![Fig. 11 Changes in cerebellum. (A, B) Cerebellar folia showing preserved myelination in white matter (*) on Luxol fast blue stain (A) with normal Purkinje neurons (B) highlighted by Nissl stain. (C, D) 28 days postinjury showing focal demyelination in white matter (C, *) and several Purkinje neurons are shrunken, dark, and pyknotic (D, arrows) on Nissl stain in rats exposed to high blast pressures of 210 kPa. (magnification = scale bar).](image-url)
in rodents: toward a greater understanding of blast and the potential ramifications for injury in humans exposed to blast. Front Neurol 2012;3:32

