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Research report

Chronic post-traumatic stress disorder-related traits in a rat model of low-level blast exposure

Georgina Perez-Garcia [a,b,c], Miguel A. Gama Sosa [b,c,d], Rita De Gasperi [b,c,d], Margaret Lashof-Sullivan [e], Eric Maudlin-Jeronimo [e], James R. Stone [f,g], Fatemeh Haghighi [b,c,d,h], Stephen T. Ahlers [e], Gregory A. Elder [a,c,d,i,*]

[a] *Department of Neurology, Icahn School of Medicine at Mount Sinai, One Gustave Levy Place, New York, NY 10029, USA*
[b] *Research and Development Service, James J. Peters Department of Veterans Affairs Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468, USA*
[c] *Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, One Gustave Levy Place, New York, NY 10029, USA*
[d] *Department of Psychiatry, Icahn School of Medicine at Mount Sinai, One Gustave Levy Place, New York, NY 10029, USA*
[e] *Department of Neurotrauma, Operational and Undersea Medicine Directorate, Naval Medical Research Center, 503 Robert Grant Avenue, Silver Spring, MD 20910, USA*
[f] *Department of Radiology and Medical Imaging, University of Virginia, Charlottesville, VA 22908, USA*
[g] *Department of Neurosurgery, University of Virginia, Charlottesville, VA 22908, USA*
[h] *Fishberg Department of Neuroscience, Icahn School of Medicine at Mount Sinai, One Gustave Levy Place, New York, NY, 10029, USA*
[i] *Neurology Service, James J. Peters Department of Veterans Affairs Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468, USA*

HIGHLIGHTS

- Blast-related traumatic brain injury (TBI) has been common in veterans of the recent conflicts in Iraq and Afghanistan.
- Blast-related mild TBI (mTBI) has been frequently associated with post-traumatic stress disorder (PTSD).
- Rats exposed to repetitive low-level blast develop PTSD-like behavioral traits in the absence of a psychological stressor.
- The presence of such traits 28–35 weeks after blast exposure suggests that blast induces chronic behavioral effects.
- These observations have implications for understanding the relationship of mTBI to PTSD in dual diagnosis veterans.

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ABSTRACT

The postconcussion syndrome following mild traumatic brain injuries (mTBI) has been regarded as a mostly benign syndrome that typically resolves in the immediate months following injury. However, in some individuals, symptoms become chronic and persistent. This has been a striking feature of the mostly blast-related mTBIs that have been seen in veterans returning from the recent conflicts in Iraq and Afghanistan. In these veterans a chronic syndrome with features of both the postconcussion syndrome and post-traumatic stress disorder has been prominent. Animal modeling of blast-related TBI has developed rapidly over the last decade leading to advances in the understanding of blast pathophysiology. However, most studies have focused on acute to subacute effects of blast on the nervous system and have typically studied higher intensity blast exposures with energies more comparable to that involved in human moderate to severe TBI. Fewer animal studies have addressed the chronic effects of lower level blast exposures that are more comparable to those involved in human mTBI or subclinical blast. Here we describe a rat model of repetitive low-level blast exposure that induces a variety of anxiety and PTSD-related behavioral traits including exaggerated fear responses that were present when animals were tested between 28 and 35 weeks after the last blast exposure. These animals provide a model to study the chronic and persistent behavioral effects of blast including the relationship of PTSD to mTBI in dual diagnosis veterans.

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1. Introduction

Worldwide between 2000 and 2014 more than 320,000 US service members sustained TBIs [1]. Public awareness of TBI in

* Corresponding author at: James J. Peters VA Medical Center, Neurology Service (3E16), 130 West Kingsbridge Road, Bronx, NY 10468, USA.
E-mail address: gregory.elder@va.gov (G.A. Elder).

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the military increased recently due to the conflicts in Iraq and Afghanistan [2] where TBI has been frequent with estimates that 10–20% of returning veterans suffered a TBI [3,4]. As in civilian life, TBIs in the military result from various mechanisms including vehicular accidents and falls. However in Iraq and Afghanistan because of the wide spread use of improvised explosive devices, blast-related TBI was the most common cause [3,4].

Acutely, TBI may be associated with a combination of cognitive, somatic and affective symptoms referred to as the postconcussion syndrome. The postconcussion syndrome associated with mild TBI (mTBI) has historically been regarded as a relatively benign syndrome that typically resolves in the immediate months following injury [5]. However, in some individuals, including many veterans, symptoms become chronic and persist for months to years [6–10].

Animal modeling of blast-related TBI has developed rapidly over the last decade leading to advances in the understanding of blast pathophysiology [11–14]. However most studies have focused on the acute to subacute effects of blast on the nervous system. Moreover, most animal studies have focused on relatively powerful blast exposures delivered at levels high enough to induce significant pulmonary and intracranial pathology [11,13]. Few studies have addressed the chronic and persistent effects attributable to lower level blast exposures.

Here we describe our experience with an animal model of low-level blast exposure in which rats are subjected to three low-level blast exposures delivered one per day for three consecutive days [15]. In prior studies we established that blast-exposed animals exhibited a variety of anxiety and PTSD-related behavioral traits as well structural, biochemical and epigenetic changes in brain [16–20]. Here we extend this prior work by showing that anxiety and PTSD-related behavioral traits are present between 28 and 35 weeks following the last blast exposure. These animals provide a model in which to study the chronic behavioral effects of blast including the relationship of PTSD to mTBI.

2. Methods and materials

2.1. Animals

Adult male Long Evans Hooded rats (250 g–350 g; 10 weeks of age; Charles River Laboratories International Inc., Wilmington, MA, USA) were used as subjects. All studies were reviewed and approved by the Institutional Animal Care and Use Committees of the Walter Reed Institute of Research/Naval Medical Research Center and the James J. Peters VA Medical Center. Studies were conducted in compliance with the Public Health Service policy on the humane care and use of laboratory animals, the NIH Guide for the Care and Use of Laboratory Animals, and all applicable Federal regulations governing the protection of animals in research.

2.2. Blast overpressure exposure

Rats were exposed to overpressure injury using the Walter Reed Army Institute of Research (WRAIR) shock tube, which simulates the effects of air blast exposure under experimental conditions. The shock tube has a 0.32-m circular diameter and is a 5.94 m-long steel tube divided into a 0.76-m compression chamber that is separated from a 5.18-m expansion chamber. The compression and expansion chambers are separated by polyethylene terephthalate Mylar ™ sheets (Du Pont Co., Wilmington, DE, USA) that control the peak pressure generated. The peak pressure at the end of the expansion chamber was determined with piezoresistive gauges specifically designed for pressure-time (impulse) measurements (Model 102M152, PCB, Piezotronics, Inc., Depew, NY, USA). This apparatus has been used in multiple prior studies to deliver blast overpressure injury to rats [15–23].

Individual rats were anesthetized using an isoflurane gas anesthesia system consisting of a vaporizer, gas lines and valves and an activated charcoal scavenging system adapted for use with rodents. Rats were placed into a polycarbonate induction chamber, which was closed and immediately flushed with a 5% isoflurane mixture in air for two minutes. Rats were placed into a cone shaped plastic restraint device and then placed in the shock tube. Movement was further restricted during the blast exposure using 1.5 cm diameter flattened rubber tourniquet tubing. Three tourniquets were spaced evenly to secure the head region, the upper torso and lower torso while the animal was in the plastic restraint cone. The end of each tubing was threaded through a toggle and run outside of the exposure cage where it was tied to firmly affix the animal and prevent movement during the blast overpressure exposure without restricting breathing. Rats were randomly assigned to sham or blast conditions with the head facing the blast exposure without any body shielding resulting in a full body exposure to the blast wave. Further details of the physical characteristics of the blast wave are described in Ahlers et al. [15]. Blast-exposed animals received 74.5 kilopascal (kPa) exposures equivalent to 10.8 pounds per square inch (psi). One exposure per day was administered for three consecutive days. Sham exposed animals were treated identically including receiving anesthesia and being placed in the blast tube but did not receive a blast exposure. Subjects received blast overpressure exposure at the Naval Medical Research Center (Silver Spring, MD, USA) and were transferred on the day following the last blast exposure to the James J. Peters VA Medical Center (Bronx, NY, USA) where all other procedures were performed.

2.3. Animal housing for behavioral testing

Animals were housed at a constant 70–72 °F temperature with rooms on a 12:12 h light cycle with lights on at 7 A.M. All subjects were individually housed in standard clear plastic cages equipped with Bed-O'Cobs laboratory animal bedding (The Andersons, Maumee, OH, USA) and EnviroDri nesting paper (Sheppard Specialty Papers, Milford, NJ, USA). Access to food and water was *ad libitum*. Subjects were housed on racks in random order to prevent rack position effects. All behavioral testing was performed by the same investigator (G.P.G.).

2.4. Locomotor activity and open field

General locomotor activity and open field behavior was examined in 40.6 cm × 40.6 cm Versamax activity monitors (Accuscan, Columbus, OH, USA), each outfitted with a grid of 32 infrared beams at ground level and 16 elevated 7.6 cm above ground level. Locomotor activity was recorded during 60 min and analyzed with VersaData Software (Accuscan), which automatically calculates move time, move distance and center time based on beam breaks. The center of the chamber was defined as a square of 25.4 cm × 25.4 cm (7.6 cm from each side wall) and virtually drawn with VersaMap software (Accuscan). Center entries and center rest time were defined based on the centroid of the rat being in the center of the chamber with rest time defined as time when the centroid was in the center of the chamber but during which no beam breaks were generated. Samples were recorded in 1 min bins and summed into 5 min intervals for presentation.

2.5. Light/dark emergence

A light/dark emergence task was run in Versamax activity monitors with opaque black Plexiglas(R) boxes enclosing the left half of the interiors so that only the right sides were illuminated.

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