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TITLE: Brain Vulnerability to Repeated Blast Overpressure and Polytrauma

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
It is likely that the mild TBI and cognitive impairments observed among many of the troops returning from OIF and OEF result from repeated exposures to blast overpressure. Although the clinical symptoms of concussion are typically transient, there is both a cumulative risk for persistent damage due to repeated concussions, and a post-concussion period of greatest vulnerability to a second impact. Specific risk assessments and guidelines should be established for exposure to blast overpressure. We are using a preclinical model of blast overpressure in rats to investigate the cumulative effects of multiple blast exposures on neurologic status, neurobehavioral function, and brain histopathological endpoints. Repeated exposures to blast overpressure with varied inter-blast intervals are used to characterize and define the temporal window of brain vulnerability to repeated blast overpressure. We anticipate that these data will provide a critical first step in establishing rational risk guidelines and developing mitigation strategies.

15. SUBJECT TERMS
Traumatic Brain Injury (TBI), blast exposure, blast overpressure

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INTRODUCTION:

It is likely that the mild TBI and cognitive impairments observed among many of the troops returning from OIF and OEF result from repeated exposures to blast overpressure. Although the clinical symptoms of concussion are typically transient, mild concussive brain injury can also result in persistent alterations in cognitive and emotional status. Based upon observations among athletes in contact sports, there is both a cumulative risk for persistent damage due to repeated concussions, and a post-concussion period of greatest vulnerability to a second impact, which may elicit subdural hematoma, vasospasm, brain swelling, elevated intracranial pressure, and occasionally death. Specific guidelines have been developed and periodically revised to establish when an athlete can resume their sport, based upon concussion severity and number. Similar risk assessments and guidelines should be established for exposure to blast overpressure. We are using a preclinical model of blast overpressure in rats to investigate the cumulative effects of multiple blast exposures on neurologic status, neurobehavioral function, and brain histopathological endpoints. Repeated exposures to blast overpressure with varied inter-blast intervals are used to characterize and define the temporal window of brain vulnerability to repeated blast overpressure. Along with vestibulomotor assessments on a rotating pole, spatial learning and memory is assessed using the Morris water maze on days 1-10 post-BOP. Following training, latencies to find the submerged platform are recorded along with swim patterns while doing so. Following injury, the platform is repositioned to a new location on each test day to increase the challenge of the test and its sensitivity to distinguish impairments. Brains are then prepared for histopathological analysis to establish the extent of brain injury and to determine whether the brain injury severity increases with repeated exposure to blast, and diminishes with increased inter-BOP intervals. We anticipate that these data will provide a critical first step in establishing rational risk guidelines and developing mitigation strategies.

BODY:

Overview**:** A preclinical model of air blast injury in rats has been biomechanically validated and is being used to investigate the cumulative effects of repeated blast exposures on neurological status, neurobehavioral function, visual acuity, and brain histopathological endpoints. Varied inter-BOP intervals are used to identify the temporal window of brain vulnerability to repeated BOP. We anticipate that these data will provide a critical first step in establishing rational risk guidelines and developing mitigation strategies.

KEY RESEARCH ACCOMPLISHMENTS:

During this reporting period, we:

- Utilized a custom designed gauged rat holder to subject rats to single blast overpressure (BOP) exposures (11, 14, and 16 psi peak pressures) as well as repeated BOPs separated by 24 hr (specific aim 1). For all BOP exposures, rats

were pre-trained in neurobehavioral tasks, and on the day of the experiment they were secured and suspended in mesh netting 2.5 ft within the mouth of the tube (fig 1). Piezoresistive gauges within the holder monitor the side-on and total pressures encountered by each experimental subject with this blast simulation (fig 2). Based upon both neurobehavioral and neuropathological data, the modifications we implemented to yield a higher fidelity simulation of blast appears to have resulted in diminished TBI relative to what we observed with rats positioned at the mouth of the shock tube in a metal holder. Consequently, modifications in the beam-walking and Morris water maze (MWM) tasks were implemented to increase the sensitivities of these assessment tools to detect subtle perturbations in performance resulting from the mild traumatic brain injuries caused by BOP. Specifically, we incorporated a rotating pole for the beam walking task. Pre-BOP, rats were trained to ambulate across the cylindrical pole rotating in a clockwise direction (4.5 rpms), and after BOP, rats were tested daily on the pole with rotations in both clockwise (4.5 rpms) and counterclockwise (6 rpms) directions. This modification provides an improved neurobehavioral task which is much more sensitive for detection of BOP-induced cerebellar and vestibulomotor perturbations. Similarly, with the MWM, rats were trained during 3 days pre-BOP to locate a platform in a fixed location. After BOP, the location of the platform in the MWM changed daily, so that rats were required to daily learn a new location, yielding a more complex, demanding, and sensitive neurobehavioral test.

Despite the improvements in these functional tests, injuries produced with these exposure conditions were mild (fig 3) and the associated functional impairments were quite modest (figs 4-7). Abilities to ambulate across the rotating pole were largely unaffected by 11 psi BOP exposures, and showed decrements only following exposure to 14 psi peak overpressures, either as a single (results not shown) or repeated (fig 7) BOP . Curiously, in contrast to expectation, with a 24 hr interblast interval, MWM performance appeared to actually be improved following repeated BOP exposures relative to that seen after a single exposure. This unexpected finding has been made by other investigators with a short interblast interval (≤24 hrs) and prompts speculation about a possible interplay between evolving neuroprotective and neurodegenerative mediators within this timeframe. Although we do not yet have adequate subject populations to draw conclusions, ongoing experiments with the longer interblast intervals (e.g. 3 and 5 days, specific aims 2 and 3) will be informative to sort out the basis for this unexpected finding. We expect these important comparisons to be possible within the next 6 months.

- We have discerned BOP-induced neuropathological changes with the 11, 14, and 16 psi peak pressure exposure conditions, although the severities of the injuries appear to have diminished as the exposure methodologies have been refined to more snuggly secure rats in the mesh of the holder. High speed videography of BOP exposures within the shock tube along with measurements with accelerometers point to appreciable displacement and acceleration of

experimental subjects with airblast, which diminish with increased constraint within the mesh. The association of diminished injuries with better subject constraint suggests that acceleration/displacement substantially contribute to the brain injuries produced following BOP exposures. Although neuropathological changes are less striking and widespread, they are similar in nature; brains of rats exposed to airblast typically are devoid of any obvious cell loss or injury, and instead most typically show fiber degeneration that is evident in silver-stained sections of the brain. Silver impregnation of fibers is fairly routinely evident in the cerebellum (fig 3), optic tracts, and in the internal capsule. These neuropathological changes are much less pronounced than those previously documented for similar intensity airblasts with rats positioned at the mouth of the tube or in a rigid metal holder.

- Visual discrimination procedures have been developed, refined, and implemented to distinguish blast-induced impairments in the ability of a rat to bar-press for food in response to different visual cues. Rats with 24 hr interblast intervals are currently under evaluation. This procedure tests both visual acuity and visually-based cognitive performance.

- Telemetric EEG recordings were performed to detect post-traumatic seizure activity and ensuing blast-induced EEG anomalies. Unfortunately, we discovered that EEG electrodes implanted by the rat vendor caused appreciable damage to the cerebral cortex, rendering data collected from these subjects unusable. This was most unfortunate as it compromised concomitant neurobehavioral and neuropathological data that were collected along with the EEG recordings.

REPORTABLE OUTCOMES:

Abstracts/Presentations

Shoge, R.O. Pressure profiles of shock tube exposures. Invited platform presentation at the Advanced Technology Applications for Combat Casualty Care (ATACCC) Meeting, St. Pete's Beach FL, Aug, 2010.

Riccio C., Bentley T.B., and Long J.B. Blast overpressure injury in rats produces widespread fiber degeneration. Poster at the Advanced Technology Applications for Combat Casualty Care (ATACCC) Meeting, St. Pete's Beach FL, Aug, 2010.

Long, J.B. Experimental Evaluation of Blast-induced Traumatic Brain Injury in Rats Using a Cylindrical Shock Tube. Invited speaker at the Third DOD Brain Injury Computational Modeling Expert Panel Meeting, Johns Hopkins University Applied Physics Laboratory, Laurel MD, Dec 2010.

Shoge, R.O. Experimental Evaluation of Blast-induced Traumatic Brain Injury in Rats Using a Cylindrical Shock Tube. Invited speaker at the National Capital Region TBI Research Symposium. April 5, 2011.

CONCLUSION:

Single or repeated BOP exposures to 11, 14, or 16 psi peak overpressures using the mesh holder positioned 2.5 ft within the shock tube yield mild TBI that is associated with modest neurobehavioral disruptions. The severities of these disruptions are not consistently worsened with repeated blasts with a 24 hr interblast interval. These findings point to a need to examine greater BOP intensities and longer interblast intervals, as is planned.

REFERENCES: NONE

APPENDICES: NONE

SUPPORTING DATA: BELOW

Fig 1. Instrumented rat holder records static and total pressure in the vicinity of the experimental subject which is secured in mesh.

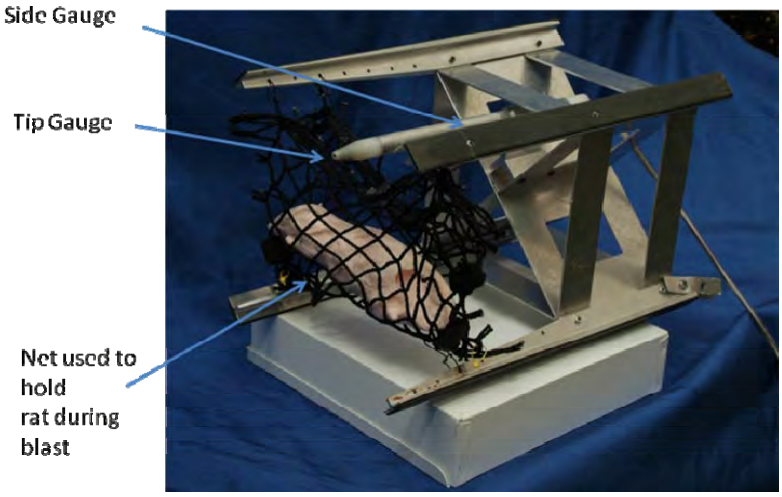

Fig 2. Flow conditions vary along the length of the shock tube, particularly at the mouth.

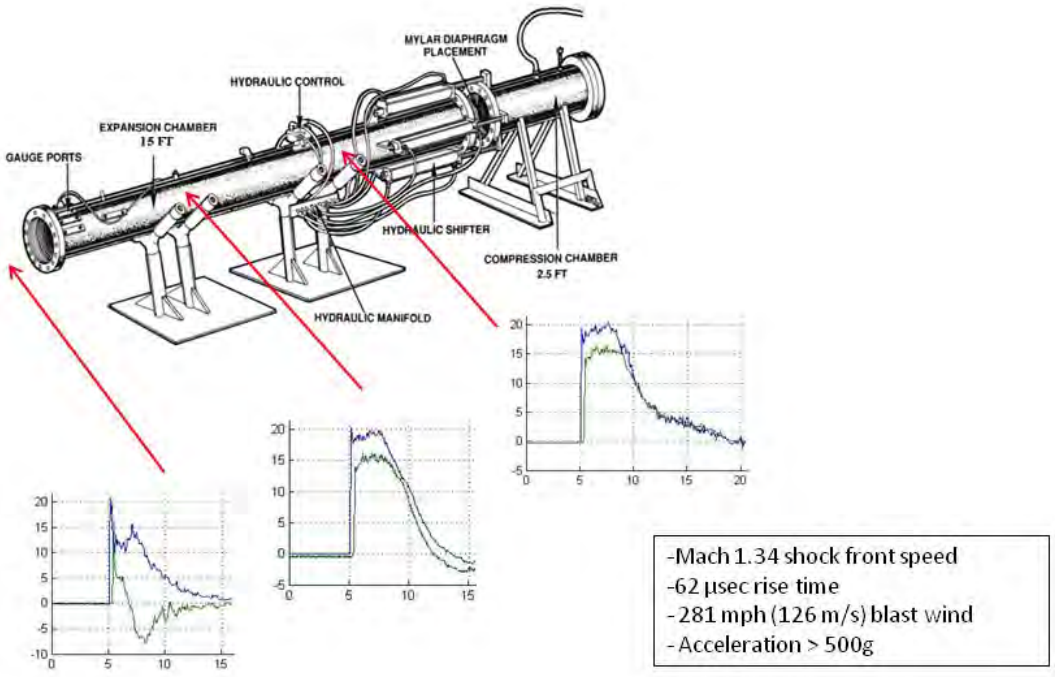

Fig 3. Blast-induced fiber degeneration

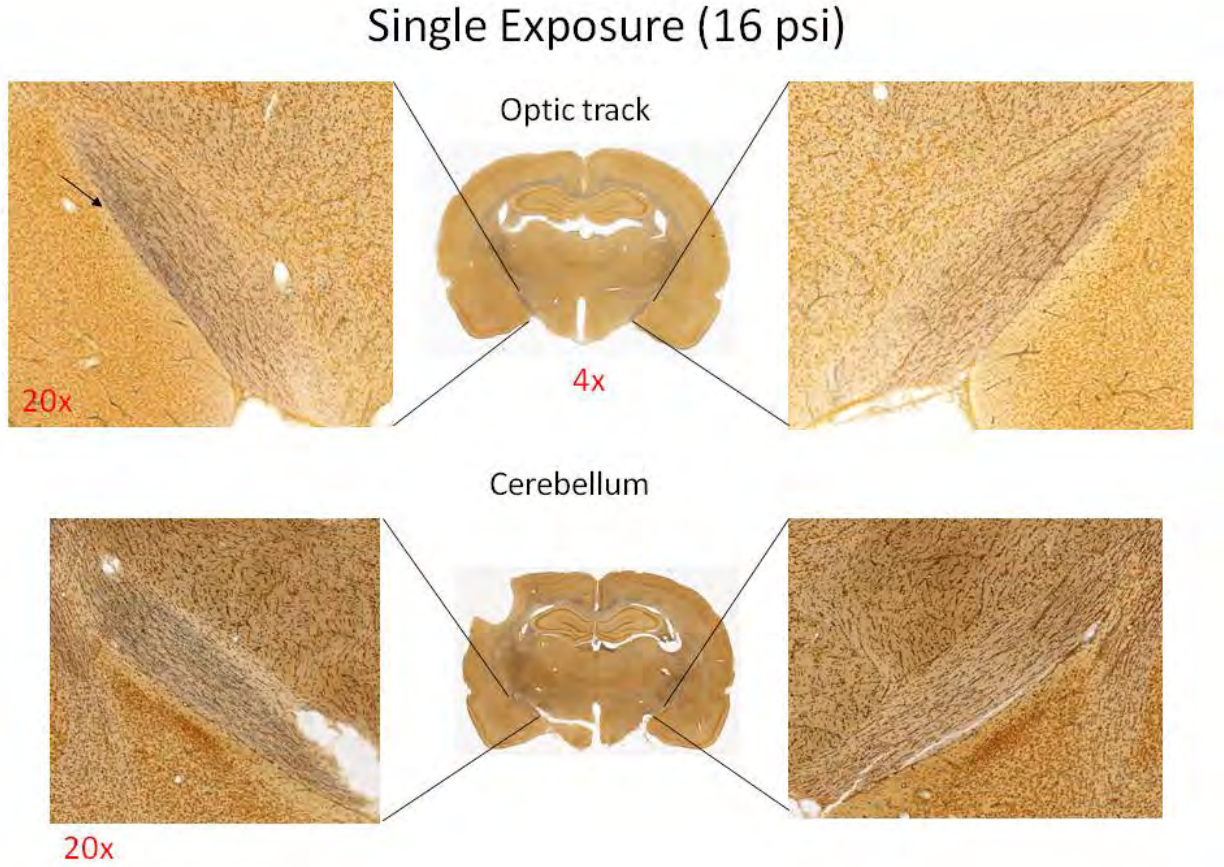

Fig 4. Morris water maze performance after single (squares) or repeated (triangles) 11 psi BOP exposures (24 hr interblast interval). After retention testing on day 1 post-BOP, acquisition was tested by daily relocating the platform. N= 6-8 rats per gp.

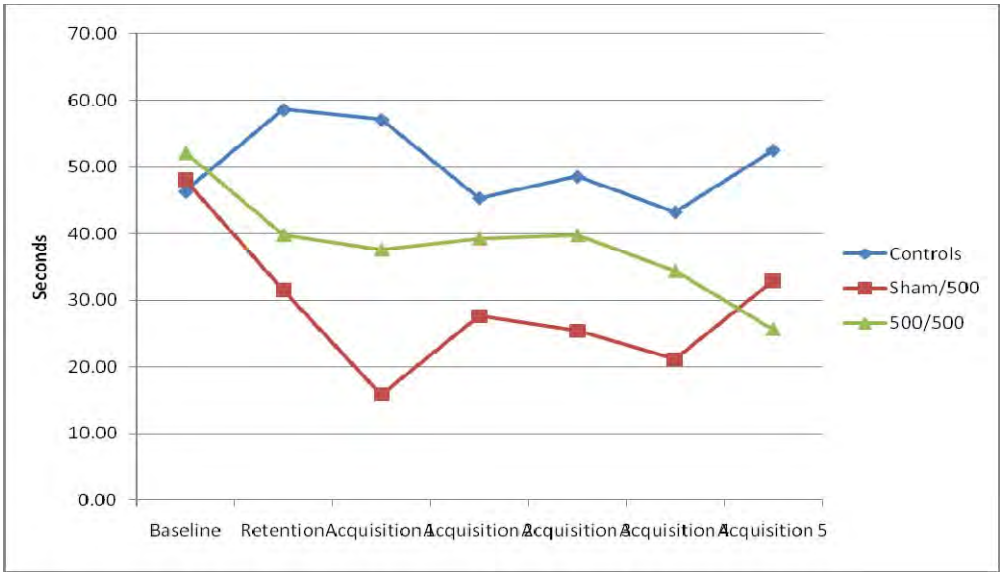

Fig 5. Morris water maze performance after repeated BOP exposures (11 psi &11 psi, squares; 11 psi & 14 psi, triangles). BOP exposures were separated by 24 hr. After retention testing on day 1 post-BOP, acquisition was tested by daily relocating the platform. N= 6-8 rats per gp.

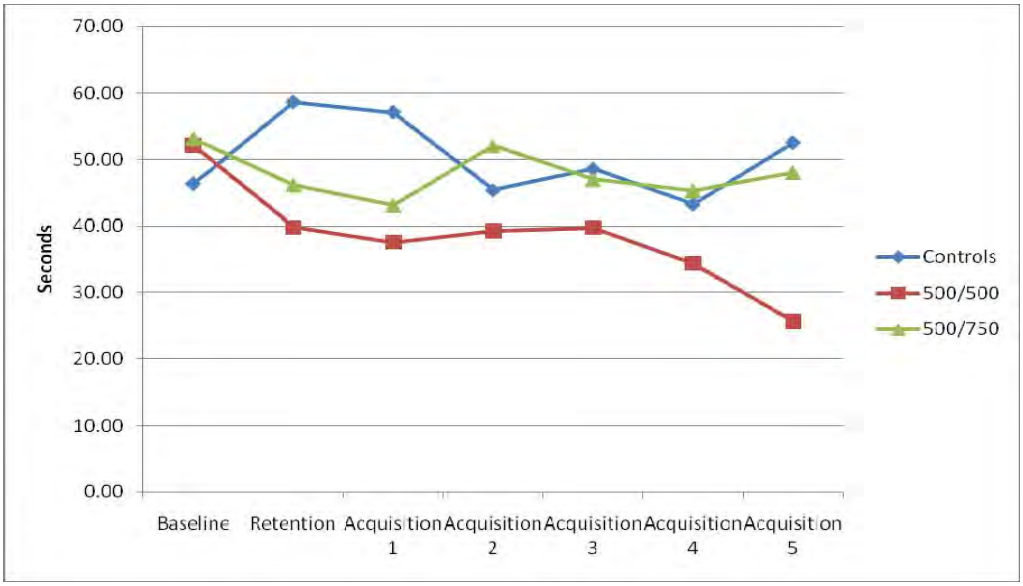

Fig 6. Rotary pole performance after single (squares) or repeated (triangles) 11 psi BOP exposures (24 hr interblast interval). N= 6-8 rats per gp.

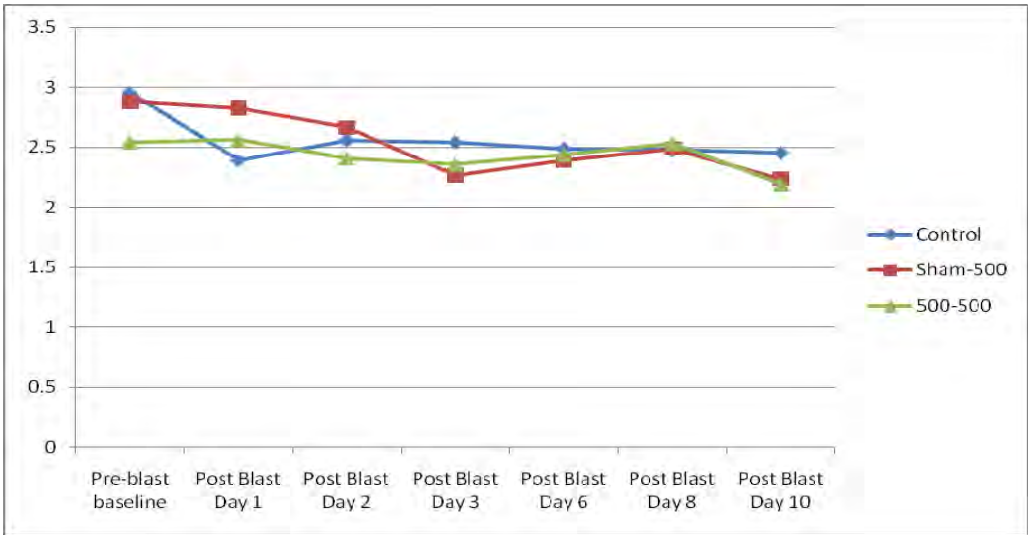

Fig 7. Rotary pole performance after repeated BOP exposures (11 psi &11 psi, squares; 11 psi & 14 psi, triangles). BOP exposures were separated by 24 hr. N= 6-8 rats per gp.