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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.					
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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 (MWM) 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-blast overpressure (BOP) intervals. We anticipate that these data will provide a critical first step in establishing rational risk guidelines and developing mitigation strategies.

BODY

Overview

An air-driven shock tube is used to simulate BOP and study the cumulative effects of repeated blast exposures on neurological status, neurobehavioral function, visual acuity, and brain histopathological endpoints. After biomechanical validation of this model, varied inter-BOP intervals are used to identify the temporal window of brain vulnerability to repeated BOP.

Progress toward this objective was hampered during the previous reporting period by substantial personnel changes, prompting the need to request a no-cost extension (NCE) period to complete the project. Notably, two Ph.D. contractors departed and 5 federal government employees were lost as a result of a reduction in force (RIF) action. We have largely recovered from the turbulence associated with these disruptive changes, but are not yet caught up and will require continuation of our NCE period.

In particular, in the original statement of work we proposed evaluation of rats exposed to single or repeated blasts separated by 1, 3, or 5 day intervals. Despite a number of experimental refinements yielding a high fidelity simulation of blast overpressure that we believe is well suited for our purpose, we failed to observe the hypothesized vulnerabilities and associated worsened outcomes with the blast overpressure exposures repeated with these intervals. From our own ongoing observations in related projects, and from discussions with investigators at other research institutions, we anticipate that the hypothesized vulnerabilities and worsened outcomes require shorter interblast intervals and will be readily apparent with the outcome measures we are employing.

Notably, in a closely related project, we have recorded appreciable exacerbation of blast injury outcomes when the rat is exposed to 2 closely coupled BOPs relative to what is seen with a single BOP exposure. Moving forward, we have revised our milestones accordingly, and have begun to use the rotating pole, MWM and free field tests to reveal vestibulomotor impairments, spatial navigation disruptions, and anxiety in rats exposed to single or repeated blasts separated by 1, 4, or 24 h intervals. In addition to neurobehavioral assessments, EEG is telemetrically recording pre- and post-BOP to distinguish electrophysiological consequences of blast and repeated blast. Neuropathological microscopic evaluations with silver stained sections are used to assess and compare the extent and location of fiber degeneration within the brains of experimental subjects exposed to single and repeated blasts. Finally, we have corroborated these light microscopic assessments using diffusion tensor imaging (DTI) magnetic resonance imaging (MRI), which provides a powerful comprehensive quantitative analysis of the brains from which morphological comparisons can be derived.

As a result of modifications in blast exposure conditions that we implemented to improve the fidelity of our blast simulation, the TBI and functional impairments resulting from BOP exposures in our shock tube have diminished substantially. It is very likely that the vulnerability to a subsequent BOP exposure was also affected by these modifications. Despite these apparent setbacks to achieving our milestones, the shock tube exposure modifications represent critical improvements to ensure that we are working with injuries in the laboratory that approximate conditions associated with blast injuries to Warfighters. In particular, we have eliminated artefactual biomechanical loading associated with a collimated jet as it exits the tube as well as the appreciable, uncontrolled acceleration and displacement otherwise occurring with less movement restriction.

Task 1:

Using rats pretrained on neurological and neurobehavioral procedures, determine if re-exposure to a mild BOP 24 hrs following the first BOP exposure significantly worsens acute physiological responses, visual acuity, and neurobehavioral and histopathological outcome measures relative to those seen in shams and in single insult subjects.

During this reporting period, we have successfully implemented all experimental procedures required to fulfill all specific aims. Data collection employing a 1 day inter-BOP interval (specific aim 1) was completed and failed to reveal a consistent, significant worsening of outcome by a second BOP exposure relative to that seen following a single BOP exposure. Rats were evaluated using several functional neurobehavioral outcome assessments. We have utilized ambulation across a rotating cylindrical pole as a neurobehavioral task which is sensitive for detection of BOP-induced cerebellar and vestibulomotor perturbations (fig 2). Rats were trained to negotiate the pole pre-BOP and were then tested at varied intervals post-BOP using a scoring scheme based upon distance travelled, velocity, and balance, with 3 being the maximum achievable score (fig 3,4).

Rats were also evaluated in the MWM to evaluate BOP effects on spatial learning and memory (figs 5-8), and in open field tests to monitor exploratory behavior and general activity. Rotarod tests were used to assess motor coordination, balance, and motor learning, and visual discrimination procedures were developed, refined and implemented to test visual acuity and visually based cognitive performance and reaction time. Telemetric EEG recordings have been implemented to distinguish blast-induced EEG anomalies. Our initial focus was to detect post-traumatic seizure activity which proved to be infrequent and variable. Algorithms have been developed to monitor for other EEG anomalies over a more prolonged timeframe.

Neuropathological changes resulting from a single BOP exposure are modest and largely consist of limited fiber degeneration that is evident in silver-stained sections and is most prominent in the cerebellum, optic tracts, and in the internal capsule. Brains of rats exposed to BOP are typically devoid of any obvious cell loss or injury. Recent collaborative DTI imaging of brains exposed to BOP corroborates the light microscopic observations and provides a powerful means to comprehensively and quantitatively compare these subtle neuropathological features.

Task 2

Determine if vulnerability to worsened outcome diminishes with the inter-BOP interval extended to 3 days.

Not initiated due to the absence of worsened outcome with a 1 day inter-BOP interval.

Task 3

Determine if vulnerability to worsened outcome diminishes with the inter-BOP interval extended to 5 days.

Not initiated due to the absence of worsened outcome with a 1 day inter-BOP interval.

KEY RESEARCH ACCOMPLISHMENTS

- Shock tube BOP exposure conditions have been carefully characterized and refined to create a reproducible high fidelity simulation of blast and repeated blast TBI.
- A battery of sensitive neurobehavioral assessments have been carefully developed to distinguish functional disruptions resulting from single or repeated BOP exposures.
- Neuropathological and neurochemical consequences of shock tube BOP exposures of varied intensities have been characterized.
- Telemetric EEG recordings have been established to distinguish electrophysiological consequences of single and repeated blast exposures.
- An advanced blast simulator (ABS) was procured to further refine assessments of BOP-induced brain injuries.

REPORTABLE OUTCOMES

Based in part upon the work supported by this award, during this reporting period additional funding was sought through research preproposals and proposals submitted to the CDMRP and DMRP. In addition, funding from this project supported the following presentations:

Oral presentation

P Arun. Role of tissue non-specific alkaline phosphatase in the etiology of tauopathy and chronic traumatic encephalopathy. *National Capital Region Traumatic Brain Injury Research Symposium* held at National Institutes of Health, MD – April 30, 2013

Poster presentation

P. Arun, R. Abu-Taleb, S. Oguntayo, A. Edwards, C. Riccio, S. VanAlbert, I. Gist, Y. Wang, M.P. Nambiar, J.B. Long. Tissue non-specific alkaline phosphatase in the etiology of tauopathy and chronic traumatic encephalopathy after traumatic brain injury. *USUHS Research Day* held at Bethesda, MD – May 13, 2013

CONCLUSION

As the result of substantial refinement, under carefully controlled experimental conditions, the biomechanical perturbations of the brain that yield blast-induced mild TBI in injured Warfighters can be recreated with reasonable fidelity to reproduce characteristic sequelae of blast-induced mild TBI. Results to date are consistent with the hypothesis that BOP generates a mild insult to the brain (and other organs as well). With the refined exposure conditions used to date, the severity of these disruptions has not been consistently worsened with repeated blasts with a 24 hr interblast interval. These findings point to a need to alter our experimental plan and examine consequences of BOP repeated with shorter intervals (1, 4, and 24 h). The end-product repeated BOP model will provide an invaluable tool to define underlying neurobiological mechanisms and rationally establish effective guidelines (e.g. return-to-duty) and countermeasures to lessen short-term impairments as well as chronic debilitation (e.g. chronic traumatic encephalopathy).

SUPPORTING DATA

Fig 1. BOP exposure: The shock tube consists of a 2.5 ft long compression chamber that is separated from a 15 ft long expansion chamber by polyester Mylar membranes (DuPont, Wilmington, DE). Both chambers are 1 ft in diameter. The compression chamber is pressurized with air, causing the Mylar membrane to rupture at a pressure that is linearly dependent upon its thickness. For whole body exposures, anesthetized rats were suspended in a transverse prone position in a tightly secured coarse mesh pouch secured 2.5 ft within the mouth of the shock tube. The critical biomechanical loading to the experimental subject is determined from both the static (P_s) and dynamic pressure (P_d) of the associated with the shock wave, which are established by the combination of side-on and head-on pressure gauges mounted in the rat holder as shown in fig 2.

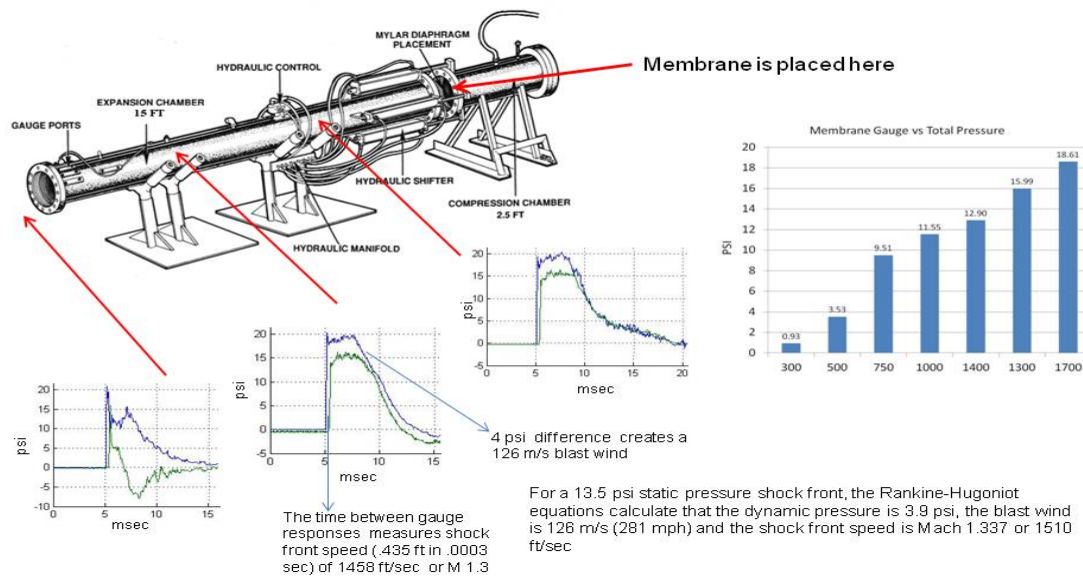


Fig 2. Rats are secured in a holder equipped with Endevco piezoresistive pressure transducers that measure total (tip gauge) and static (side gauge) air pressures in the shock tube.



BOP flow conditions were mapped throughout the shock tube and exposure conditions defined and recorded using piezoresistive pressure transducers mounted in the rat holder. Based upon these measurements, isoflurane anesthetized rats were positioned 2.5 ft within the mouth of the shock tube for all experiments. After discovering appreciable acceleration of experimental subjects in association with BOP exposures, care was taken to tightly secure each rat to maintain consistency with this potentially confounding biomechanical variable.

Rats were exposed to BOPs of varied peak amplitudes (70 to 140 kPa) either singly or repeated with a 24 h interblast interval. Over this range, injuries and functional outcome impairments were modest and were not detectably exacerbated by a subsequent exposure to BOP 24 hr later. In contrast, exposure to BOP 1 min following the first significantly impacted neuropathological changes and functional outcome impairments on the rotating pole (fig 5), prompting the conclusion that the hypothesized vulnerabilities and worsened outcomes require shorter interblast intervals and will be readily apparent with the outcome measures we are employing. Consequently, we request revision of milestones to make outcome comparisons in rats exposed to single or repeated blasts separated by 1, 4, or 24 h intervals.

Fig 3. Rotating pole scores following single or repeated BOP exposures separated by 24 h (n=8-10 rats/gp).

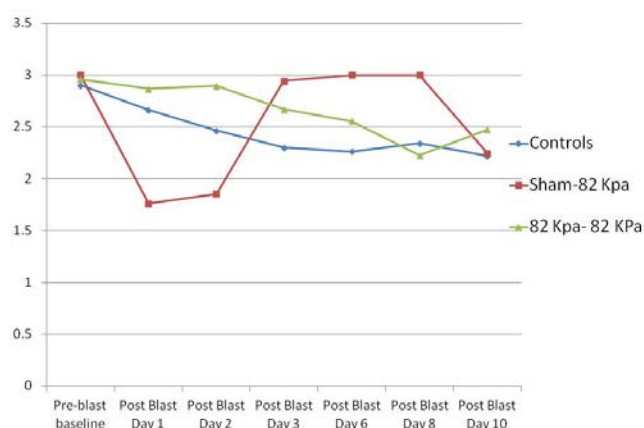


Fig 4. MWM latencies with single & repeated BOP separated by 24 h (n=8-10 rats/gp). Platform was relocated on each trail day.

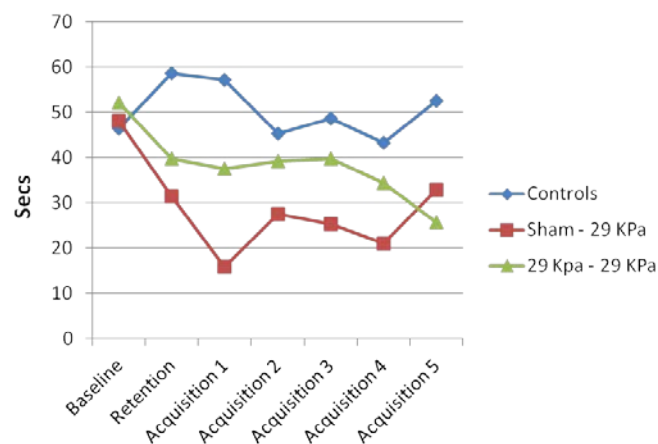


Fig 5. Rotating pole scores following single or repeated BOP exposures separated by 1 min (n=8-10 rats/gp).

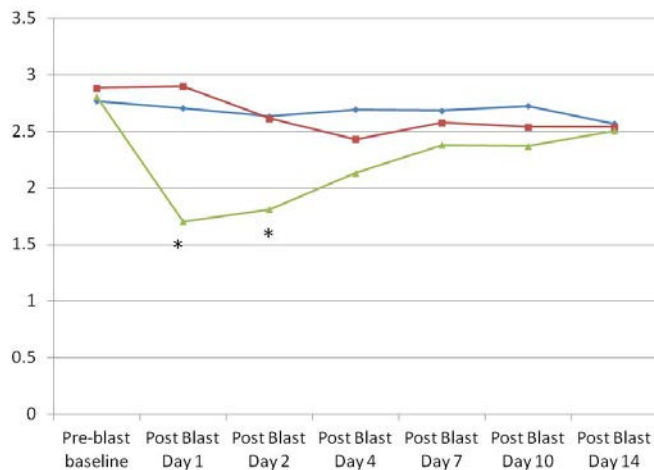


Fig 6. MWM latencies with single & repeated BOP separated by 1 min (n=8-10 rats/gp). Platform was not relocated.

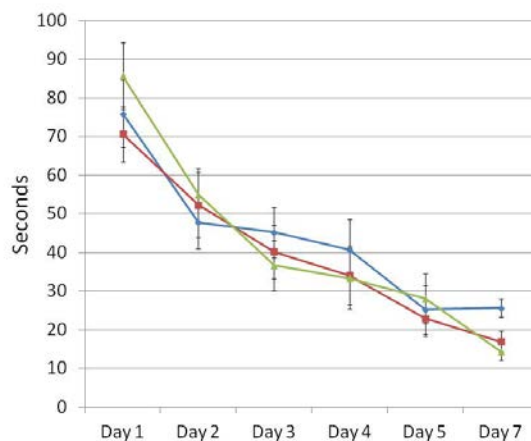
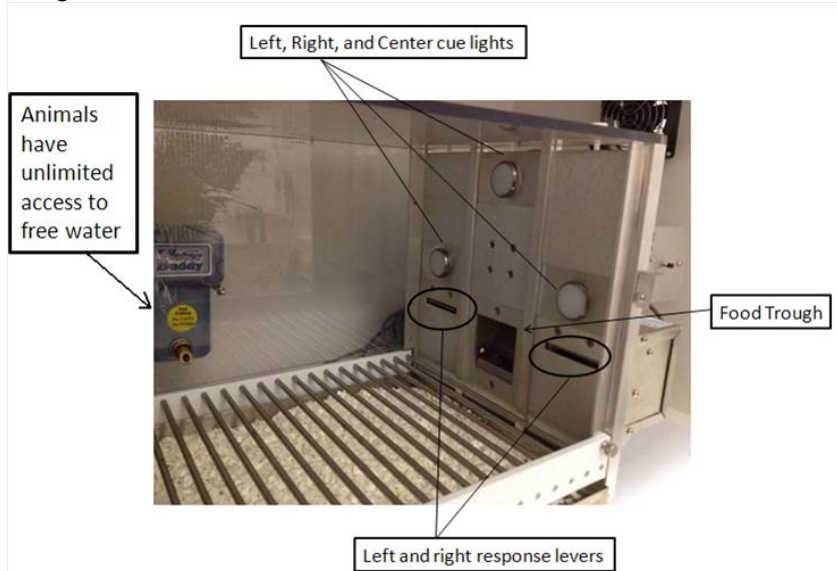


Fig 7. Visual discrimination box. One wall of the chamber contains a food trough where reward pellets are delivered for correct responses. A response lever with an indicator light above it is positioned on each side of the trough. Rats were required to press the lever in response to the light cue.



Although visual discrimination performance was unaltered by single BOP (figs 8 & 9) or 2 BOPs separated by 24 hr (not shown), closely coupled repeated BOP exposure increased reaction times (fig 11). Significantly worsened performance on the rotarod was also observed with closely coupled repeated BOP but not following a single BOP exposure (fig 12 & 13).

Fig 8 & 9. Immediately after a successful left lever press, the right light was illuminated and the time required for the rat to press the right lever was recorded, with a 30 sec cutoff. Failure to lever press within 30 sec resulted in a 30 sec timeout during which the chamber was completely darkened before testing resumed. Number of timeouts is reciprocally related to accuracy. N=4 and 5 rats/gp.

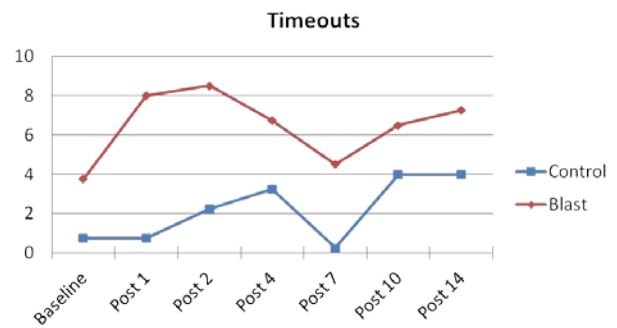
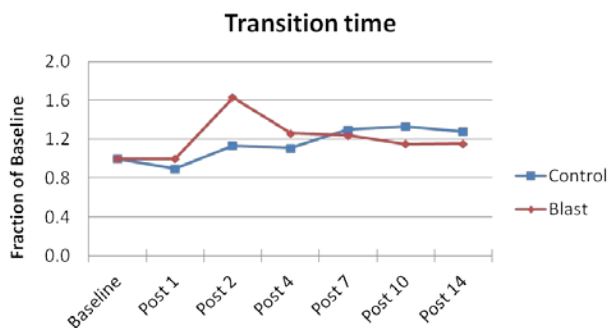


Fig. 10 & 11. Visual discrimination performance after closely coupled BOPs (1 min interval, 19 psi). Accuracy was unchanged but reaction time was increased (n= 4 and 5 rats/gp).

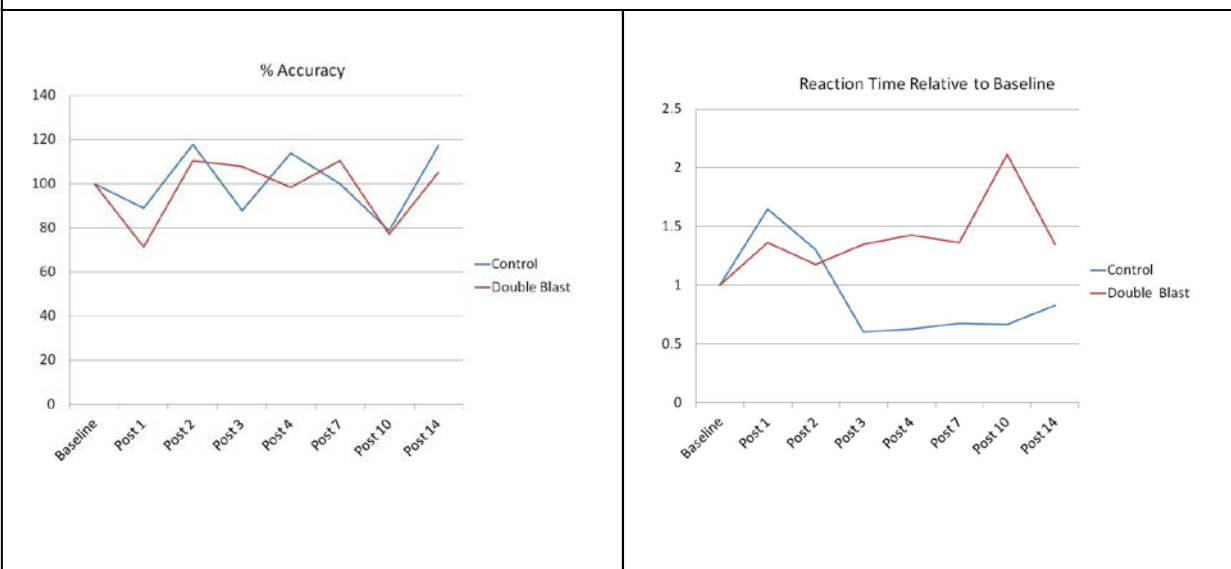


Fig 12. Rotarod performance after single or closely coupled repeated BOP.

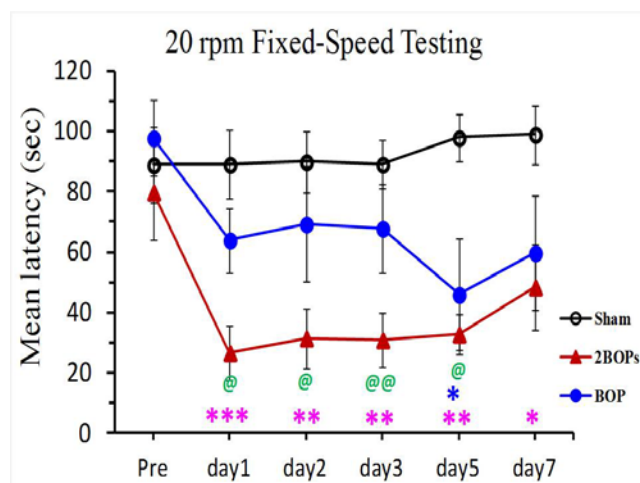
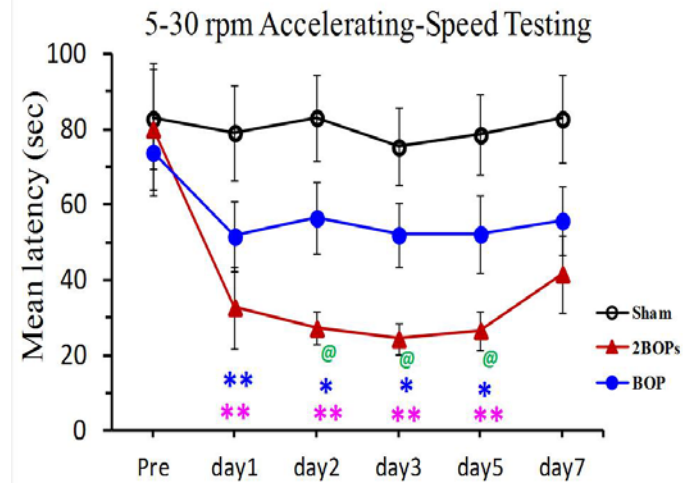
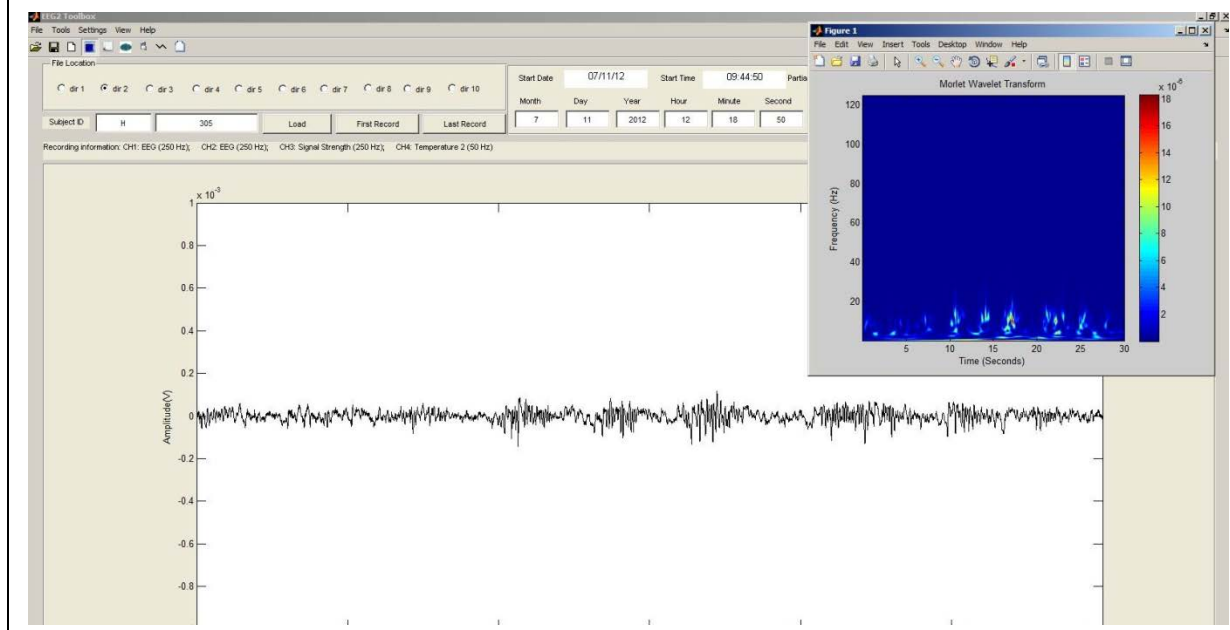


Fig 13. Rotarod performance after single or closely coupled repeated BOP.



EEG analyses were disrupted by the personnel changes identified above, but are now reestablished. Continuous bi-hemispheric EEG acquisition, before, during and after injury, enables capture and characterization of pre-seizure synchronicity in addition to

Fig 14. Epileptiform discharges recorded 1.5 hr after a single 19 psi BOP exposure



the seizures present directly after injury and those occurring throughout a 15-day post injury period. With development of techniques for the evaluation of large data sets, a comprehensive and dynamic analysis is being implemented. Results to date show that seizure activity is sparse, cortical epileptiform discharges are not typically seen after blast but are detected after weight drop injuries (in a closely related study) with dominant frequencies in the delta and theta band. While epileptiform discharges have been observed, in the absence of consistently robust seizurogenic responses to BOP, current analyses are focusing on interhemispheric frequency coherence, phase delay, and amplitude asymmetry between hemispheres.

We have collaboratively applied diffusion tensor imaging to expand our histopathological assessments of brain injuries resulting from BOP. Working with investigators at the Center for In Vivo Microscopy at Duke University School of Medicine, we have successfully applied quantitative ex vivo analysis and comparison of sham & BOP-exposed brains. After tensor estimation and computation of DTI parametric maps (i.e. fractional anisotropy [FA], radial diffusivity [RD], axial diffusivity [AD], mean diffusivity [MD]) were carried out, image data were spatially normalized using non-linear, diffeomorphic image registration to create an average brain template for voxelwise comparisons. Voxelwise analysis of DTI parameter changes were performed using SurfStat MATLAB tools and generated statistical significance maps using false discovery rate (FDR) to correct for multiple comparisons. These analyses corroborated conventional light microscopic histopathological findings (fig 14), and

revealed significant microstructural damage in rats exposed to closely coupled repeated BOP exposures, but no consistent and significant changes in subjects exposed to a single BOP relative to sham handled subjects (fig 15).

Fig 14. Coronal silver stained sections reveal sparse fiber degeneration following single BOP in contrast to much more substantial injuries following closely coupled repeated BOP.

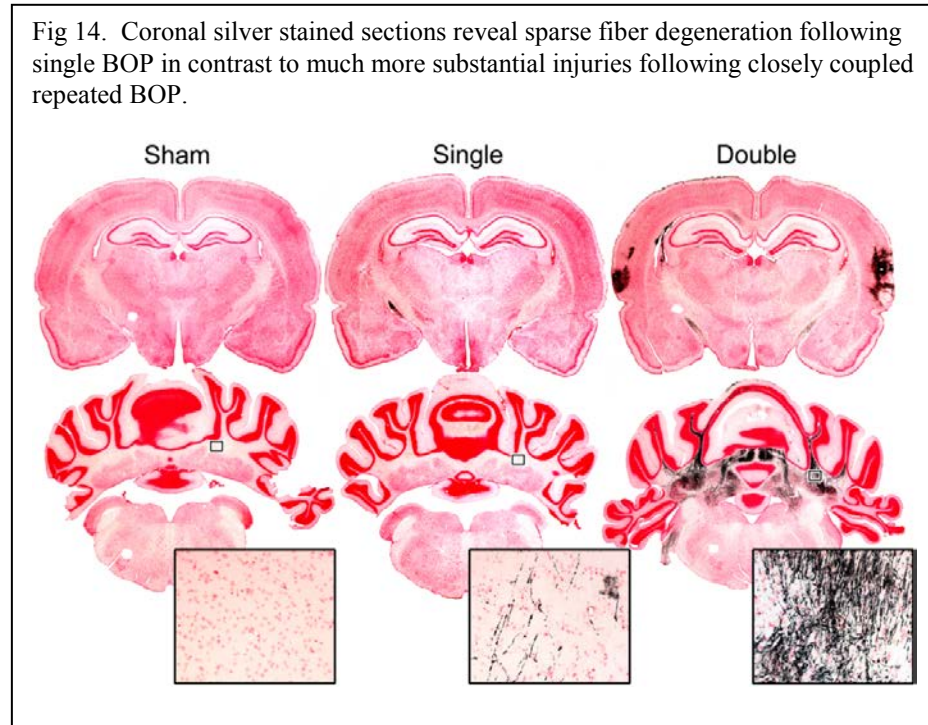


Fig 15. Ex vivo DTI parameter comparisons across experimental groups reveal statistically significant differences of repeated blast subjects relative to either shams or single blast subjects 72 hrs after BOP exposures; N= 9 rats per group.

