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TITLE: Brain Vulnerability to Repeated Blast Overpressure and Polytrauma

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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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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: Research accomplishments associated with each task outlined in the approved Statement of Work are described below.

Overview**:** An air-driven shock tube is used to simulate blast overpressure (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. We anticipate that these data will provide a critical first step in establishing rational risk guidelines and developing mitigation strategies. Progress toward this objective was hampered during this 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 sweeping, abrupt changes and now anticipate completing the project within the NCE period. Also, as detailed below, we have not yet seen a consistent significant worsening of outcome by a second BOP exposure relative to that seen following a single BOP exposure. Consequently, we will propose modifying the SOW to look at a

shorter BOP interval (4 hrs) in addition to looking at a 3 day inter-blast interval during the NCE. Additionally (or alternatively), we can combine BOP exposure with impact/acceleration-induced TBI, varying the interval between the two insults.

The diminished TBI and functional impairments we now observe relative to those seen in our earlier work (Long et al., 2009) are likely due in large part to the substantial modifications in blast exposure conditions that were implemented to improve the fidelity of our blast simulation. Previously, rats were positioned (but not secured) at the mouth of the shock tube in a rigid metal holder for BOP exposure which we subsequently learned in all likelihood caused extremely different loading and injury phenomena due to the conversion of flow energy to a collimated jet as it exits the tube. Working closely with a blast physicist, we modified both the holder and the positioning of the rat in the tube (fig 1). Rats are now exposed to a shock wave when snuggly suspended in a tautly drawn course mesh netting 2.5 ft within the tube and are substantially less injured than were those exposed to the exit flow conditions at the mouth of the tube. As a result of these "cleaner" improved BOP exposure conditions, TBI is much less severe and it has been difficult documenting persistent neurobehavioral deficits after either single or repeated BOP exposures, despite attempts to improve the sensitivities of these tests to distinguish mild TBI. In addition to the position of the rat in the tube (mouth vs 2.5 ft within the tube), we also discovered that the manner in which the rats are suspended appears to impact their sensitivities to BOP. In particular, high speed videography and accelerometer recordings revealed sizeable displacements and large accelerations of rats impacted by BOP within the tube (fig 1) which is influenced by the tautness or play of the netting in which the rats are suspended. By more tightly securing the netting, we have reduced exposure variability (i.e. levels of acceleration and displacement) which has in turn further lessened the severity of the resultant injury.

**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 overcome several procedural impediments summarized in our last report (e.g. EEG) and have successfully implemented all experimental procedures required to fulfill all specific aims. Specific aims 1, 2 and 3 are to use these procedures to establish if outcomes are worsened following re-exposure to BOP 1, 3, and 5 days, respectively. During this reporting period, all work was performed with a 1 day inter-BOP interval (specific aim 1). Surprisingly, with the data collected using these outcome measures, we have not yet seen a consistent significant worsening of outcome by a second BOP exposure relative to that seen following a single BOP exposure.

Rats have been evaluated using several functional 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). After being trained during 3 days pre-BOP to locate a platform in a fixed location, after BOP the location of the platform in the MWM was changed daily so that rats were required to daily learn a new location, yielding a more complex, demanding, and sensitive neurobehavioral test.

Despite the added challenges imposed by the refinements of these functional tests (i.e. pole rotation and varied platform location), functional impairments produced with these injury conditions were generally quite modest and survivable brain injuries were typically mild. The unexpected absence of worsened outcome following a second BOP exposure prompts speculation about a possible interplay between evolving neuroprotective and neurodegenerative mediators within this timeframe. Evaluations with both shorter and longer interblast (e.g. 3 and 5 days, specific aims 2 and 3) will be informative to sort out the basis for this unexpected finding. Attempts to achieve greater blast-induced brain injury using a machstem wedge to provide body protection while concentrating shock wave exposure on the head (fig 9) were largely unsuccessful. Brain injuries were not substantially worsened and the wedge did not hold up to repeated BOP exposures.

Limited neuropathological changes were widespread and somewhat similar in nature (figs 10, 11). Brains of rats exposed to BOP typically were devoid of any obvious cell loss or injury, and instead most typically show fiber degeneration that was evident in silver-stained sections of the brain. Silver impregnation of fibers is fairly routinely evident in the cerebellum, optic tracts, and in the internal capsule. As noted previously, these neuropathological changes were 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 (fig 12). This procedure tests both visual acuity and visually-based cognitive performance. Rats injured by a single 19 psi BOP showed transient deficits with eventual recovery (figs 13, 14). Repeated BOP exposures will now be assessed.

Telemetric EEG recordings were successfully initiated to detect post-traumatic seizure activity and ensuing blast-induced EEG anomalies. Immediately after isoflurane anesthesia, cortical EEG was suppressed and highly synchronized (fig 15). Although the number of experimental subjects is limited, it appears that in contrast to sham rats, which have not yet presented any signs of epileptiform discharges, injured rats occasionally present immediate epileptiform EEG activity with occasional additional discharges through 9 days postinjury (figs 16, 17).

**Task 2:** Determine if vulnerability to worsened outcome diminishes with the inter-BOP interval extended to 3 days.

Not initiated.

**Task 3:** Determine if vulnerability to worsened outcome diminishes with the inter-BOP interval extended to 5 days.

Not initiated.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

- Shock tube BOP exposure conditions have been further characterized and refined to create a high fidelity simulation of blast and repeated blast TBI.
- Neurobehavioral, neuropathological, and neurochemical consequences of shock tube BOP exposures of varied intensities have been described and are ongoing.
- EEG recordings have been initiated to distinguish electrophysiological consequences of individual and combined blast- and weight drop-induced brain insults.
- Visual discrimination procedures have been established to quantitatively assess visual acuity and cognitive performance.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

Based in part upon the work supported by this award, funding was sought through research preproposals and proposals submitted to the CDMRP and DMRP, which included:

- Imaging biomarkers for mild blast-induced traumatic brain injury
- Blast-induced acceleration in a shock tube: distinguishing primary and tertiary blast injury mechanisms in rat TBI
- Roles of polyunsaturated fatty acids in traumatic brain injury vulnerabilities and resilience: evaluation of salutary effects of DHA supplementation using neurolipidomics and functional outcome assessments
- Diagnostic and Therapeutic Targeting of Neuroinflammation in Blast TBI
- Novel nitroxide-based therapy to optimize 100% oxygen use in critical traumatic brain injury resuscitation and transport

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 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 examine greater BOP intensities and alternative interblast intervals, as is planned. The endproduct 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).

REFERENCES: Long JB, Bentley TL, Wessner KA, Cerone C, Sweeney S, and Bauman RA. Blast overpressure in rats: recreating a battlefield injury in the laboratory. J. Neurotrauma 2009 26:827-840.

SUPPORTING DATA: BELOW

SUPPORTING DATA:

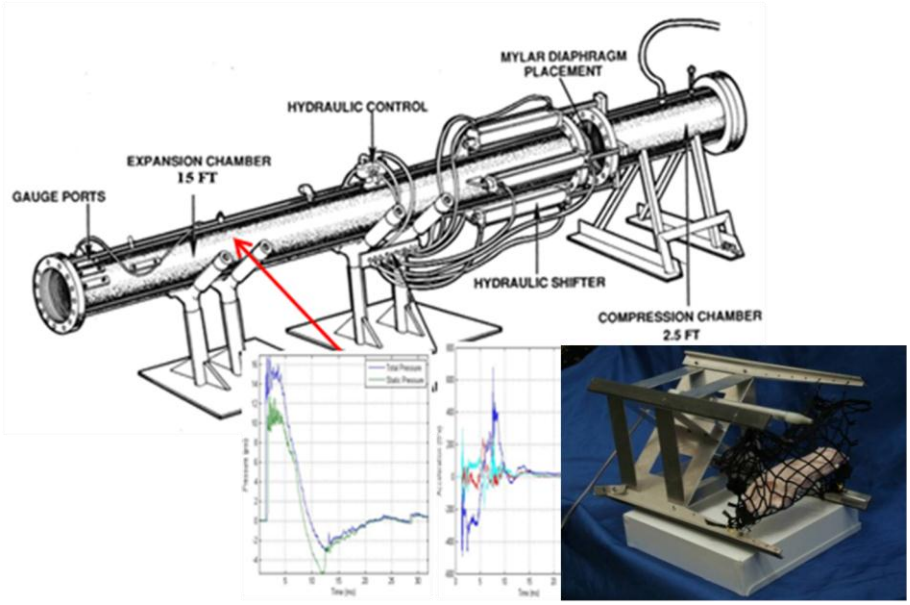



**Fig 1. BOP exposure:** The shock tube consists of a 2.5 ft long compression chamber that is separated from a 15 ft long expansion chamber by polyester Mylar membranes (DuPont, Wilmington, DE). Both chambers are 1 ft in diameter. The compression chamber is pressurized with room air, causing the Mylar 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 mesh pouch positioned 2.5 ft within the mouth of the shock tube. The critical biomechanical loading to the experimental subject is determined from both the static (Ps) and dynamic pressure (Pd) of the blast wave, which are fully recorded by the combination of side-on and head-on pressure gauges (left tracing). Accelerometers also reveal appreciable acceleration of the rat during BOP exposure (right tracing).

**Fig 2. Rotating Pole Test:** Rats are trained to traverse a rotating pole. After TBI they are tested and scored based upon distance travelled, velocity and balance with 3 being the maximum score.

**Fig 3.** Rotating pole scores following a single BOP exposure

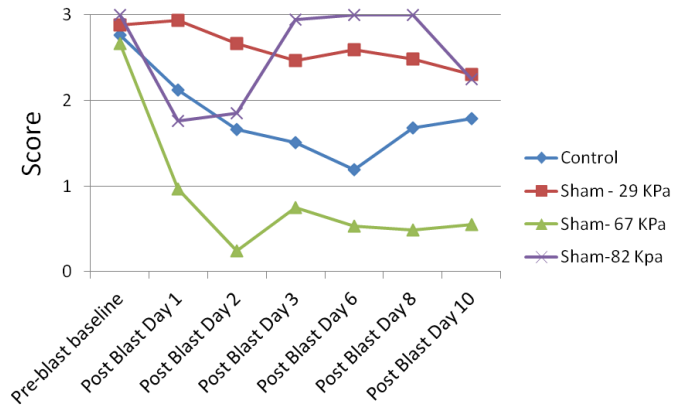



**Fig 4.** Rotating pole scores following repeated BOP exposures

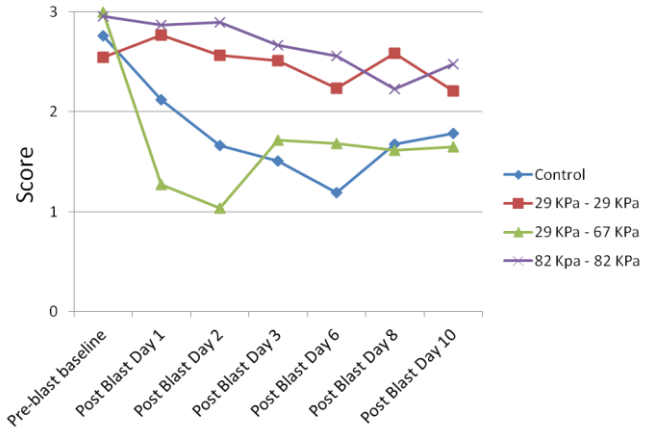



**Fig 5.** MWM latencies with single & repeated BOP

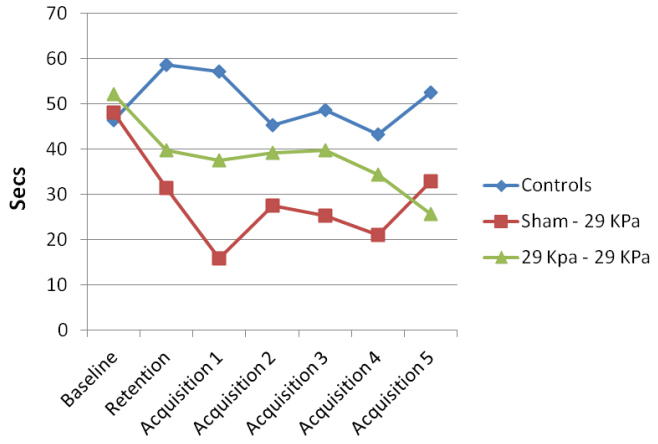



**Fig 6.** MWM latencies with repeated BOP of varied intensities

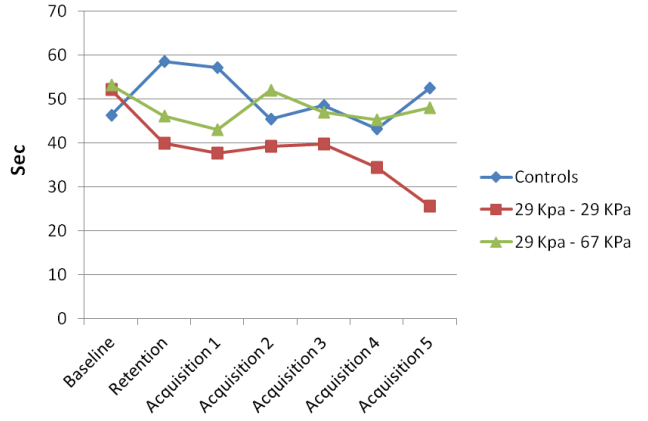



**Fig 7.** MWM latencies with repeated BOP of varied intensities

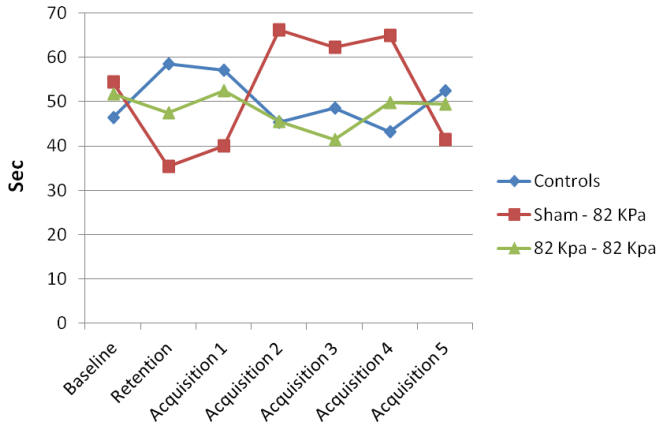



**Fig 8.** MWM latencies with repeated BOP of varied intensities

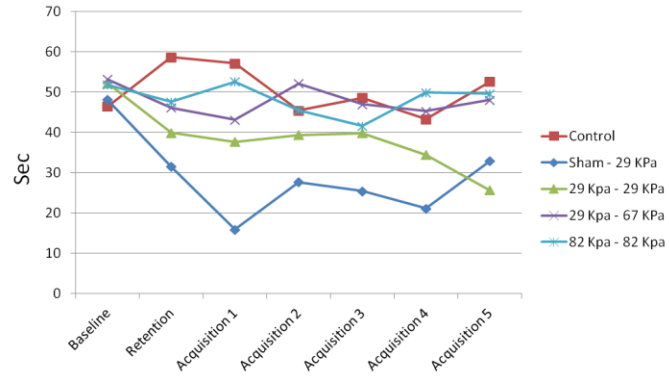
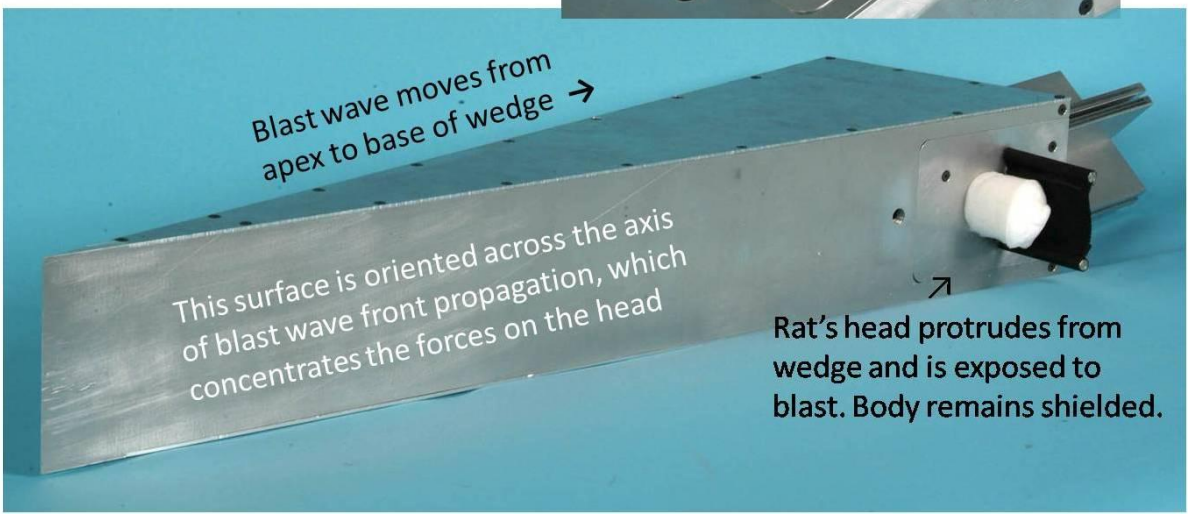
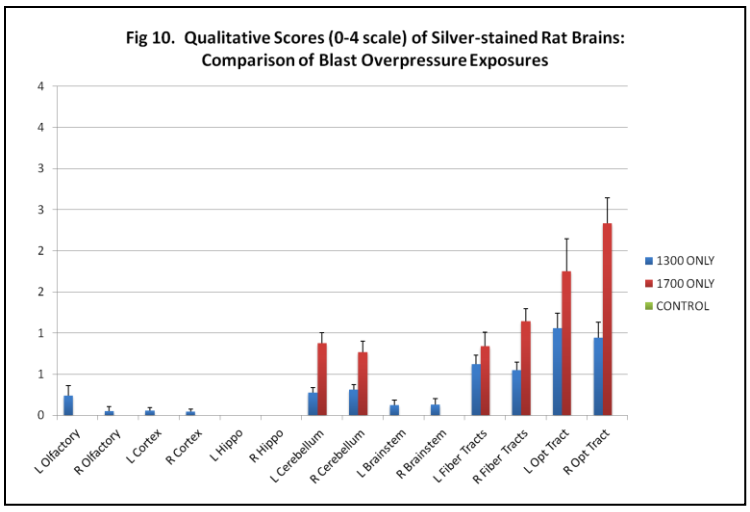


**Fig 9.**

A mach stem wedge is used to position and constrain the rat within the blast tube. The wedge protects the body while the angled surface concentrates blast forces on the head, allowing careful control over bTBI severity. A pneumatically driven piston or miniature captive bolt gun (A) will be mounted on the wedge to generate closely-timed controlled impact acceleration injuries.

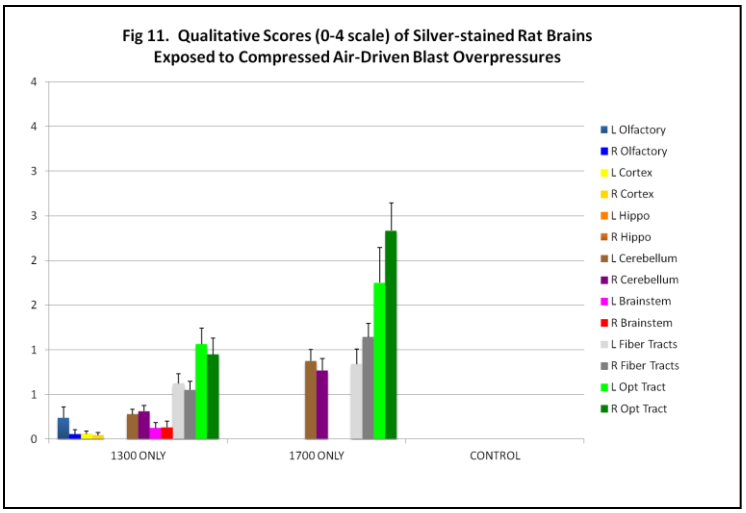



Fig 12. Visual discrimination box. One wall of the chamber contained a food trough where reward pellets were delivered for correct responses. On each side of the trough was a response lever with an indicator light above it (Figure 2). The animals were required to press the lever as indicated by the light. Initially, the left light was illuminated to signal a left lever press.

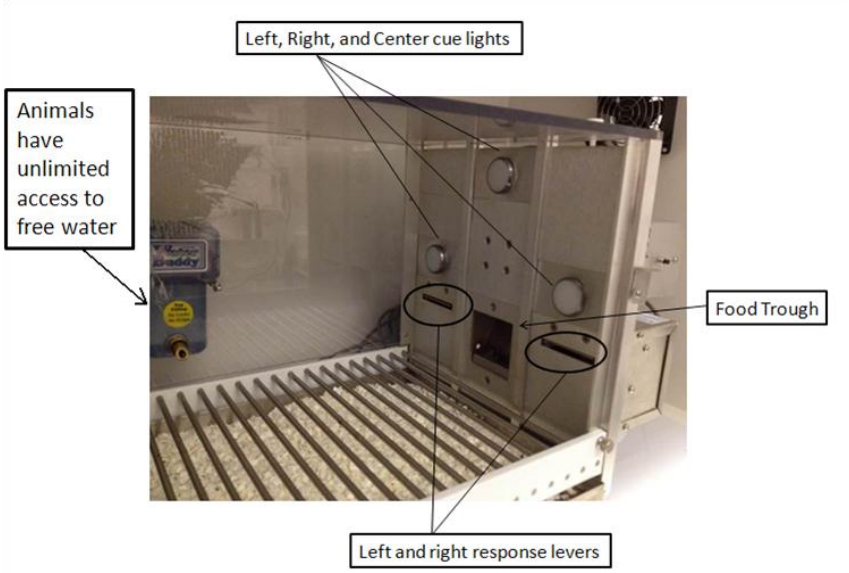
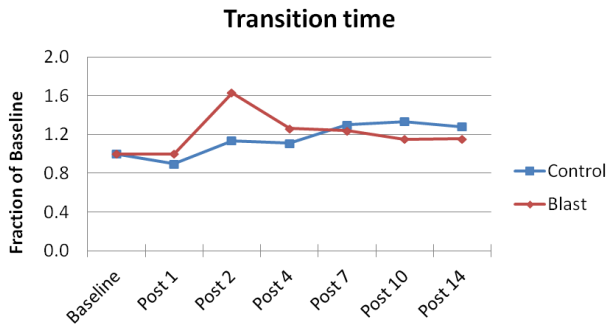



Fig 13 & 14. Immediately after a successful left lever press, the right light was illuminated and the time required for the rat to press the right lever was recorded, with a 30 sec cutoff. Failure to lever press within 30 sec resulted in a 30 sec timeout during which the chamber was completely darkened before testing resumed.



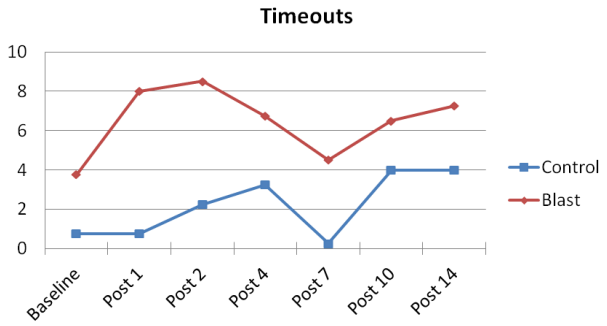



Fig 15. Synchronized suppressed EEG following isoflurane administration.

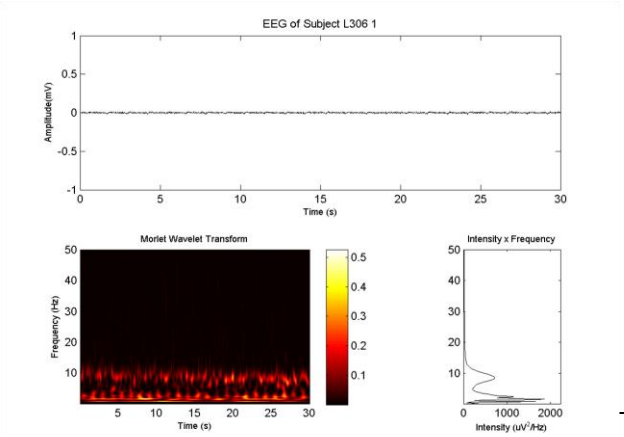



Fig 16. Epileptiform activity immediately postinjury.

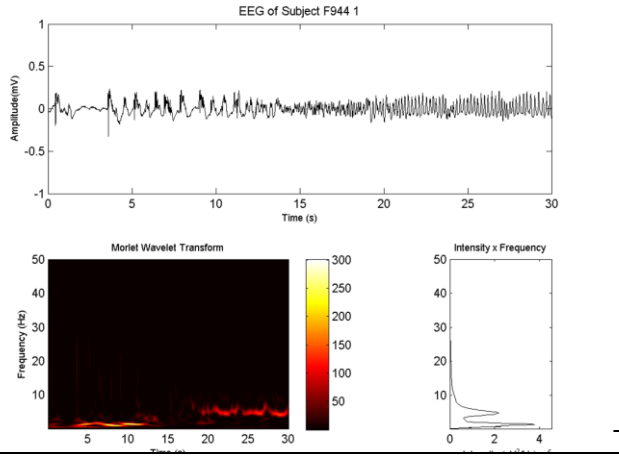



Fig 17. Epileptiform discharges 9 days postinjury.