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Award Number: W81XWH-08-2-0017

TITLE: Kevlar Vest Protection Against Blast Overpressure Brain Injury: Systemic
Contributions to Injury Etiology

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REPORT DATE: May 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE 31 May 2009		2. REPORT TYPE Annual		3. DATES COVERED 1 May 2008 – 30 April 2009	
4. TITLE AND SUBTITLE Kevlar Vest Protection Against Blast Overpressure Brain Injury: Systemic Contributions to Injury Etiology				5a. CONTRACT NUMBER W81XWH-08-2-0017	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Joseph B. Long, Ph.D. M.D. Email: joseph.long@na.amedd.army.mil				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Geneva Foundation Lakewood, WA 98496				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The etiology of blast-induced traumatic brain injury (TBI) is largely undefined. Along with reducing mortality, in preliminary experiments Kevlar vests significantly protected against BOP-induced neuropathological changes in rats. We postulate that: 1) much of the blast-induced fiber degeneration in brain results from pressure surges transmitted through the vasculature that elicit a series of intracranial disruptions, and 2) Kevlar vests are neuroprotective by uncoupling this pressure transmission following exposure to blast. Using a compression driven shock tube, we compare external, systemic (e.g. vascular arterial and venous), and central (e.g. intracranial pressure) BOP-induced pressure changes, and assess the impact of Kevlar vests on these changes. We seek to: 1) determine if measured pressure changes are blast severity-dependent and correspond with outcome measures, and 2) assess the impact of Kevlar vests on measured BOP-induced pressure changes and outcome measures and establish whether a protective vest encasing the thorax ameliorates blast-induced brain injury, pointing to a significant contribution of the effects of blast on the thorax to brain injury. These studies will provide critical insights into the etiology of blast-induced brain injury, and will advance the development of mitigation strategies.					
15. SUBJECT TERMS Traumatic Brain Injury (TBI), Kevlar Vests, Neuroprotection					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	13	19b. TELEPHONE NUMBER (include area code)

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INTRODUCTION:

Body armor has made blast injuries survivable; consequently, we speculate that to a large extent blast-induced head injuries have emerged among troops who without body armor would have simply been killed in action as a result of injury to more vulnerable organs such as the lung. Serendipitously, in a preliminary experiment we noted that along with reducing mortality, lung injury, and cardiovascular disruptions by blast overpressure (BOP), Kevlar vests protected against BOP-induced neuropathological changes in rats. These preliminary findings suggested that a protective vest encasing the thorax might ameliorate blast-induced brain injury, pointing to a significant contribution of the effects of blast on the thorax to brain injury pathophysiology. We hypothesize that much of the blast-induced fiber degeneration in brain results from pressure surges transmitted through the vasculature (venous as well as arterial) that elicit a series of intracranial disruptions, and that Kevlar vests are neuroprotective by uncoupling this pressure transmission following exposure to blast.

BODY:

Description of work to be done (Statement of work):

To address how BOP effects on the thorax contribute to brain injury and to evaluate how Kevlar vests protect the brain, we propose to measure, compare, and correlate external, systemic (e.g. vascular arterial and venous), and central (e.g. intracranial pressure) BOP-induced pressure changes, and assess the impact of Kevlar vests on these changes. In particular, we will, use a compression driven shock tube to: 1) determine if measured pressure changes are blast severity-dependent and correspond with neuropathological and neurobehavioral outcome measures, and 2) assess the impact of Kevlar vests on measured BOP-induced pressure changes and outcome measures. As detailed below, in addition to neuropathological and neurobehavioral evaluations, these outcome measures will include assessments of blood-brain barrier integrity and cerebral blood flow measurements, since we postulate that the cerebrovasculature plays a pivotal role in blast-induced brain injury pathophysiology, and is likely to be disrupted by blast-induced perturbations.

Specific Aims/Milestones:

1. Using a compression-driven shock tube, measure, compare and correlate external (i.e. shock tube), systemic (i.e. vascular arterial and venous), and central (i.e. intracranial pressure) effects of BOP of varied intensities (114, 126 and 147 kPa; requires 80 rats).
2. Determine if measured pressure changes in the experimental subject are blast severity-dependent and correspond with neuropathological and neurobehavioral outcome measures (requires the same 80 rats as specific aim 1).
3. Assess impact of Kevlar vests on measured BOP-induced pressure changes and neurobehavioral and neuroanatomical outcome measures (requires 160 rats).
4. Assess impact of Kevlar vests on measured BOP-induced pressure changes and acute cerebrovascular measures (CBF and BBB measurements, requires 160 rats).

Methods:

As previously described, BOP will be generated using a compression-driven shock tube with mylar membranes rupturing at predetermined pressure thresholds. Using piezoelectric free field blast probes, Millar pressure transducers and /or fiber optic pressure sensors, external and physiological compartmental pressure changes evoked by different BOP intensities (126 and 147 kPa) will be recorded in isoflurane-anesthetized rats wearing or not wearing protective Kevlar vests. Vests are made from Kevlar neck shields and completely wrap around the rat's thorax, leaving the head fully exposed. Rats are placed in a transverse prone position in a holder that is secured across the mouth of the shock tube. Arterial and central venous pressures will be recorded through PE 50 catheters implanted into the carotid artery and external jugular vein, respectively. ICP will be recorded using cannulae stereotaxically implanted into the lateral cerebral ventricle. Data will be captured using LabView software. In addition to establishing the effects of Kevlar vests on pressure recordings and mortality, surviving rats will be evaluated for neurological function and spatial learning using kinematic analysis of beam walking and Morris water maze, respectively. Rats will be kinematically evaluated 1-5 days post-BOP, and will be tested in the Morris water maze on days 8-12 post-BOP. At 14 days post-BOP, rats will be perfused transcatheterially and euthanized. After fixation, serial coronal sections will be prepared using fluoro-jade, cresyl violet, and silver stains, and microscopically evaluated to assess the extent and location of neuronal injury and fiber degeneration. Based upon indications of fiber degeneration in preliminary studies, histopathological assessments will include optical density quantitation of fiber degeneration in silver-stained sections.

In a parallel series of experiments, BOP-induced changes in cerebral blood flow, cerebral oxygen tension, and blood-brain barrier permeability will be determined. The effects of Kevlar vests on these perturbations will also be examined. Cerebral blood flow and cerebral oxygen tension will be monitored during the first 2 hrs post-BOP using Perimed PF5010 LDF units and Licox A3 oxygen electrode systems, respectively. Blood-brain barrier disruption will be evaluated using quantitation of extravasated Evans Blue dye, at 1 and 4 hrs post-BOP.

KEY RESEARCH ACCOMPLISHMENTS:

During this initial reporting period, we:

- Interviewed, hired and trained 3 research technicians who are now skilled in the animal handling, surgical, and experimental data collection procedures required for this study.
- After researching telemetric physiological data collection systems from competing vendors, selected and purchased a system from Data Sciences International. This EEG recording capability is now established and in place for data collection after airblast exposure.

- After researching experimental means to perform visual assessments in rats, we ordered visual discrimination equipment from Med Associates, Inc.

During this initial reporting period, we also recognized several significant technical challenges for our proposed research, and responded with experimental solutions. Notably:

- After gaining a better appreciation of blast physics, we recognized that it is critical to be able to vary the position of rats within the shock tube using a holder that is minimally intrusive regarding rat exposure to shock wave and associated air movement. Realizing that our existing rat holder is inadequate, we designed a new rat holder that is under production that will enable us to record the static and dynamic pressures each rat is exposed to in a non-rigid restraint device that neither shields the experimental subject nor contributes to the injury. The new holder will also better accommodate instrumentation required for physiological and internal pressure recordings. Although this unforeseen requirement has slowed our data collection from our original time projections, we are confident that we can complete the study within the overall time schedule with vastly improved, artefact-free data.
- During the course of exploring the different transducer technology options we have considered for intravascular and intracranial recordings, we encountered difficulties with the durability and fidelity of fiber optic recordings. Although to date we have not yet established a completely problem-free alternative, we have discovered an alternative that we optimistically believe will be both durable and capable of recording artefact-free pressure changes in the brief timeframe required.

Despite these significant challenges that interrupted our planned pressure recordings, we did nevertheless complete work to establish that, although the protective vest did not significantly reduce the duration of apnea (Fig. 1), it did blunt the depressor and bradycardic responses to a 147-kPa airblast (Figs. 2 and 3). In addition, the protective vest significantly improved survival 24 h following exposure to 126- or 147-kPa airblast exposure (Table 1).

Brains removed from these survivors at 2 weeks postairblast revealed characteristic neuropathological changes. Notably, the brains from rats exposed to a 126-kPa airblast typically were devoid of any obvious cell loss or injury, and instead most typically showed widespread fiber degeneration that was clearly prominent in silver-stained sections throughout all levels of the brain (Fig. 4). Fiber degeneration was observed bilaterally and did not appear to be more prominent in either hemisphere. Axonopathy was strikingly evident in commissural fibers and other fiber tracts, and was also evident at higher magnification in small-caliber fibers as well.

When the brains of rats exposed to airblast with and without vests were compared, a clear pattern of limited protection is evident. The brains of rats wearing vests when exposed to a 126-kPa airblast were strikingly different from those not wearing vests.

The widespread fiber degeneration that was conspicuous in the latter group was largely absent in the former. Optical density comparisons as well as neuropathological categorization by a blinded trained observer revealed that protective vests significantly protected brains from axotomy following airblast exposure (Fig. 5), pointing to a significant contribution of the systemic effects of blast to the associated brain injury pathophysiology. More specifically, these findings directly associate the systemic influences of airblast with axonopathy and fiber degeneration, since this dominant neuropathological feature was largely eliminated by the protective vest at this airblast intensity.

In addition to causing intensity-dependent neuropathological changes in the brain, airblast also disrupted neurobehavioral performance. Beam-walking latencies were significantly elevated the day following airblast, and progressively returned to pre-injury levels over 4 days (Fig. 6). As also seen in Fig. 6, spatial navigation in the MWM was significantly impaired 8 days following airblast exposure (126 kPa). Latency to locate the submerged platform was significantly prolonged on the first 2 days of testing, and improved to sham latencies by days 3 and 4. To minimize the impact of transient motor impairments (e.g., weakness) and neurological disruptions on water maze performance, the rats were not evaluated in the MWM until 8 days after airblast exposure, by which time beam ambulation times had returned to pre-injury levels, indicating a sizeable recovery of motor function. Spatial navigation in the water maze was subsequently significantly albeit transiently disrupted, with latencies to find the platform among airblasted rats recovering to those seen in sham injured rats by day 3 of testing. In addition to motor function, since this test of acquisition and retention also requires visual acuity sufficient to recognize and navigate relative to the visual cues placed around the pool, airblast injuries to the eyes and visual system could also account for performance decrements. Although it cannot be completely ruled out, the rapid recovery of performance by day 3 of testing argues against this possibility, since visual impairments and resultant performance decrements would in all likelihood be much more persistent.

REPORTABLE OUTCOMES:

Manuscript and presentation:

Long, J.B., Bentley, T.L., Wessner, K.A., Cerone, C., Sweeney, S. and Bauman, R.A.. Blast Overpressure in Rats: Recreating a Battlefield Injury in the Laboratory. *Journal of Neurotrauma*, In press.

Long, J.B. Blast Overpressure in Rats: Recreating a Battlefield Injury in the Laboratory. Presentation at the International State-of-the-Science Meeting on Non-Impact, blast-induced mild traumatic brain injury in Herndon, VA, May 12-14, 2009.

CONCLUSION:

In summary, blast-induced TBI has emerged as a major threat to warfighters, and presents a daunting challenge for those responsible for their medical care. Optimization of this medical care will require appreciable preclinical support to recreate novel aspects of these injuries, along with the rapid development of interventions and mitigation strategies. Although its fidelity to these warfighter injuries is not yet clearly established, our preliminary work with shock tube-generated airblast injury clearly points to interplay between the brain and the periphery as important pathophysiological considerations to factor into these strategies.

SUPPORTING DATA:

Tables, figures and legends:

Table 1.

24 hour survival

Intensity	Vest	No Vest
126 kPa	8/8	5/8
147 kPa	6/6	4/11

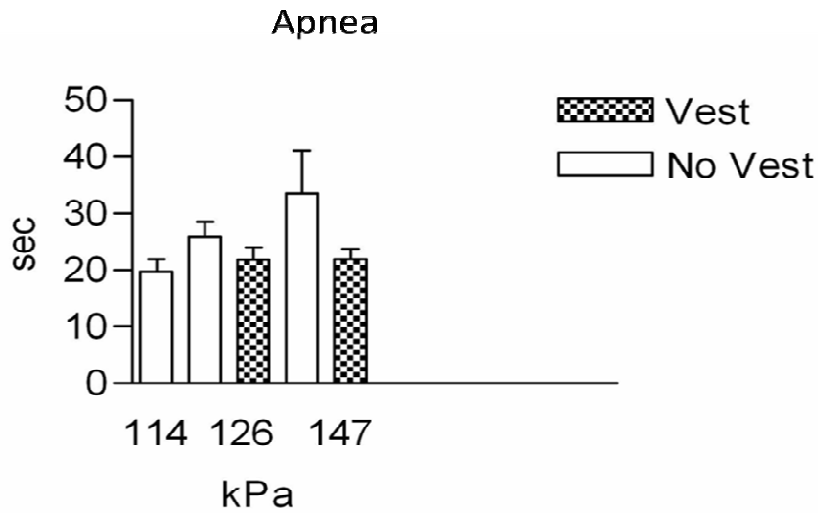


FIG. 1. Duration of apnea recorded after 114, 126, or 147 kPa airblast exposures. At the 2 higher intensities, apneic responses were compared among rats exposed to airblast with or without protective vests, and did not differ. Protective vests were not applied to any rats exposed to 114 kPa airblast exposure.

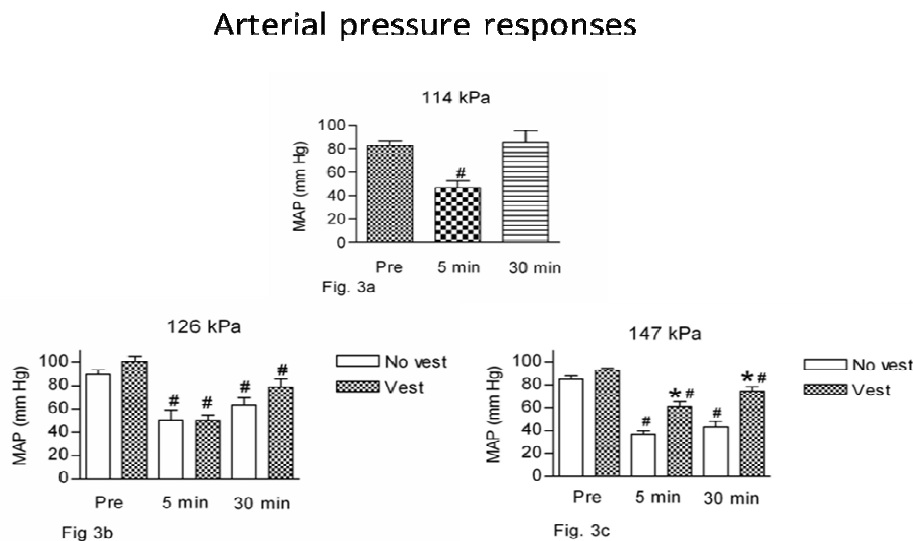


FIG. 2. Arterial pressure responses to airblast. Immediate depressor responses were seen with all 3 airblast intensities. MAP returned to baseline by 30 min following 114

kPa airblast, but rats remained hypotensive through 30 min following higher intensity exposures. The protective vest ameliorated the depressor response to 147 kPa airblast.

* $p < 0.01$ when compared to no vest subjects

$p < 0.01$ when compared to pre-airblast measurements

Heart rate responses

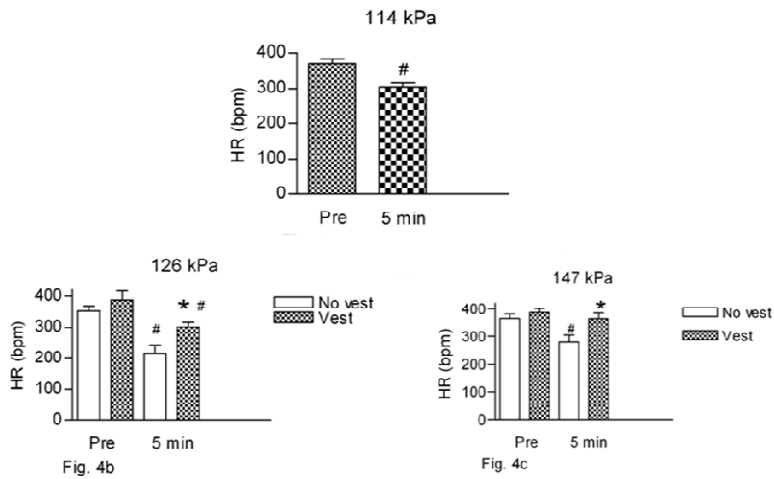


FIG. 3. HR responses to airblast. Immediate bradycardia was seen with all 3 airblast intensities. Bradycardia was significantly attenuated in rats exposed to airblast while wearing the protective vest. Heart rates recovered to baseline by 30-45 min post-airblast (not shown).

* $p < 0.01$ when compared to no vest subjects

$p < 0.01$ when compared to pre-airblast measurements

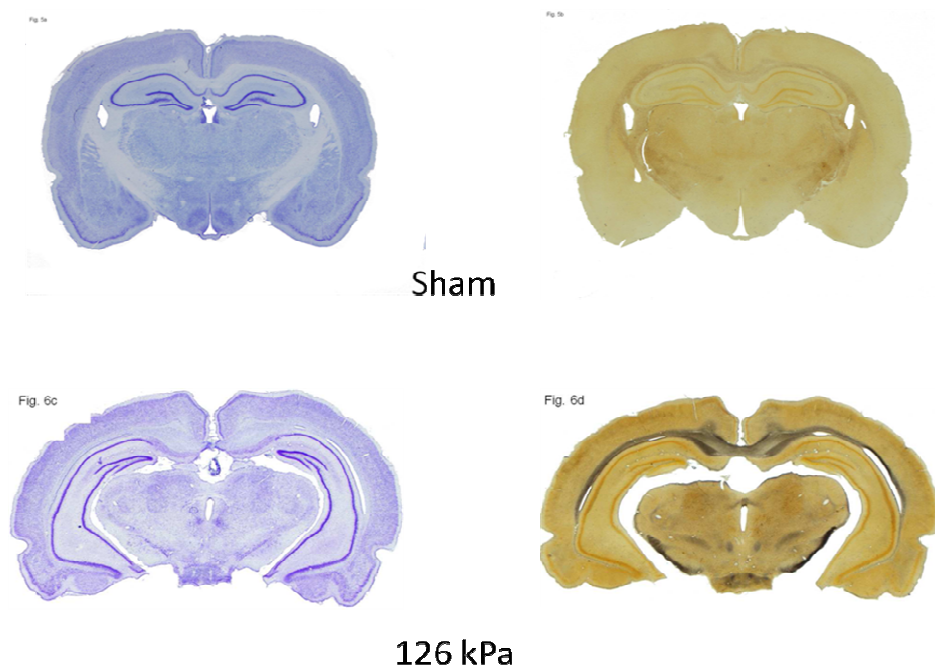


FIG. 4. In contrast to sham rats (top), brains of rats exposed to a 126-kPa airblast (bottom) showed extensive axonopathy, which was evident as blackened fibers in silver-stained sections (bottom right). Despite extensive fiber degeneration, the brains were devoid of any obvious cell loss (bottom left).

Fiber degeneration following 126 kPa airblast

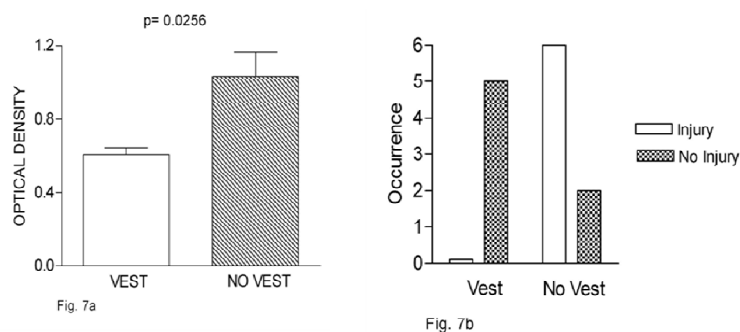


FIG. 5. Protective vests significantly eliminated fiber degeneration resulting from a 126-kPa airblast. Comparisons of optical densities (A) and occurrence of neuropathological injury (B) reveal significant neuroprotection afforded by the vests.

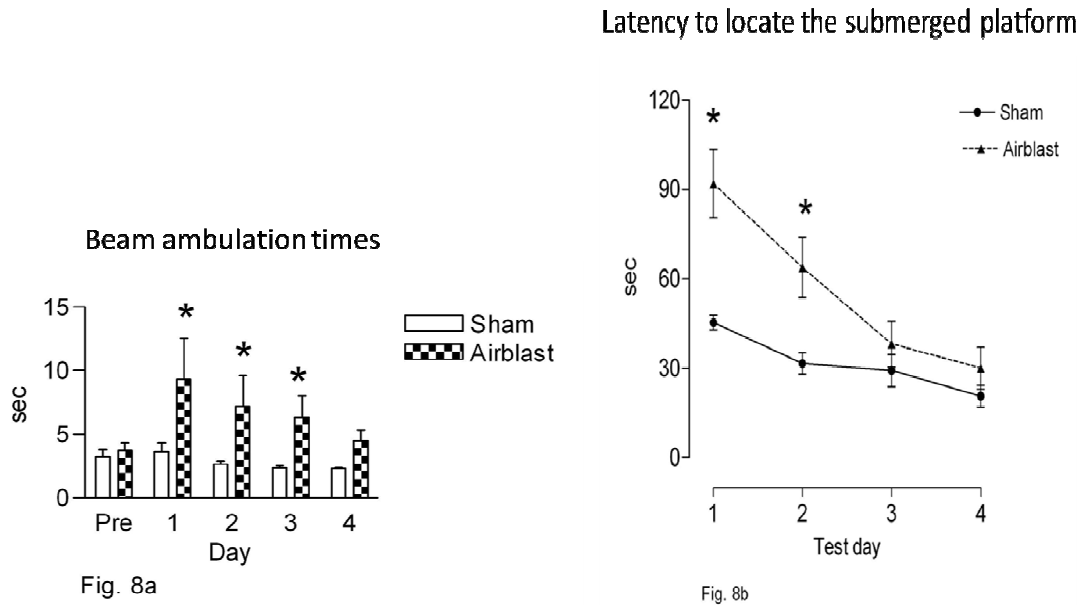


FIG. 6 . Beam ambulation (left) and MWM latencies (right) were recorded on days 1-4 and days 8-11 following 126 kPa airblast, respectively. Beam ambulation time was significantly increased through the first 3 days postinjury and returned to preinjury latencies by day 4. MWM latencies were significantly increased on the first 2 test days, and did not differ from latencies seen in sham treated rats by test days 3 and 4.

* $p < 0.05$ when compared to sham-treated rats