

Characterising Recovery of Long Term Potentiation following Repeated Blast-induced Traumatic Brain Injury in Rat Brain Organotypic Hippocampal Slice Cultures

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I. INTRODUCTION

The Department of Defense has recorded over 385,000 cases of traumatic brain injury (TBI) in United States military personnel worldwide, including TBI due to blast exposure [1]. Although exposure to blast during combat is a major concern for military personnel, some service members, like breachers, who use explosives to penetrate closed facilities, and others who fire heavy weapons are regularly exposed to blast during training [2]. There is a concern that military personnel who are exposed to repeated low intensity shock waves may suffer neurocognitive deficits. Previously, we have shown in rat organotypic hippocampal slice cultures (OHSCs) following a single and repeated mild blast TBI, long term potentiation (LTP), a neuronal correlate for learning and memory was impaired [3-4]; repeated blast injuries delivered less than six days apart resulted in aggravated LTP deficits. However, there was generally a recovery of LTP six days following the second blast exposure [5]. To better inform return-to-duty criteria and further determine the optimal recovery period, in this study we varied the blast levels, the inter-blast-intervals, and recovery periods following repeated injury.

II. METHODS

OHSCs (400 μm thick) were excised from the brains of rapidly decapitated P8-10 Sprague Dawley rat pups, cultured on porous Millipore Millicell cell culture membranes, and fed every 3-4 days for 10-12 days. After verifying minimal cell death (<5%) by staining with propidium iodide (PI), healthy brain slices were placed into a sterile plastic bag within the warmed (37°C) fluid-filled receiver, and exposed to either sham or blast injuries using our previously described shock tube injury model [6-7]. Cultures were subjected to a variety of blast levels and inter-blast intervals (Table 1).

TABLE I
MATRIX OF EXPERIMENTS CONDUCTED DURING THIS STUDY

	Overpressure Duration Impulse	Inter-Blast Interval	Post-Blast Recovery
Group 1 -Double Blast	Level 2 - 93 kPa 1.4 ms 39 kPa•ms	4 days	
		5 days	5 days
		6 days	6 days
Group 2-Double Blast	Level 1 - 106 kPa 0.25 ms 9.2 kPa•ms	1 day	
	Level 2 - 93 kPa 1.4 ms 39 kPa•ms	3 days	4 days
	Level 3 - 190 kPa 1.2 ms 73 kPa•ms	6 days	
Group 3-Triple Blast	Level 1 - 106 kPa 0.25 ms 9.2 kPa•ms		3 days
	Level 2 - 93 kPa 1.4 ms 39 kPa•ms	1 day	6 days

After the recovery period, OHSCs were once again stained with PI for cell death, and then neuronal function, like LTP, was measured with a 60 channel microelectrode array (MEA, MultiChannel Systems, Reutlingen, Germany). The slices were stimulated in the pyramidal cells of the Schaffer Collateral (SC) in the CA3 region of the hippocampus from 0-200 μA (10 μA increments). The recordings from each electrode in the CA1 region were fit to a sigmoidal curve and the current necessary to elicit a half-maximal response (I_{50}) was determined. Baseline measurements were established by stimulating the slices at I_{50} once a minute, for 30 minutes. LTP was induced by using a 100Hz tetanic constant-current (I_{50}) stimuli. Post-induction recordings were then captured by stimulating at I_{50} once a minute for 60 minutes. Potentiation was calculated from the electrodes in the CA1 region as the percent change in the average response over the last 10 minutes of the post-LTP and baseline recordings.

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III. INITIAL FINDINGS

In all the blast and sham groups, the slices exhibited less than 5% PI staining, indicating minimal cell death. There was non-significant LTP loss five days after a four or five day interval between two Level 2 blasts; for all intervals tested, there was recovery of LTP six days after the second blast injury (Figure 1A). There were LTP losses in all levels of blast delivered at one day inter-blast intervals (Figure 1B). There was a recovery of LTP to sham values in the Level 1 blast group after increasing the inter-blast interval to three days, but there was some LTP loss in the Level 2 and 3 groups, in accordance with previous findings [5]. LTP was restored in both the Level 2 and 3 blast severities after extending the inter-blast interval to six days, but there did not appear to be LTP recovery in the Level 1 group, which was surprising. There were some LTP deficits following both blast levels in the one day inter-injury interval group at three days following the third blast exposure (Figure 1C). At six days following the third blast exposure, LTP fully recovered after a Level 1 or Level 2 blast.

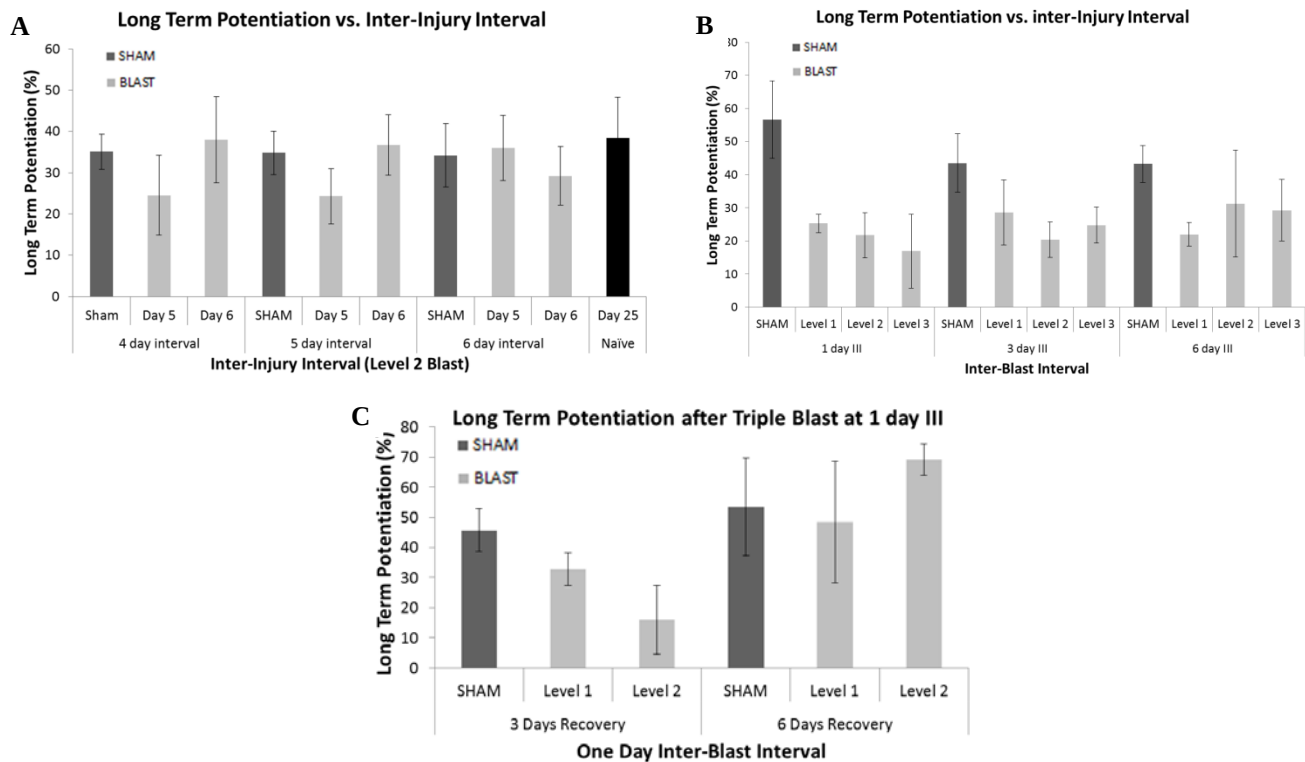


Fig. 1. The mean potentiation ($\pm$ SEM) in the CA1, following SC stimulation, for each group after blast exposure ($n = 5-10$ slices).

IV. DISCUSSION

To better inform return-to-duty criteria, a variety of blast levels, inter-blast intervals, and recovery times were tested (Table 1). As previously reported [5], we found aggravated LTP deficits after repeated blast, but found no worsening of LTP after a third blast injury when compared to two repeated exposures. Although there are still LTP deficits four days after blast, there was full recovery of LTP to sham values after six days in all tested groups. This spontaneous recovery confirms previous findings that a minimum of six days is needed for LTP-recovery. As the interval between blast injuries increased, there was an improvement in LTP, with the most recovery in groups with an inter-blast interval of six days.

V. REFERENCES

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