Recovery of long-term potentiation in rat brain organotypic hippocampal slice cultures following repeated blast-induced traumatic brain injury

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I. INTRODUCTION

Traumatic brain injury (TBI) has been recorded by the Department of Defense over 375,000 times in US military personnel worldwide [1]. These injuries are incurred by service members participating in recreational activities, during training, and during active deployment. Some of these reported TBI cases are the result of exposure to primary blast waves, which interact with the brain tissue. While many service members can be at risk of exposure to blast-induced traumatic brain injury (bTBI), some service members, like breachers, who use explosives to penetrate closed facilities, are prone to repeated blast exposures during both training and deployment [2]. Military personnel who are exposed to a bTBI are subject to return-to-combat criteria that largely depend on when the affected service member becomes asymptomatic [3]. Previous studies have shown deficits in long-term potentiation (LTP), a neuronal correlate for learning and memory, in rat organotypic hippocampal slice cultures (OHSCs) following a single mild bTBI [4-5], and additional deficits in LTP as a result of a second, mild bTBI less than six days after the first blast. After repeated blast injury, there was some recovery of LTP at six days following the second blast exposure [6]. Previously collected data suggested a more objective recovery period following bTBI that could inform return-to-combat criteria. In order to further determine the optimal recovery period, we varied the interval times between blast injuries and recovery periods following the second injury.

II. METHODS

Following the rapid decapitation of P8-10 Sprague Dawley rat pups, hippocampi were excised and immediately sectioned into 400 µm thick slices. The OHSCs were cultured onto porous Millipore Millicell cell culture membranes and fed every 3–4 days for 10–12 days. At 10–12 days after culturing, the OHSCs are stained with propidium iodide to verify minimal cell death (<5%). Following this verification, the healthy tissues were exposed to either sham or blast injuries using our previously described shock tube injury model [7-9]. The OHSCs on the Millipore membranes were placed into a sterile plastic bag within the warmed (37°C) fluid-filled receiver of the shock tube and exposed to either a mild blast or sham. The slices were exposed to two repeat mild blasts (peak pressure 93 kPa, duration 1.4 m/s, impulse 39 kPa•m/s). Following the first blast, the Millipore membranes were removed from the receiver and placed into fresh full serum media for 4, 5 or 6 days. The slices were once again exposed to either the same mild blast or to sham injury following this interval. At 5 or 6 days following the second injury, the slices were stained with propidium iodide for cell death analysis and then neuronal function, like LTP, was measured with a 60 channel microelectrode array (MEA, MultiChannel Systems).

Initially, the slices were stimulated at the pyramidal cells of the Schaffer Collateral (SC) in the CA3 region of the hippocampus from 0-200 µA (n 10 µA increments). The recordings from each electrode in the CA1 region was fit to a sigmoidal curve and the current necessary to elicit a half-maximal response (I_{50}) was determined. For LTP measurements, the slices were stimulated at the same electrodes in the SC of the CA3 at I_{50} once a minute, for 30 minutes, in order to establish baseline measurements. Subsequently, LTP was induced by stimulating the slice at the I_{50} with three successive 100 Hz pulse trains for 1 second, with 10 second intervals between each train. Then, slices were again stimulated at the SC of the CA3 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-induction and baseline recordings.

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III. INITIAL FINDINGS

In all the blast and sham groups, the slices exhibited less than 5% propidium iodide staining, indicating minimal cell death. The mild blast exposure generally reduced LTP in the experimental groups when compared to the sham slices for the 4, 5 and 6 day inter-injury intervals at both 5 and 6 days recovery following the second blast. Interestingly, as the inter-injury interval increased to 6 days and the recovery period increased to 6 days, LTP appears to recover to sham levels.

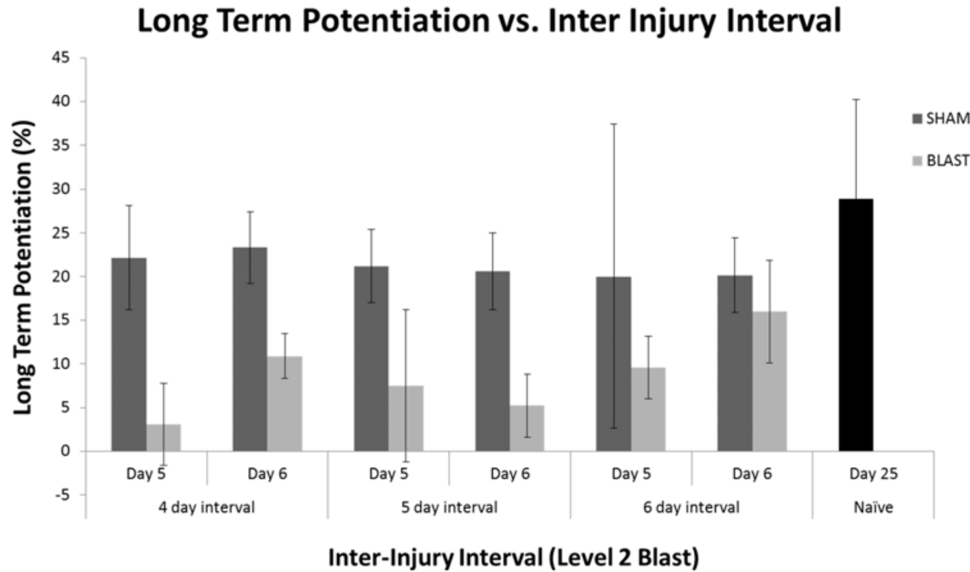

Fig. 1. The mean potentiation (±SEM) in the CA1, following SC stimulation, for each blast exposure group. LTP potentiation was lowered in all inter-injury intervals and both recovery days following the second injury, as compared to the sham slices.

IV. DISCUSSION

In order to better inform return-to-combat criteria, the inter-injury interval was varied and LTP at 5 and 6 days following the final injury was measured. Following a single mild blast injury, Vogel III [4] had provided evidence for the recovery of LTP between 6 and 10 days and in multiple blast experiments, while Effgen [6] had reported that LTP only recovered once the inter-injury interval was extended to 6 days. From the current study, an inter-injury interval of 6 days and a recovery period of 6 days after the second blast resulted in the greatest recovery of the LTP when compared to sham. While more data need to be collected to determine statistical significance, it appears that 6 days is the minimum time needed for LTP recovery following a repeated blast.

V. REFERENCES

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