Delayed inhibition of long-term potentiation in rat brain slice cultures caused by primary blast exposure

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I. INTRODUCTION

Traumatic brain injury (TBI) has been diagnosed in over 320,000 US service personnel since 2000, with 82.5% of cases classified as mild [1]. Common symptoms of these injuries include loss of consciousness, loss of spatial navigation, behavior/mood changes and memory loss [2-3]. Blast-related TBI poses a significant problem for military personnel both in combat and in training. Primary blast loading, which is the loading caused by the interaction of the shock-wave with the skull and brain tissue, remains poorly understood. Great emphasis has been placed on identifying operational thresholds for blast-induced TBI. Previous studies have quantified alterations in electrophysiological activity within acute or cultured neurons/slices following blast [3]. A specific electrophysiological process of interest is long-term potentiation (LTP), which is the functional correlate of memory formation [4]. Our previous research has reported that primary blast can inhibit LTP [5]. This short communication investigates the time course of blast-induced LTP-inhibition within rat brain organotypic hippocampal slice cultures (OHSCs).

II. METHODS

Rat OHSCs (400 µm thick) were excised from the brains of P7-P10 Sprague-Dawley pups, cultured on porous Millipore Millicell cell culture inserts, and fed every two days for 10–14 days. Blast injury was then initiated using a compressed-gas driven shock tube, with the cultures placed in a fluid-filled receiver directly below the shock tube exit. Cultures were subjected to either a sham injury or to one of two blast conditions: 336 kPa/0.84 ms/87 kPa·ms (mild); or 424 kPa/2.31 ms/248 kPa·ms (moderate).

Electrophysiological recordings were acquired at 1 hour, 1, 2, 4, 6 or 10 days following injury. At the time of recording, slices were placed onto 60-channel microelectrode arrays and perfused with artificial cerebral spinal fluid. Stimuli from 0-200 µA (in 10 µA increments) were applied across the Schaffer collateral (SC) pathway to generate S/R curves. Each electrode's response was fit to a sigmoidal curve, defined by specific parameters with physiological meaning: the maximum amplitude of the evoked response (R_{max}), the current necessary to generate a half-maximal response (I_{50}), and the synchronicity of the firing neurons (m). Pre-LTP recordings were captured by application of I_{50} stimuli once a minute for 30 minutes. LTP was induced in CA1 via the SC pathway using 100Hz tetanic constant-current (I_{50}) stimuli. Post-LTP recordings were captured by application of I_{50} stimuli once a minute for 60 minutes post-induction. Potentiation was calculated as the change in average response over the last 10 minutes of post-LTP recordings divided by the average response over the last 10 minutes of pre-LTP recordings.

An observed lack of blast-induced cell death was confirmed through propidium iodide staining prior to moderate blast or sham exposure and at 10 days post-exposure. To verify the slice culture viability after blast, a subset of cultures was exposed to moderate blast injury and subsequently to an excitotoxic injury (10mM of glutamate for 3 hours) 10 days following blast exposure and imaged for cell death 24 hours later.

III. INITIAL FINDINGS

At 60 minutes post-blast exposure, there was no significant difference in potentiation between sham (58 ± 11%), mild (50 ± 10%) and moderate blast (61 ± 22%) exposure levels. At 1 day post-blast exposure, both mild (27 ± 7%) and moderate (18 ± 13%) blast exposures caused a significant decrease in potentiation as compared to sham exposure (65 ± 7%). This significant LTP deficit remained present at intermediate time points of 2, 4 and 6 days. At 10 days post-injury, LTP was no longer significantly depressed in cultures exposed to mild blast (43 ± 14%), but LTP remained depressed in cultures exposed to the moderate blast (11 ± 7%) as compared to sham (60 ± 12%). Cell death was measured in the day 10 cultures and was not significantly different from sham-exposed cultures, remaining low in all regions (< 11%). When 10 days post-moderate blast cultures were exposed to glutamate, cell death was significantly elevated in all regions (> 80%), indicating the presence of living cells 10 days after moderate blast.

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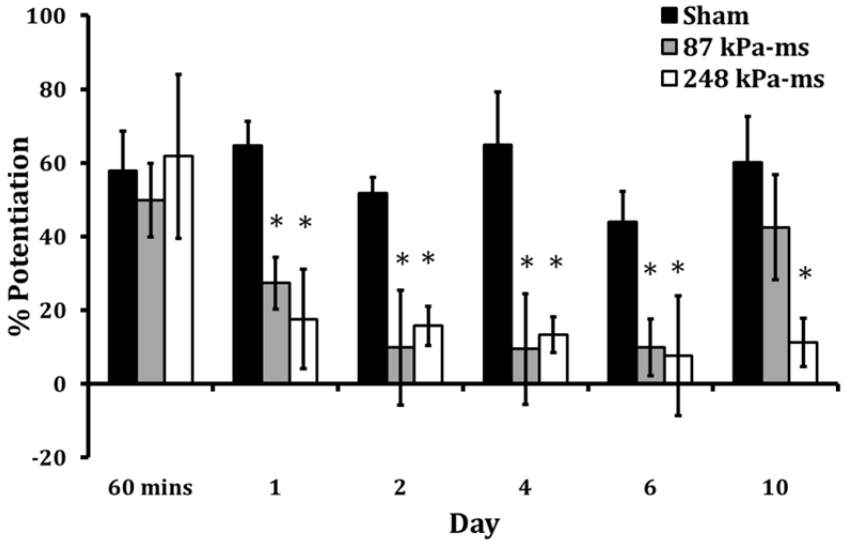

Fig. 1. Time course of mean potentiation (±SEM) in the CA1, following SC stimulation, for each blast exposure group. LTP was significantly decreased at 1 day post-mild [Pressure: 336 kPa, Duration: 0.84 ms, Impulse: 87 kPa-ms, n ≥ 5 slices] and post-moderate [Pressure: 424 kPa, Duration: 2.31 ms, Impulse: 248 kPa-ms, n ≥ 5] blast as compared to the age-matched sham slices (n ≥ 5). The deficit remained significant until day 10 for only the moderately injured group. A univariate general linear model was used at each time point, with percent potentiation as the unique dependent variable and experimental group (sham and both blast injury levels) as the fixed factor (*p< 0.05).

IV. DISCUSSION

We report that primary blast injury did not acutely (within 1 hour) reduce LTP for either injury level. However, a significant deficit developed between 1 and 24 hours for both injury levels. This LTP deficit persisted after both mild and moderate injury for at least 6 days. At 10 days post-injury, LTP recovered in the mild injury group, but LTP remained significantly depressed after moderate blast-exposure at the same time point. Although LTP has not been previously studied following primary blast injury, fluid percussion injury in rodents depresses LTP as early as 2–4 hours [6-7] and for as long as 15 days [8] after injury. Substantial (but not complete) LTP recovery was demonstrated in rats at 7 days following unilateral cortical impact injury [9]. Although the injury biomechanics are different between our blast model and the non-blast TBI models used in previous studies, it is evident that TBI depresses LTP shortly after injury, but can naturally recover after a certain period of time. These deficits occurred in the absence of substantial cell death, which indicates diminished neuronal function within living cells. Future work will focus on identifying the molecular mechanisms responsible for blast-induced LTP deficits.

V. REFERENCES

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