Primary blast injury erases long term potentiation in rat brain organotypic hippocampal slices

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I. INTRODUCTION

Traumatic brain injury (TBI) has been diagnosed in nearly 300,000 members of the US armed forces since 2000 [1]. Common symptoms of these injuries include concussion, 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, the blast 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 attempted to observe the effect of blast-induced TBI on cognitive/behavioral changes in mice following exposure [4]. Another approach is to quantify alterations in electrophysiological activity within acute or cultured neurons/slices following blast [5]. These in vitro approaches may have utility for determining tolerance criteria to primary blast in terms of changes in neural function. This short communication investigates the effect of primary blast loading on multiple electrophysiological measures 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 plastic culture-well inserts placed into sterile bags within a fluid-filled receiver directly below the shock tube exit. Cultures were subjected to either a sham injury or one of two blast conditions: 336 kPa/0.84 ms/87 kPa·ms (mild) or 424 kPa/2.31 ms/248 kPa·ms (moderate).

Functional recordings were acquired four to six days following injury and, prior to recordings, cell death was measured by propidium iodide staining. At the time of recording, slices were placed onto 60-channel microelectrode arrays and perfused with artificial cerebral spinal fluid. Three functional measures (stimulus-response [S/R], paired-pulse [PP], long-term potentiation [LTP]) were recorded from the exposed tissue slices. Stimuli from 0-200 µA (in 10 µA increments) were applied in specific locations 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). PP stimulation, a measure of short-term plasticity, was produced by injecting two successive electrical stimuli, at a magnitude of I_{50}, with increasing interstimulus intervals (ISIs), ranging from 20-2000ms. PP ratios were reported as the amplitude of the second response divided by the first, at each ISI. These parameters were measured in each region of the hippocampus (DG, CA3, and CA1) in response to the two major stimulation pathways (mossy fibers [MF] or Schaffer collaterals [SC]). LTP, a functional correlate of memory formation, was induced in CA1, via the SC pathway, using 100Hz tetanic constant-current (I_{50}) stimuli. 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.

III. INITIAL FINDINGS

After blast injury, in response to mossy fiber (MF) pathway stimulation, S/R activity was slightly reduced in CA1 and CA3 regions, whereas DG was unaffected. In these cases, injury decreased R_{max} and increased I_{50}. No substantial S/R changes were observed with SC stimulation. Mild blast injury did not significantly alter the PP response. When stimulating across MF pathway, PP ratios at shorter ISIs (20-100ms) were marginally increased, in CA1, after moderate blast injury only. When stimulating across the MF pathway at ISIs between 140-500ms, PPR decreased in DG. When stimulating across SC pathway at ISIs between 140-500ms, PPR decreased in all three regions. When stimulating across either the MF or SC pathways, there was no effect of blast injury at ISIs above 500ms. In contrast with the previous measures, long-term potentiation was completely disrupted following blast injury. Both mild (-8 ± 3%) and moderate blast (8 ± 5%) eliminated LTP as compared to sham exposure (53 ± 9%).

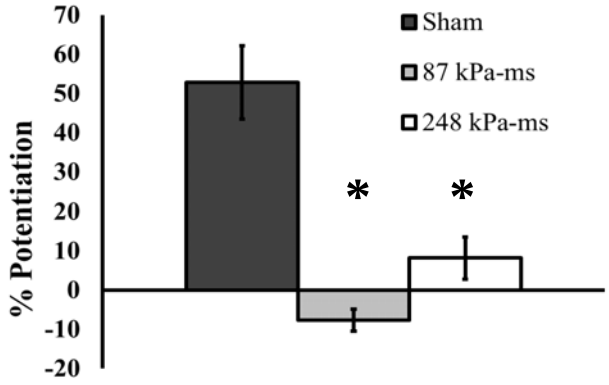

Figure 1. Mean potentiation (±SEM) in the CA1, following SC stimulation, for each blast exposure group. LTP potentiation was significantly lowered in mild [Pressure: 336 kPa, Duration: 0.84 ms, Impulse: 87 kPa-ms, n= 5 slices] and severe [Pressure: 424 kPa, Duration: 2.31 ms, Impulse: 248 kPa-ms, n= 5] injured slices, as compared to the sham slices (n= 5). A univariate general linear model was used with percent potentiation as the unique dependent variable and experimental group (sham and both blast injury levels) as the fixed factor (significance * $p< 0.05$).

IV. DISCUSSION

We conclude that primary blast injury does not significantly affect S/R or PP evoked activity; however, exposure to primary blast wholly disrupts long-term potentiation. Following MF stimulation, excitability was marginally decreased in CA3 and CA1 at both injury levels, as was the response amplitude. Larger deficits in hippocampal excitability and response amplitude have been reported previously following lateral fluid percussion injury [2]. In our study, PP ratios were slightly increased at shorter ISIs, where the response is governed by γ-aminobutyric $acid_A$ ($GABA_A$) receptors [6]. PP ratios decreased at mid-length ISIs, where the response is governed by $GABA_B$ receptors [6]. Unlike the S/R and PP protocols, LTP was completely reduced following either blast exposure level. Previous studies have shown that the mild blast exposure used in the current study did not cause cell death [3]. Our study highlights that LTP was fully eliminated at blast levels that do not induce cell death. Similar results have been reported within CA1 following inertially-driven injuries [7]. Our findings suggest that blast-induced TBI preferentially affects cellular mechanisms that control LTP compared to those that control other functional responses (S/R, PPR). The functional deficits that occur in the absence of cell death suggest that neuronal death is not a cause for the observed changes, as well, for the levels of blast that were tested. LTP deficits may explain memory loss commonly observed in TBI patients. When attempting to identify a safe blast threshold, LTP may be a more sensitive outcome measure than other functional measurements. Future research will elucidate the mechanisms responsible for the disruption of LTP following primary blast injury.

V. REFERENCES

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