# Primary Blast Exposure Reduces Brain Tolerance to Subsequent Blast

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## I. INTRODUCTION

An increasing number of soldiers are diagnosed with traumatic brain injury (TBI) following blast-exposure [1]. As many as 20,000 US soldiers were diagnosed with at least one mild TBI (mTBI) in 2012 [2]. Blast-exposure is comprised of multiple phases. Primary blast-loading results from the shockwave and is unique to blast; higher order blast-loading mechanisms (secondary, tertiary, etc.) have known potential to cause brain injury [3-4].

Some US soldiers can experience multiple primary blast-exposures in isolation without concomitant secondary or tertiary exposure. Among such soldiers are included breachers, who use explosives to break perimeters, such as locked buildings [5]. Breachers can experience as many as 20 explosive detonations over five days during practical training [5-6], and symptoms reported after repetitive blast-exposure have been informally referred to as "breacher's brain" [5-6]. These symptoms are similar to those of multiple non-blast concussions and include difficulty concentrating, sleep disturbances and difficulty forming new memories [5-6]. Previously we have reported that isolated primary blast alters electrophysiological activity of brain slice cultures with minimal cell loss [7]; however it is unclear whether multiple primary blasts interact to influence worse outcomes for soldiers. Therefore, we set out to study the effect of repetitive primary blast on the brain.

In previous studies, mild non-blast injury reduced the tolerance of brain cells to subsequent injury, resulting in a synergistic increase in neurodegeneration and cell death [8-9]. It is unclear, however, if primary blast alters tissue tolerance to subsequent blast, resulting in increased vulnerability to subsequent primary blast-loading. Evaluating the potential of primary blast to reduce the tolerance of brain tissue to subsequent exposure could inform the design of improved head protection for soldiers to guard against lower intensity blasts. This could also inform preventative measures for soldiers exposed to isolated, primary blast, removing them from active duty to prevent worse outcomes from repetitive primary blast exposure.

## II. METHODS

Organotypic hippocampal slice cultures (OHSCs) were generated from P8-10 Sprague-Dawley rat pups, as previously described [7]. At the time of injury, OHSCs were placed in a custom fluid-filled receiver that was positioned directly at the exit of a 76 mm diameter shock tube producing shockwaves with nitrogen or helium [7]. Sham cultures were treated identically, except the shock tube was not fired.

For repetitive blast studies, cultures received 0, 1 or 2 blast exposures of varying intensities (9–87 kPa•ms), either 24 or 72 hours apart. Samples not receiving a blast at a given time point received the sham injury. A subset of OHSCs received three consecutive high intensity blasts (248 kPa•ms). Cell death was evaluated three days following the final exposure. Electrophysiological function was assessed on days three through five following the final exposure.

Cell death was quantified by propidium iodide (PI) fluorescence prior to and three days following final exposure. At the end of the experiment, and as a positive control for total cell death, OHSC were treated with 10 mM glutamate for three hours, and samples were PI-stained 24 hours later. Tissue damage was quantified as the percentage area of a specific region of interest (ROI) (DG, CA3, CA1) exhibiting fluorescence above a threshold [7].

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Electrophysiological function was evaluated by collecting stimulus-response (S-R) and long-term potentiation (LTP) data. All recordings were performed with 60-channel microelectrode arrays (MEAs). S-R curves were generated by applying a constant current, bi-polar, biphasic stimulus of increasing magnitude (0-200 µA in 10 µA increments) to electrodes located in the Schaffer collaterals (SC). Evoked responses were recorded from all electrodes simultaneously and assigned to an anatomical region (DG, CA3, CA1) based on an image of the slice on the MEA. As described in the literature, each electrode's recording was fit to a sigmoidal curve with the following equation [10]:

$$R(S) = \frac{R_{max}}{1 + e^{m(I_{50} - S)}}$$

$R_{max}$ is the maximum voltage response. $I_{50}$ is the current needed to generate a response that is 50% of $R_{max}$. The term *m* is the slope of the linear and increasing portion of the sigmoidal fit [10]. LTP was immediately evaluated following S-R. A baseline response was recorded for 30 minutes by stimulating the SC at $I_{50}$ every 60 seconds prior to LTP induction. LTP was induced by stimulating the SC with three successive trains of 100 Hz at $I_{50}$ for 1 second with 10-second intervals between trains. The post-induction response was recorded for 60 minutes, with stimulation in the SC at $I_{50}$ every 60 seconds. Potentiation was quantified as the percent increase in response (average peak-peak voltage) of the post-induction recording as compared to the baseline recording for each electrode. Data was calculated for electrodes within the CA1 [11-12].

## III. INITIAL FINDINGS

There was no significant difference among OHSCs receiving one blast of 37 kPa•ms (Level 2) or the sham. However, there was a significant decrease in LTP for samples receiving two Level 2 blasts delivered 24 hours apart as compared to sham. This deficit persisted even when the interval between exposures was increased to 72 hours. There was no significant difference in LTP among OHSCs receiving one or two blasts of 9 kPa•ms (Level 1) delivered 24 hours apart. There was a significant decrease in LTP for one or two blasts of 89 kPa*ms (Level 4) delivered 24 hours apart.

Cell death did not increase significantly following repetitive primary blast exposure as compared to sham injury (<5% cell death in all ROI). Cell death was minimal in sham-injured controls (<5% cell death in all ROI). Cell death induced by glutamate exposure (10 mM) following repetitive blast indicated that OHSC contained viable cells that were not killed by these injury paradigms (>37% cell death in all ROI).

There was no significant difference among OHSC receiving two Level 2 blasts delivered 24 hours apart for the S-R parameters m, $R_{max}$ or $I_{50}$ in any region as compared to sham.

## IV. DISCUSSION

These studies suggest that primary blast may reduce brain tissue tolerance such that multiple Level 2 primary blasts resulted in super-additive deficits in LTP. Changes in LTP were observed in the absence of significant cell death or changes in S-R parameters, which suggests that single and repetitive primary blasts do not cause gross structural damage or alter neuron firing. Taken together, these data suggest that repetitive primary blast may disrupt pathways specific to the hippocampus's ability to remodel. Given that LTP is an *in vitro* correlate for learning and memory, our data suggests that the brain's ability to form new memories or to learn could be altered by repetitive, low-level primary blast exposure. This is the first study to demonstrate that isolated primary blast reduces brain tolerance to subsequent primary blast.

A threshold for repetitive blast-induced LTP deficits was observed between Levels 1 and 2. Additionally, a single Level 2 blast initiated a period of heightened vulnerability that persisted for at least three days, such that subsequent exposure resulted in significant LTP deficits. These results, in conjunction with those of future *in vivo* studies, could be used to improve the design of helmets to protect the brain against shockwave loading, as well as inform safe return-to-duty guidelines for soldiers exposed to primary blast.

## V. REFERENCES

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