In Vitro Primary Blast Injury Induces Cell Death in the Hippocampus

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I. INTRODUCTION

An increasing number of Warfighters are diagnosed with traumatic brain injury (TBI) following blast-exposure [1-2]. Blast-exposure resulting in injury is multiphasic: primary blast-loading results from the shock wave and is the only injury mechanism unique to blast. Higher order injury mechanisms have been studied previously with known potential to induce TBI, including penetrating (secondary) or inertial-driven (tertiary) TBI. Therefore, the complexity of blast confounds interpretation of the resultant pathology, and there exists controversy in the literature as to whether a shock wave alone can injure brain. Exposure of *in vitro* brain slice cultures to isolated primary blast would allow for the resolution of this controversy without these dilemmas.

II. METHODS

Organotypic hippocampal slice cultures (OHSCs) from P8-10 Sprague-Dawley rat pups were generated as previously described [3]. OHSC were grown for 10-14 days before injury. At the time of injury, OHSC were placed in a custom fluid-filled receiver directly at the exit of the tube [4-5].

OHSC were secured in a custom receiver, and a 76 mm diameter shock tube pressurized with helium or nitrogen generated a shock wave [4-5]. In a previous study, finite element analysis predicted that tissue-strain during blast exposure was below 5% for this set-up [4]. OHSC were immediately removed from the receiver and returned to the incubator until the prescribed time point. Sham cultures were treated identically except the shock tube was not fired. For repetitive blast, OHSC were exposed to sham or blast injury on day 0 and 1 and received 0, 1 or 2 total blast injuries. Propidium iodide (PI) fluorescence was used to quantify cell death prior to and following injury. Tissue damage was quantified as the percentage area of a specific region exhibiting fluorescence above a threshold.

III. INITIAL FINDINGS

Cell death was evaluated up to 4 days following a 248 kPa·ms impulse, 424 kPa peak pressure and 2.31 ms duration blast. Cell death increased significantly at day 4 as compared to time-matched controls but was not elevated prior to this time point. For a range of blast severities, cell death most highly correlated with impulse, and the threshold for a significant increase of cell death was between 151 and 184 kPa·ms as compared to sham-injured controls. Blast of 184 kPa·ms delivered twice 24 hours apart did not significantly increase cell death compared to sham-injured cultures or those exposed to a single blast at the same level.

IV. DISCUSSION

These data suggest that primary blast results in cell death and that impulse may be the most relevant basis for a tolerance criterion. The threshold for significant primary blast-induced cell death was between 151 kPa·ms and 184 kPa·ms. The pattern of induced cell death suggested that principal excitatory neurons were more vulnerable to blast. Given the increased use of improvised explosive devices in combat, Warfighters are exposed to multiple blasts. Our data suggest that repetitive injury synergy (RIS) from two primary blasts does not occur at 184 kPa·ms, which we identified as a supra-threshold exposure for cell death; future studies are needed to identify relevant thresholds for blast-induced RIS.

V. REFERENCES

[1] US DOH, Blast Injuries, 2009. [2] Faul M e al, US DOH, 2010. [3] Morrison B 3 e al, Stapp, 2003. [4] Panzer M B e al, Frontiers, 2012. [5] Effgen G E e al, Frontiers, 2012.

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