Interval-Specific, Blood-Brain Barrier Disruption *In Vitro* After Repetitive Primary Blast Injury

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I. INTRODUCTION

Studies of repetitive brain injury (non-blast) have shown heightened susceptibility to vascular dysfunction and neuropathological changes after the initial insult [1]. However, effects of repetitive primary blast injury on the blood-brain barrier (BBB) are not well understood. This study demonstrates a temporal window of vulnerability to repetitive blast injury in an *in vitro* BBB model by transendothelial electrical resistance (TEER).

II. METHODS

A mouse brain endothelial cell line (bEnd.3) was used to generate *in vitro* cultures representing the BBB. Blast injuries (402 kPa overpressure, 0.92 ms duration, and 118 kPa*ms impulse) were delivered in repeated fashion using a shock tube [2]. Cultures were placed in a fluid-filled receiver simulating the surrounding brain and skull. TEER was measured with an Endohm-12 electrode chamber and EVOMX Voltohmmeter (WPI).

III. INITIAL FINDINGS

Following exposure to repeated blast injury separated by 24 hours, TEER of the double injury group decreased to 44 ± 7 %, compared to TEER of 71 ± 4 % in the single injury group and 101 ± 5 % in sham (Fig. 1A). Repeated injury also resulted in delayed recovery of TEER (3 days) compared to cultures exposed to a single blast (1 day; Fig. 1B). After repeated blast delivered 72 hours apart, the double injury group exhibited a consistent 25-30 % difference in TEER compared to age-matched shams on both days 1 and 4 (Fig. 1C).

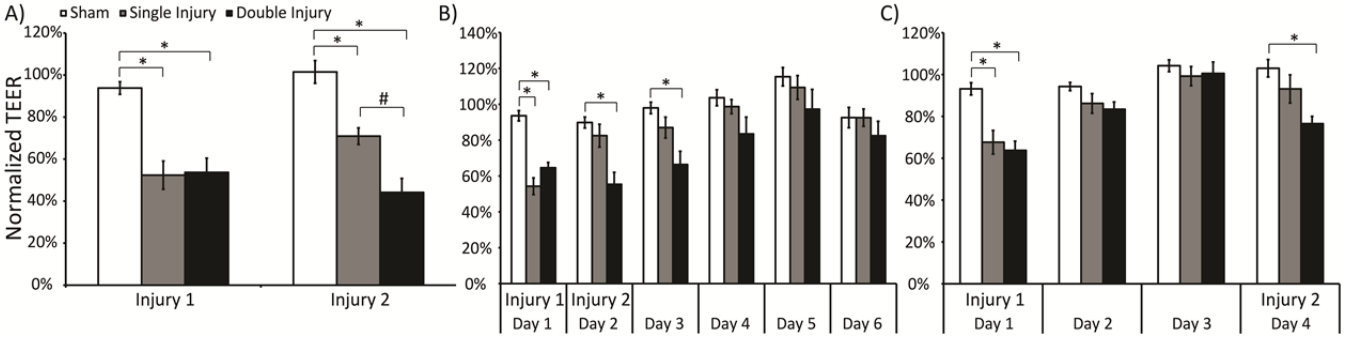

Fig. 1: Interval-specific effects of repetitive blast on an *in vitro* BBB model. A) TEER response after repeated blast delivered 24 hours apart. B) Delayed TEER recovery in cultures exposed to repeated blast injury. C) Elimination of cumulative TEER depression associated with 72 hour inter-injury interval (*$p < 0.05$, comparison to sham; # $p < 0.05$, comparison between single and double injury groups; SEM; Sham n ≥ 6; Injured n ≥ 6).

IV. DISCUSSION

Cultures exposed to repeated blast injury separated by 24 hours exhibited delayed recovery of TEER compared to single injury exposure. An expanded 72 hour inter-injury interval eliminated cumulative effects on TEER depression, as demonstrated by similar differences in TEER between the double injury and sham groups. These results reveal a window of heightened vulnerability to repetitive blast injury in an *in vitro* BBB model and may suggest a minimum mandatory rest-period to prevent cumulative effects.

V. REFERENCES

[1] Fujita M et al, J Neurotrauma, 2012; [2] Effgen GB et al, Front Neurol 3, 2012

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