

## CUMULATIVE EFFECTS OF REPEATED PRIMARY BLAST IN RATS

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**Background:** The recent emergence of evidence linking multiple mild traumatic brain injuries to progressive, long-term debilitations and neurodegeneration has prompted concern over the cumulative deleterious effects of blast exposures and the need to define exposure limits and standards to mitigate this risk among Warfighters. To understand cumulative effects of blast on neurobehavioral status and associated pathology, a preliminary study was conducted to assess the extent of lung injury, spontaneous exploratory activity, and brain levels of TDP-43 and piezo2 (proteins associated with neurodegeneration and a mechanotransduction channel, respectively) using an advanced blast simulator (ABS) that closely mimics “free-field” blast. Isoflurane-anesthetized rats (n=6/group) were either longitudinally (frontal) or transversely (side-on) restrained in an ABS, exposed to 1-4 daily blasts (single, 2X, 3X, or 4X) at a peak incident pressure of 131 kPa with a positive pressure duration of 5 msec. Control animals underwent identical handling with the exception of blast exposure. Righting times were recorded immediately and open field behavior testing was performed 24hr following each blast exposure. Animals were euthanized 24hrs following the final blast exposure, lungs were insufflated and fixed with paraformaldehyde, and brains were flash frozen until further analyses. Yelverton score and a custom Matlab code were used to identify the extent of pulmonary injury. The whole right cerebrum was homogenized to measure TDP-43, piezo2 and  $\beta$ -actin with Western blotting technique. Pulmonary contusion and hemorrhage were significantly evident in single and repeated exposure frontal and side-on groups when compared to shams ( $p < 0.05$ ), while righting times increased in only side-on exposure groups. Animals exposed to a single blast or 2X blasts did not show a significant alteration in brain TDP-43 levels. However, rats exposed to either 3X or 4X repetitive blasts did have a significant increase in TDP-43 in the brain compared to sham animals (3X: 16%,  $p < 0.05$ ; 4X: 38%,  $p < 0.01$ ). These rats also showed parallel significant increases in piezo2 (3X: ~30%,  $p < 0.01$ ; 4X: ~35%,  $p < 0.05$ ). Open field activity decreased significantly in animals exposed to either 3X or 4X, whereas no changes were observed in the single blast or 2X treatment groups. These findings reveal that cumulative effects of repeated daily exposures to blast can lead to changes in behavior and pathophysiological changes in the brain and systemic organs. In particular, the increase in TDP-43 levels after 3 and 4 high pressure blasts indicates that repetitive blast injury can alter a protein associated with CTE and the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). This finding demonstrates a possible link between blast injury and neurodegenerative disease and provides an important first step in understanding how to prevent these diseases in soldiers exposed to blast.