Neuroinflammatory and behavioral consequences of shockwave-induced traumatic brain injury in mice

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Background

Traumatic brain injury (TBI) is a significant problem worldwide, including for combat military personnel, and is deemed a signature injury of recent wars[1]. Proximity to improvised explosive devices can cause blast-related TBI ("bTBI")[2-3]. Although survival has improved[2], TBI increases the risk for developing neuropsychiatric complications post-injury, including depression, anxiety, and post-traumatic stress disorder[3-6]. Inflammation, a key feature of TBI, is linked to mood disorders and may be one mechanism responsible for TBI-induced neuropsychiatric consequences[7-10]. There is a critical need to define the temporal relationship between inflammatory and behavioral consequences of TBI. Defining this relationship is likely to help guide targeted immune-based treatments, which is the long-term goal of our laboratory.

Research Aims

Aim 1: Define acute and chronic inflammatory consequences in a mouse model of bTBI.

Aim 2: Define acute and chronic behavioral consequences in a mouse model of bTBI.

Methods

Animals. Adult male C57BL6/J mice were subjected to TBI or sham injury and assigned to either acute (7 days post-injury) or chronic (28 days post-injury) cohorts, as follows: 1) Sham injury – Acute, 2) TBI – Acute, 3) Sham injury – Chronic, or 4) TBI – Chronic (***Figure 1***).

Injury model. Prior to injury, animals were anesthetized with a cocktail of ketamine-xylazine and received a single dose of buprenorphine for pain management. TBI: Primary, shockwave-induced injuries can be experimentally induced in rodents using a shock tube (ORA, Inc., ***Figure 2***). Injury parameters for the current study included repeated injury (3x) with a 2 and 30 minute inter-blast interval, blast pressures of ~131 kPa (~19.2 psi), and prone positioning (i.e., facing the shockwave) without body shielding. We chose this model due to its clinical relevance and neuroinflammatory consequences. Specifically, blast pressures in this range induce diffuse axonal injury, astrogliosis, microgliosis, blood-brain-barrier disruption, central cholinergic alterations, neurodegeneration, and chronic motor deficits in mice[11-17]. Due to the similarity of these neuropathological findings to TBI consequences in humans, we consider this a reasonable model of human moderate-severe bTBI. Sham injury animals received the same anesthesia without exposure to the shockwave. ***Figure 3*** depicts blast overpressure recordings previously obtained from Sensors #1 and #3, demonstrating similar peak overpressure and impulse values. Blast tracings also show the secondary shock wave observed at ~10 ms and the minor secondary shock thereafter. These reflective waves are characteristic of our blast tube model due to the constraints of the size of the end wave eliminator[18]. Note that recordings were collected from previous experiments that used single sheets of 14 mil Mylar. The current study employed two sheets of 7.5 mil Mylar to achieve target blast pressures.

Behavioral assessments. Cognitive deficits (novel object recognition), anxiety-like (elevated plus maze, open field test), and depressive-like behavior (forced swim test) were assessed with standardized assays throughout the study (***Figure 1***). Novel objects were presented at +2 and +24 hours after the known object was first introduced.

Tissue collection. At study end and under anesthesia, the meninges overlying the cisterna magna were exposed. A small glass capillary tube was used to puncture the arachnoid membrane and collect cerebrospinal fluid (CSF) by capillary action. Peripheral blood was collected via cardiac puncture and animals were perfused with PBS and 4% paraformaldehyde (PFA). Skulls were post-fixed overnight in 4% PFA, following which, brains were excised and cryopreserved. Frozen brain sections (10 µm) were cut and immunofluorescence was used to identify resident and recruited immune cells, as follows: microglia (TMEM-119), astrocytes (GFAP), immune cells (CD45), and cell nuclei (DAPI) in cortical and hippocampal brain regions. Cytokine protein levels in biofluids were assessed using a multiplex Luminex xMAP assay (Mouse Chemokine Panel 23-plex; Bio-Rad, USA).

Methods, Continued

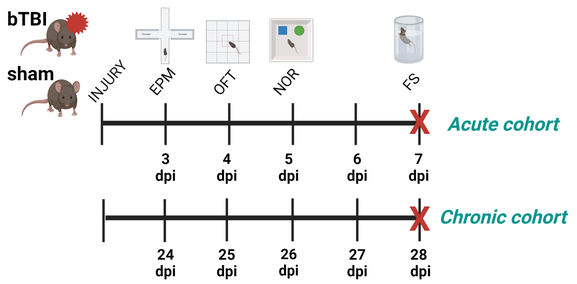


Figure 1. Study timeline. Standardized behavioral assays were performed throughout the studies in the order of least to most aversive. Assays included the elevated plus maze (EPM), open field test (OFT), novel object recognition test (NOR), and forced swim test (FS). Assessment points (days post-injury; dpi) are indicated for each cohort. Animals were euthanized at 7 dpi (Acute) or 28 dpi (Chronic). *Figure created with BioRender.com.*

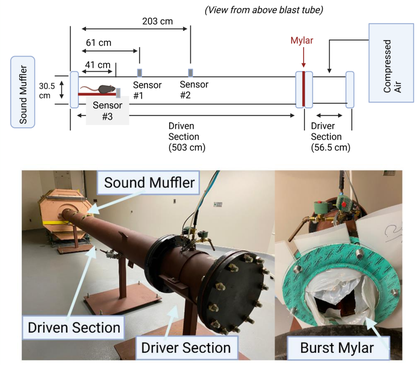


Figure 2. White River Junction VA Healthcare System blast tube apparatus and dimensions.

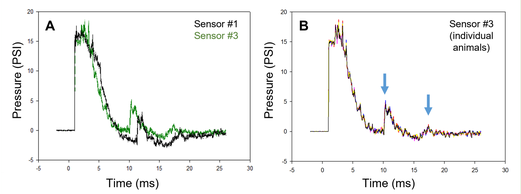
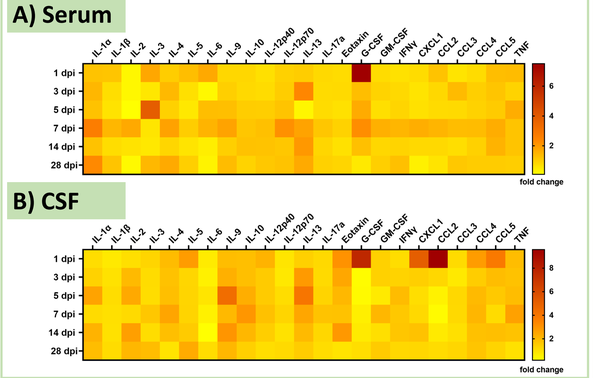
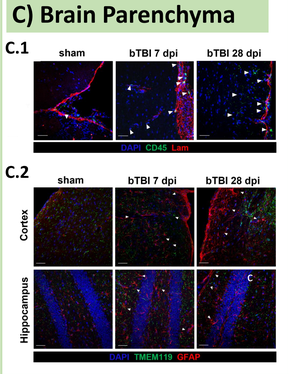


Figure 3. Representative blast overpressure tracings from previous blast injuries (14 mil Mylar) demonstrated the reproducibility of achieving target blast pressures. **A**. Average recordings obtained from pencil probe (Sensor #3) overlaid on recordings obtained from Sensor #1 (*n*=5 per sensor). **B**. Individual blast tracings of overpressures obtained from Sensor #3 (*n*=5). Area under the curve depicts peak overpressure duration (~8-10 ms), with secondary shock waves identified by arrows.

Results





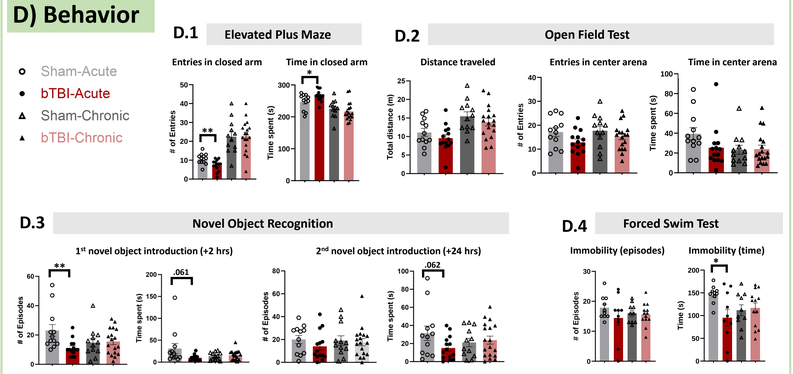


Figure 4. A-B. Heat map displaying temporal mean fold change of each cytokine in **(A)** serum and **(B)** CSF in bTBI (*n*=10-13 per timepoint) compared to sham (*n*=12 per timepoint). **C.** bTBI-induced neuroinflammation. Representative z stack compilations of brain sections from sham and bTBI mice at 7 dpi and 28 dpi (*n*=4 per time group). **(C.1)** Immunostaining with DAPI (blue), CD45 (green), and laminin (lam; red) in the cortex. CD45+ cells are observed in the perivascular, meningeal, and parenchymal space at 7 dpi (white arrows), with increased CD45 immunostaining present in the parenchyma at 28 dpi (white arrows). Scale bar=20 µm. **(C.2)** Immunostaining with DAPI (blue) TMEM119+ microglia (green) and GFAP+ astrocytes (red) in cortical and hippocampal regions. Following bTBI, astrogliosis is observed in both acute and chronic timepoints (white arrows). TMEM119+ microglia immunoreactivity was increased during chronic timepoints (white arrows). Scale bar=30 µm. **D.** bTBI-induced behavioral consequences. **(D.1)** Elevated plus maze. In the acute phase, bTBI mice had fewer entries and spent more time in closed arms compared to sham. **(D.2)** Open field test. We did not detect significant gross motor impairment (distance traveled) or entries/time spent in the center arena between injury groups at either timepoint. **(D.3)** Novel object recognition. In the acute phase, bTBI mice were less attentive to novel objects compared to sham. **(D.4)** Forced swim test. In the acute phase, bTBI mice demonstrated less time in immobility compared to sham.

Discussion and Ongoing Work

- bTBI induced acute inflammatory and behavioral consequences and chronic neuroinflammation in mice.
- Although chronic behavioral deficits are observed up to ten months post-injury in rats[19], our study did not demonstrate chronic behavioral deficits in mice. These dissimilarities may reflect inherent species differences or differences in injury parameters that could be considered in future treatment models.
- Future studies will define how neuroinflammatory changes within key brain regions influence behavioral outcomes. It is anticipated that this knowledge will inform targeted immunotherapy and treatment delivery.

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References

[1] Flanagan SR. (2015). Arch Phys Med Rehabil.
[2] Taylor PA, Ludwigsen, JS, & Ford, CC. (2014). Brain Injury.
[3] Reid MW, et al. (2014). J Neurotrauma.
[4] Twamley EW, et al. (2018). Psychiatry Research.
[5] Thomas MM, et al. (2017). Prim Care Companion CNS Disord.
[6] Yurgil KA, et al. (2014). JAMA Psychiatry.
[7] Hernandez A, et al. (2018). Molecular Brain.
[8] Broussard JI, et al. (2018). Brain Injury.
[9] Simon DW, et al. (2017). Nature Reviews Neurology.
[10] Fenn AM, et al. (2014). Biological Psychiatry.
[11] Cernak I, et al. (2011). Neurobiology of Disease.
[12] Skotak M, et al. (2019) Front Neurol
[13] Koliatsos VE, et al. (2011). Journal of Neuropathology and Experimental Neurology.
[14] Meabon JS, et al. (2016). Science Translational Medicine.
[15] Valiyaveettil M, et al. (2012). Neuroscience Letters.
[16] Valiyaveettil M, et al. (2013). Chem Biol Interact.
[17] Wang Y, et al. (2011). J Neurotrauma.
[18] Sajja VS, et al. (2018). Pre-Clinical and Clinical Methods in Brain Trauma Research.
[19] De Gasperi R, et al. (2023) J Neurotrauma.