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Electrophysiological white matter dysfunction and association with neurobehavioral deficits following low-level primary blast trauma

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ABSTRACT

There is strong evidence that primary blast injuries can cause neuropathological alterations in the brain. Clinical findings from war veterans indicating evidence of diffuse axonal injury have been corroborated by numerous primary blast models in animals. However, the effect of a subclinical blast (blast with no obvious sign of external trauma or lung injury) as a contributing factor to the neurological symptoms and neuropathology is less clear. Our group recently developed a model of low-level primary blast and characterized aberrant expression of white matter cytoskeletal proteins in the cortex and hippocampus following a subclinical wave shock exposure. Here we examined the susceptibility of the corpus callosum following subclinical blast. We also demonstrate that white matter dysfunction is associated with neurobehavioral deficits associated with anxiety and stress in rats. Anesthetized male Sprague–Dawley rats (~300 g) were exposed to a primary blast (approx. 28 kPa), below the threshold required to induce pulmonary trauma. Rats were evaluated on three behavioral outcome measures; the rotarod, the light/dark box and open field anxiety test. We used Western blotting to examine expression and degradation of axonally expressed αII-spectrin, NF200 and voltage-gated sodium channels (VGSC) in the corpus callosum. Acute slice preparations were used for electrophysiological analysis of evoked compound action potentials (CAPs) in the corpus callosum. There was evidence of αII-spectrin degradation in the corpus callosum at 48 h post-injury detectable up to 14 days post-injury, as well as increased heavy neurofilament expression. A reduction in VGSC expression was observed at 48 h post-blast as well as a reduction in the interaction between ankyrin G and intact αII-spectrin. Blast exposed rats had significantly lower rotarod latency times relative to sham rats ($p = 0.002$). Increased anxiety-related and stress-related behavior were observed in blast rats relative to sham animals as indicated by the increased frequency of fecal droppings ($p = 0.029$) and reduced exploratory activity ($p = 0.036$) in the open-field test. Blast rats had fewer transitions and time spent in lit sections of the light/dark box. Electrophysiological recordings from the corpus callosum indicated greater deficits in unmyelinated fibers of the corpus callosum relative to myelinated fibers characterized by reduced CAP amplitude response at 14 days post-injury. Analysis of the relationship between stimulation distance to evoked response indicated an underlying abnormality in N1 myelinated fibers at close stimulation distances. Collectively, our results indicate that subclinical blast exposure can result in persistent neurological changes in cerebral white matter occurring in parallel with detectable neurobehavioral deficits.

Introduction

There has been a high incidence of traumatic brain injuries associated with exposure to blast in soldiers serving in Afghanistan and Iraq. Soldiers and civilians exposed to non-lethal low-level primary blasts represent the largest group of individuals exposed to overpressure waves with sufficient energy to potentially cause mild brain trauma. It is unclear whether the symptoms affecting soldiers exposed to blasts are a manifestation of post-traumatic stress disorder (PTSD) or whether a biomechanical transduction of shockwave energy from explosives can cause mild traumatic brain injury (mTBI) (Belanger et al., 2011; Kennedy et al., 2010a, 2010b; Matthews et al., 2011). The overlap in symptoms between PTSD and mTBI makes etiological diagnosis of the disease state difficult. Clarifying the physiological effects of low-level blast exposure on brain function would be of value in the accurate identification of neurological symptoms currently being reported. Furthermore, determining whether the distinction between PTSD or mTBI from shockwaves is of significance will be critical toward prevention and treatment of symptoms.

Accordingly, in the present study we sought to clarify the susceptibility of subcortical white matter to the effects of low-level primary

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blast exposure, and determine whether white matter dysfunction is associated with neurobehavioral abnormalities in a model of low-level primary blast exposure.

There is increasing evidence from animal models of primary blast and computer simulations to suggest that shockwaves of sufficient intensity can transmit forces to the brain resulting in detectable and significant injury (Moore et al., 2009). Some of the pathological changes that have been described to date include diffuse axonal injury (Koliatsos et al., 2011; Long et al., 2009), presence of apoptotic oligodendrocytes and astrocytes (Pun et al., 2011) and vasospasm (Alford et al., 2011; Ling et al., 2009). Many of these studies have examined the effects of relatively high overpressure blast levels on brain pathology and have reported extensive white matter injury, particularly in the cerebellum (Cullen et al., 2011; Koliatsos et al., 2011; Wang et al., 2011). It remains unclear however, whether there is a direct contribution of low level shockwaves to brain changes, in particular white matter pathophysiology (Mac Donald et al., 2011).

We recently demonstrated that rats exposed to shock waves below the threshold for pulmonary trauma – defined as a subclinical blast – exhibited cytoskeletal proteolysis of αII-spectrin within the cortex and hippocampus as well as aberrant expression and proteolysis of the heavy neurofilament, NF200 (Park et al., 2011). A subclinical blast refers to scenarios in which a non-fatal exposure to shockwaves from an explosion results in an occult injury with no obvious external signs of trauma or pulmonary injury. Such a scenario would be applicable in instances where soldiers are at a sufficient distance from an explosion such that penetrating injuries from shrapnel or debris would be inconsequential. In our model of low level primary blast, we observed a higher density of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) in the white matter and the periventricular region relative to the cortex and hippocampus further supporting the notion of white matter susceptibility to shockwaves. Recent work in a murine model of low-pressure blast indicated that chronic alterations in brain state associated with behavioral impairment were a result of primary blast exposure (Rubovitch et al., 2011). An EEG study of nine war veterans with low-level blast exposure also found abnormal white matter function associated with neurocognitive disorders (Sponheim et al., 2011).

We specifically examine the effects of a subclinical shock wave exposure in rats (~28 kPa) on the behavioral, electrophysiological and proteolytic changes associated with white matter damage by shockwaves. We demonstrate three behavioral outcome measures with sufficient sensitivity to detect behavioral abnormalities associated with shockwave exposure. Moreover, we observed that unmyelinated fibers exhibit a greater susceptibility to persistent functional impairment activity at 14 days post-injury in a low level blast injury model. Co-immunoprecipitation data in conjunction with electrophysiological data strongly support a case for altered nodal structure in myelinated axonal fibers.

Methods

All surgical procedures were performed in accordance with guidelines established by the Animal Care Committee at St. Michael's Hospital in accordance with the standards set by the Canadian Council on Animal Care. A total of 124 adult male Sprague–Dawley rats were used for the study. Rats were housed in pairs in the Animal Research Facility under a 12 hour light dark cycle. Dry rat chow and water were given ad libitum.

Primary blast shock-wave model

As previously described, we used a custom designed open-ended shock tube with the ability to produce shock waves of varying intensity and amplitude based on the angle and distance from the diaphragm rupture source (Park et al., 2011). The open-ended shock tube device consisted of a cylindrical driver chamber (6 cm × 1.5 cm diameter) pressurized through a pin-hole opening fed from the pressurized reservoir containing nitrogen gas. The base of the driver chamber was sealed through a threaded mount with a diaphragm made from aluminum. A 0.5 mm aluminum disk with a rupture threshold of approximately 12,000 kPa was used to generate a target overpressure of ~28 kPa. Anesthetized male Sprague–Dawley rats (~300 g) were fitted with protective foam ear inserts to prevent tympanic membrane rupture during shock wave exposure. Rats were placed at an angle of approximately 21° from the vertical axis of the shock tube opening at a distance of approximately 19 cm from the nozzle opening. The resulting complex waveform with a single dominant overpressure event is depicted in Fig. 1a. Static pressure measurements were confirmed using a custom-built swivel-mount pencil probe fitted with a DPX101 high-frequency piezoelectric pressure sensor (Omega Engineering, Laval, QC) flush-mounted perpendicular to the length of the probe. The pressure sensor was connected to an ACC-PSI power source with raw output to an oscilloscope for data acquisition. At 21° from the vertical axis and a distance of ~19 cm to target, the static pressure at the location of the rat's head was 28.0 ± 1.6 kPa (average of 7 pressure trials). We have previously demonstrated that this level of pressure falls below the threshold for pulmonary barotraumas in rats (Park et al., 2011). Work by others also support this level of exposure as residing below the threshold for pulmonary barotraumas in unprotected rodents estimated to reside between 48.8–77.3 kPa (Pun et al., 2011). Sham animals received the same anesthetic regimen as blast exposed rats. We observed no mortalities following the blast procedure and recovery. All rats maintained their acoustic startle reflexes, further emphasizing the mild degree of shockwave exposure.

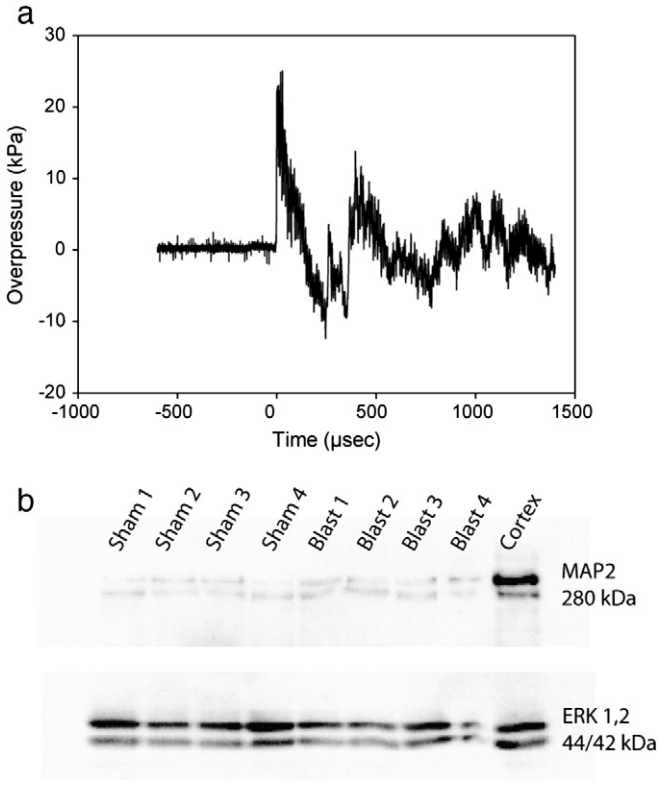

Fig. 1. a: Representative unfiltered static pressure wave recorded at the location of test rat placement. The waveform generated by the shock tube assembly was a complex wave form characterized by a dominant initial overpressure event. The initial peak overpressure event had a mean overpressure of 28.0 ± 1.6 kP. b: Representative Western blot demonstrating the predominant exclusion of MAP2 expression in corpus callosum tissue extracts dissected from the corpus callosum indicating a largely white matter homogenate.