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Repeated mild blast exposure in young adult rats results in dynamic and persistent microstructural changes in the brain

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ABSTRACT

A history of mild traumatic brain injury (mTBI), particularly repeated mTBI (rmTBI), has been identified as a risk factor for late-onset neurodegenerative conditions. The mild and transient nature of early symptoms often impedes diagnosis in young adults who are disproportionately affected by mTBIs. A proportion of the affected population will incur long-term behavioral and cognitive consequences but the underlying pathomechanism is currently unknown. Diffusion tensor imaging (DTI) provides sensitive and quantitative assessment of TBI-induced structural changes, including white matter injury, and may be used to predict long-term outcome. We used DTI in an animal model of blast rmTBI (rmbTBI) to quantify blast-induced structural changes at 7 and 90 days post-injury, and their evolution between the two time points. Young adult male rats (~P65 at injury) were exposed to repeated mild blast overpressure, or anesthetized as shams, and their fixed brains were imaged using high-field (7 T) MRI. We found that whole brain volumes similarly increased in injured and sham rats from 7 to 90 days. However, we detected localized volume increases in blast-exposed animals 7 days post-injury, mainly ipsilateral to incident blast waves. Affected regions included gray matter of the frontal association, cingulate, and motor cortex, thalamus, substantia nigra, and raphe nuclei (median and dorsal), as well as white matter of the internal capsule and cerebral peduncle. Conversely, we measured volume reductions in these and other regions, including the hippocampus and cerebellum, at 90 days post-injury. DTI also detected both transient and persistent microstructural changes following injury, with some changes showing distinct ipsilateral versus contralateral side differences relative to blast impact. Early changes in fractional anisotropy (FA) were subtle, becoming more prominent at 90 days in the cerebral and inferior cerebellar peduncles, and cerebellar white matter. Widespread increases in radial diffusivity (RD) and axial diffusivity (primary eigenvalue or E1) at 7 days post-injury largely subsided by 90 days, although RD was more sensitive than E1 at detecting white matter changes. E1 effects in gray and white matter, which paralleled increases in apparent diffusion, were likely more indicative of dysregulated water homeostasis than pathologic structural changes. Importantly, we found evidence for a different developmental trajectory following rmbTBI, as indicated by significant injury x age interactions on volume. Our findings demonstrate that rmbTBI initiates dynamic pathobiological processes that may negatively alter the course of late-stage neurodevelopment and adversely affect long-term cognitive and behavioral outcomes.

1. Introduction

Mild traumatic brain injury (mTBI) occurs annually in an estimated 42 million people worldwide (Gardner and Yaffe, 2015). More commonly known as concussions, mTBIs account for ~85% of all traumatic brain injuries (TBIs) (U.S. Department of Health and Human Services, C.f.D.C.a.P., 2009). In the military, mTBIs are typically caused by the exposure to blast overpressure generated by roadside bombs or improvised explosive devices (IEDs), thus resulting in mild blast-induced TBI (mbTBI) (Ling and Ecklund, 2011). Among civilians, concussions are mainly sustained by athletes playing contact sports (e.g., football, rugby, and boxing), as well as during falls and other accidents (Voss

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et al., 2015). Even though the acute symptoms of a single mTBI may be mild and transient, experimental and clinical studies have found that the metabolic, biochemical, and structural changes caused by mTBIs can be cumulative and have long-term consequences (MacFarlane and Glenn, 2015; Signoretti et al., 2011). Therefore, repeated head trauma is a risk factor for late-onset behavioral, cognitive, and neurodegenerative diseases, such as chronic traumatic encephalopathy (CTE) (Gavett et al., 2011; Iverson et al., 2015; McKee et al., 2015), Parkinsonism (Tansey et al., 2007), and Alzheimer's type dementia (Daneshvar et al., 2015).

In the quest for elucidating TBI pathophysiology and its neurocognitive correIates, one of the critical gaps in knowledge is the effect of age at the time of injury. Repeated mTBI (rmTBI) most frequently affects adolescents and young adults (Prins and Giza, 2012). This is an issue of particular concern for military and civilian populations because magnetic resonance imaging (MRI) studies have shown that neurodevelopmental changes take place well into adulthood (Groeschel et al., 2010; Simmonds et al., 2014; Sowell et al., 1999). While white matter microstructure reaches adult levels during adolescence, major corticolimbic association and projection tracts mature later in adulthood, particularly in males (Asato et al., 2010; Groeschel et al., 2010; Simmonds et al., 2014). Approximately 88% of the military personnel serving in Iraq and/or Afghanistan were male, and by the end of 2010, half of all those deployed were between the age of 25 and 34, with ~322,000 under the age of 25 (Institute of, M., 2013). Although civilian and military populations are equally affected, repeated low-level blast exposure can have significant implications for young service members, the military health care system, and future force readiness.

Repeated mbTBI (rmbTBI) and rmTBIs sustained during ongoing refinement of white matter integrity can effectively alter the normal course of late-phase neurodevelopment and/or aging, and affect physiologic, cognitive, and emotional function. Diffuse white matter injury is a hallmark TBI pathology (Johnson et al., 2013; Jorge et al., 2012; Mac Donald et al., 2013; Matsushita et al., 2011; Perez et al., 2014). Furthermore, brain structures are differentially vulnerable to various injury mechanisms (e.g., Purkinje cell injury and blood-brain-barrier dysfunction in the cerebellum, diffuse axonal injury in the corpus callosum) (Meabon et al., 2016; Petrie et al., 2014) with gray-white matter interfaces, peri-vascular, and peri-ventricular regions being the most injury-prone (McKee et al., 2009, 2016). Aside from MRI modalities, particularly diffusion tensor imaging (DTI), current diagnostic tests (such as functional and behavioral assessments) are neither sensitive nor specific enough to identify individuals who have sustained a mild TBI (Goldstein, 1990; Jagoda et al., 2008; Belanger et al., 2007), thereby impeding early detection and therapeutic intervention.

DTI studies have documented persistent chronic changes in white matter following one mild TBI episode; studies performed after 3 months continue to reveal white matter pathology in areas similar to those found in the acute and sub-acute phases of mTBI (Johnson et al., 2013). Accordingly, DTI can provide a sensitive and objective means for determining the relationship of cognitive deficits to TBI, even in cases where the injury was sustained years prior to the evaluation (Kraus et al., 2007). In this study, we used DTI to assess injury-induced changes in young adult rats exposed to repeated mild blast overpressure at ~P65, the equivalent of 20 years+ in humans (Semple et al., 2013). A better understanding of the microstructual dynamics of rmbTBI pathology relative to normal, late-phase neurodevelopmental changes can help predict associated cognitive impairment or recovery, and address the issue of timely, age-relevant clinical management.

2. Materials and methods

This study consisted of two identical experiments that only differed in post-injury survival time. All rats were ordered at the same weight (and corresponding age) range, underwent the same pre-procedural treatments, and were exposed to repeated blast (or sham) at the same time relative to their arrival and acclimation. Following blast or sham exposure, rats were either terminated at 7 or 90 days post-injury (or sham). At their respective termination times, rats in the 7- and 90-day survival groups were ~2 and 5 months old, respectively.

2.1. Animals and housing conditions

Seventy-two male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) were used in this study (weight at arrival: 200–225 g; age at arrival: 49–52 days, based on date of birth supplied by the vendor). Rats were pair-housed in standard cages, with built-in filters, in a reverse 12-hour light 12-hour dark cycle with food and water ad libitum. Animals were handled according to protocols approved by the Institutional Animal Care and Use Committee at the Uniformed Services University (USU; Bethesda, MD).

2.2. Experimental groups and manipulations

All animals underwent a 5-day gentling and acclimation period. Baseline open field activity was used to create two groups, *sham* and *injured*, with no statistical differences in movement, time spent in the center/margins, or horizontal/vertical activity (Kamnaksh et al., 2012). Rat numbers were identical in the 7-day ($N = 36$; sham = 18, injured = 18) and 90-day ($N = 36$; sham = 18, injured = 18) survival groups. For the duration of the studies, rats were kept in the animal facility at USU without manipulation except for behavioral testing and blast (or sham) exposures.

On the day of the exposures, all animals (sham and injured) were transported from USU to Walter Reed Army Institute of Research (Silver Spring, MD) as described earlier (Kamnaksh et al., 2011, 2014). For the duration of the exposures, rats were kept in the preparatory area of the blast room. Rats in the sham group were anesthetized in an induction chamber with 4% isoflurane (Forane; Baxter Healthcare Corporation, Deerfield, IL) in an air mixture delivered at 2 l/min. Induction times were 6 min for the 1st exposure, and 3 min for each subsequent exposure.

2.3. Injury conditions

Rats in the injured group underwent the same procedures as their respective sham controls in addition to receiving 3 mild blasts of varying pressure to mimic the variability of field blast exposures, at 20–30 min intervals. Blast overpressure was generated using a compressed air-driven shock tube that yields a single blast overpressure wave to produce a mild injury (Kamnaksh et al., 2011, 2014; Ahmed et al., 2013). Anesthetized rats in chest protection (average weight at injury: 300 g) were placed in the shock tube holder in a transverse prone position, with the right side facing the direction of the membrane and the incident blast waves and exposed to blast in the same order. *Blast no. 1*: Mylar membrane thickness = 190.5 μm (average peak total pressure = 15.54 psi or ~107.14 kPa, positive phase duration = 9.01 ms); *blast no.2*: Mylar membrane thickness = 355.6 μm (average peak total pressure = 19.41 psi or ~133.83 kPa, positive phase duration = 10.60 ms); *blast no.3*: Mylar membrane thickness = 254 μm (average peak total pressure = 17.78 psi or ~122.59 kPa, positive phase duration = 9.22 ms). Following blast (or sham), animals were moved to an adjacent bench top for observation and then transported back to the USU animal facility.

2.4. Preparation of specimens for imaging

A subset of animals from each study (7-day group: $N = 18$; sham = 9, injured = 9; 90-day group: $N = 18$; sham = 9, injured = 9) was used for the MR/DTI analyses; all other animals were used for proteomics (in preparation). At their respective termination points, rats in the 7- and 90-day survival groups were approximately 2 and