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Changes in mouse cognition and hippocampal gene expression observed in a mild physical- and blast-traumatic brain injury

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ABSTRACT

Warfare has long been associated with traumatic brain injury (TBI) in militarized zones. Common forms of TBI can be caused by a physical insult to the head–brain or by the effects of a high velocity blast shock wave generated by the detonation of an explosive device. While both forms of trauma are distinctly different regarding the mechanism of trauma induction, there are striking similarities in the cognitive and emotional status of survivors. Presently, proven effective therapeutics for the treatment of either form of TBI are unavailable. To be able to develop efficacious therapies, studies involving animal models of physical- and blast-TBI are required to identify possible novel or existing medicines that may be of value in the management of clinical events. We examined indices of cognition and anxiety-like behavior and the hippocampal gene transcriptome of mice subjected to both forms of TBI. We identified common behavioral deficits and gene expression regulations, in addition to unique injury-specific forms of gene regulation. Molecular pathways presented a pattern similar to that seen in gene expression. Interestingly, pathways connected to Alzheimer's disease displayed a markedly different form of regulation depending on the type of TBI. While these data highlight similarities in behavioral outcomes after trauma, the divergence in hippocampal transcriptome observed between models suggests that, at the molecular level, the TBIs are quite different. These models may provide tools to help define therapeutic approaches for the treatment of physical- and blast-TBIs. Based upon observations of increasing numbers of personnel displaying TBI related emotional and behavioral changes in militarized zones, the development of efficacious therapies will become a national if not a global priority.

Introduction

Traumatic brain injury (TBI) covers a range of central nervous system (CNS) injuries; such injuries can be caused by a diffuse or a focused form of tissue damage in brain. TBIs are exemplified by blunt force trauma that can occur in a car accident, a fall or in a sporting or warfare related event. TBIs can vary from mild, moderate to severe, the greater the force involved in the injury the more severe is the TBI (DeKosky et al., 2010; Hoge et al., 2008; Mendez et al., 2005). Quite often TBIs can result in the development of diffuse axonal injury (DAI), as originally described by Strich (1956) and more lately by Adams and co-workers (Adams et al., 1982, 1984). Subsequent to an injury, a series of molecular and cellular events may occur that result in changes that often proceed to neuronal cell dysfunction and death. This, in turn, manifests as changes in neurological status, exemplified by issues of irritability; headache; fatigue; memory problems and, in some cases, an increase in antisocial behavior that may lead to criminal activity (Falk, 2012; Luukkainen et al., 2012; Yang et al., 2012).

Abbreviations: TBI, traumatic brain injury; physical-TBI, physical-traumatic brain injury; blast-TBI, blast-traumatic brain injury; DAI, diffuse axonal injury; NOR, novel object recognition; EPM, elevated plus maze; PA, passive avoidance; PD, Parkinson's disease; AD, Alzheimer's disease; CNS, central nervous system; BBB, blood–brain barrier; cDNA, complementary DNA; cRNA, complementary RNA; PAGE, parametric analysis of gene set enrichment; Q-RT-PCR, quantitative reverse transcriptase PCR; GO term, gene ontology term; GOI, genes of interest.

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Typically in mild TBI, no gross tissue damage occurs at the time of trauma. However, clinical neurological consequences of the trauma develop over time, and, for the most part (~85% of cases), regress within a few months after injury (see Alexander, 1995 for a clinical perspective on diagnosis of mild traumatic brain injury).

A more recently recognized insidious form of TBI originating from warfare has become a significant medical problem; this form of brain injury has drawn considerable attention due to its prevalence in active combat zones. Primarily through the use of high explosives, and more specifically improvised explosive devices, explosive detonations are causing an increased incidence of complex TBIs. These explosive devices can cause primarily shock wave dependent damage (blast damage) to multiple organ systems, including the brain. Blast-induced brain damage and the ensuing neurological disorders caused by blast injuries are proving to be difficult to clearly diagnose without the use of extensive neurological and psychological assessments, and are an increasing problem for military and civilian healthcare providers. The consequences of these 'lesion-less' injuries often result in deficits in military personnel reaction times, spatial memory function, and an increased occurrence of headache, dizziness, sleep disturbances, and other emotional and behavioral changes (Bryan and Hernandez, 2012; Shively and Perl, 2012).

The origins of the cognitive and psychological disorders induced by blast injuries in combat zones are most likely due to undefined changes in the molecular and cellular homeostasis of different regions of the brain, and, as such, highly warrant further study. Non-blast (concussive) injuries are most likely mediated by shearing force damage to neurons causing a DAI (Adams et al., 1982, 1984; Messé et al., 2012; Strich, 1956; see Johnson and Stewart, 2012 for review), while in a primary blast injury, the role of DAI appears to be more complex (Garman et al., 2011; Risling et al., 2011; Sajja et al., 2012; Wang et al., 2011). However, it is feasible that the final end stages of neuronal damage induced by different mechanisms are mediated by the activation of shared pathways that could be identified by sensitive screening approaches. We have utilized a mild, closed head weight drop model of physical head trauma (physical-TBI) in mouse developed and extensively characterized by our laboratories (Baratz et al., 2011; Milman et al., 2005; Rachmany et al., 2012; Tashlykov et al., 2007, 2009; Tweedie et al., 2007; Zohar et al., 2003, 2011) and we have compared physical-TBI with a novel mouse model of a mild blast-TBI (Rubovitch et al., 2011). Our purpose was to compare the effects of the two different forms of mild brain injury on mouse cognitive and emotional paradigms and on hippocampal gene expression. The hippocampus was selected due to its role in memory and cognition. Behavioral changes and the hippocampal transcriptome were examined at a time point after injury where TBI related impairments are known to occur in mild physical-TBI (Baratz et al., 2011; Rachmany et al., 2012; Zohar et al., 2011).

Cognitive and emotional behavior was assessed by use of novel object recognition (NOR), Y-maze, passive avoidance (PA) and elevated plus maze (EPM) initiated 7 days after exposure to either physical- or blast-TBI. All TBI animals demonstrated deficits in novel object recognition compared to control mice, yet there were no marked differences between control and TBI mice for Y-maze, passive avoidance or elevated plus maze, thus indicating that these TBI models were mild in nature. In contrast to mouse behavioral measures, we observed marked changes in gene expression in RNA from intact TBI hippocampal tissue. There was a degree of overlap in genes co-regulated by the two mechanisms of injury. However there were a larger number of genes uniquely expressed in an injury specific manner. Consequently, molecular pathways displayed similar patterns of injury dependent regulation. While these data suggest a potentially shared injury behavioral outcome, there is a divergence in gene expression and pathway regulation that would suggest that the two types of injury are different at the molecular level. This divergence may aid in the identification of novel drug targets that may be of clinical benefit in the setting of complex human TBIs.

Methods

Animal studies

Male ICR mice weighing 30–40 g were kept five per cage under a constant 12-h light/dark cycle, at room temperature (22 ± 2 °C). Food (Purina rodent chow) and water were available ad libitum. Each mouse was used for one experiment. The Ethics Committee of the Sackler Faculty of Medicine approved the experimental protocols (M-09-055; M-07-055), in compliance with the guidelines for animal experimentation of the National Institutes of Health (DHEW publication 85-23, revised, 1995). All experimental manipulations were conducted during the light phase of the cycle.

Induction of a mild physical- and blast-TBI

A mild physical-TBI was induced using a concussive head trauma device described previously (Milman et al., 2005; Zohar et al., 2003). Briefly, mice were lightly anesthetized (Isoflurane) and placed under the weight-drop concussive head trauma instrument. The device consisted of a metal tube (inner diameter 13 mm), placed vertically over the mouse head. A metal weight (30 g) was dropped from the top of the tube (80 cm) and struck the skull at the right side temporal area between the corner of the eye and the ear. A sponge supported the head, allowing some antero-posterior motion without any rotational head movement at the moment of the impact.

Experimental conditions used to create a mild low-level blast-TBI and the subsequent model characterization, have been described in detail elsewhere (Rubovitch et al., 2011). In brief, mice were anesthetized with a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg). Once the animals were fully anesthetized they were placed at a defined distance from a detonation source, in this case 7 m. Pressure sensors were used to measure the explosion shock wave pressure (PSI) generated by the detonation (Free-Field ICP® Blast Pressure Sensor; PCB Piezoelectronics, Depew, NY, USA, Model 137). At 7 m from the source of the detonation, the animals were exposed to a maximum of a 2.5 psi (17.2 kPa) pressure shock wave. Immediately after the induction of the injury, mice were placed back in their cages. Once the animals had recovered from the anesthesia, basic neurological assessments were undertaken to identify any acute neurological dysfunction. Only animals exhibiting no evidence of acute neurological damage post injury were subsequently used in further experiments. Sham treated mouse groups were treated identically; however, they were not exposed to physical- or blast-TBI.

Cognitive and emotional behaviors

Behavioral assessments were initiated 7 days after the animals were exposed to TBI. This time point was selected based upon prior studies with these models where behavioral deficits were observed to occur from this time point. Mouse cognition and emotional behavior was assessed using the NOR; Y maze; PA and EPM paradigms. For a diagram of the experimental time course see Fig. 1A. The NOR and the Y maze behavioral paradigms have been described in detail elsewhere (Baratz et al., 2010, 2011; Edut et al., 2011; Rachmany et al., 2012; Rubovitch et al., 2010). Mouse treatment groups utilized were as follows: sham, n = 27–42; physical-TBI, n = 22–34; blast-TBI, n = 23–30. All equipment used for behavioral testing was cleaned with a 70% ethanol solution between testing sessions and animals to minimize any olfactory dependent cognitive influences on mouse behavior.

Novel object recognition paradigm

The NOR task was used to evaluate recognition memory; typically rodents display a natural inquisitiveness to explore new objects in their environment. This characteristic can be used to evaluate visual recognition memory function in rodents, and more interestingly it