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Sex-Dependent Effects of Mild Blast-induced Traumatic Brain Injury on Corticotropin-releasing Factor Receptor Gene Expression: Potential Link to Anxiety-like Behaviors

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Abstract—Traumatic brain injury (TBI) affects 1.7 million people in the United States every year, resulting in increased risk of death and disabilities. A significant portion of TBIs experienced by military personnel are induced by explosive blast devices. Active duty military personnel are especially vulnerable to mild blast-induced (mb)TBI and the associated long-term effects, such as anxiety disorders. Additionally, females are at an increased risk of being diagnosed with anxiety-related disorders. The mechanism by which mbTBI results in anxiety disorders in males and females is unknown. The sexually dimorphic corticotropin-releasing factor (CRF) is a brain signaling system linked to anxiety. CRF and its family of related peptides modulate anxiety-related behaviors by binding to CRF receptor subtypes 1 and 2 (CRFR1, CRFR2, respectively). These receptors are distributed throughout limbic structures that control behaviors related to emotion, memory, and arousal. Therefore, the aim of this study was to understand the link between mbTBI and anxiety by examining the impact of mbTBI on the CRFR system in male and female mice. mbTBI increased anxiety-like behaviors in both males and females ($p < 0.05$). In the present study, mbTBI did not alter CRFR1 gene expression in males or females. However, mbTBI disrupted CRFR2 gene expression in different limbic structures in males and females. In males, mbTBI increased baseline CRFR2 gene expression in the ventral hippocampus ($p < 0.05$) and decreased restraint-induced expression in the anterior bed nucleus of the stria terminalis (aBNST) and amygdala ($p < 0.05$). In females, mbTBI decreased restraint-induced CRFR2 gene expression in the dorsal hippocampus ($p < 0.05$). The inherent sex differences and the mbTBI-induced decrease in restraint-induced CRFR2 gene expression may contribute to anxiety-like behaviors. The results of the present study show that the response to mbTBI within the limbic structures modulates anxiety in a sex-dependent manner. The studies further suggest that CRFR2 may serve as a potential target to mitigate mbTBI effects. Published by Elsevier Ltd on behalf of IBRO.

Keywords: anxiety, traumatic brain injury, corticotropin-releasing factor, receptor, sex differences.

INTRODUCTION

1.7 million people in the United States experience a traumatic brain injury (TBI) causing physical, cognitive, emotional and behavioral symptoms (Faul et al., 2010). Clinically, TBIs are classified as severe, moderate or mild based on duration of loss of consciousness, post-traumatic amnesia, Glasgow Coma Scale (GCS) rating and neuroimaging findings. A mild (m)TBI is diagnosed when trauma causes transient loss of consciousness (< 30 min), transient post-traumatic amnesia (< 24 h) and limited impairment in verbal, motor and eye responses as rated on the GCS (O'Neil et al., 2013). The majority (~75%) of TBIs are diagnosed as mild (2018). In military populations, it is increasingly common for soldiers to experience TBI resulting from exposure to explosive blast waves emanating from bombs or improvised explosive devices (Hoge et al., 2008). Blast-induced (b)TBI is a unique entity, differing from other forms of non-bTBI because of the physics of the blast wave, number of exposures, and sequelae of events that follow blast exposure (Chapman and Diaz-Arrastia, 2014).

Of note, TBI patients are prone to anxiety, impaired memory, irritability, sleep disturbances and post-traumatic stress disorder (PTSD) (Whelan-Goodinson et al., 2009, Nelson et al., 2015). Specifically, military

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members exposed to mbTBI may be at a higher risk of developing neuropsychiatric disorders (Heltemes et al., 2012, Rosenfeld et al., 2013). These disorders are long-lasting, potentially persisting for years after the TBI occurred (Fleminger, 2008). Interestingly, females are more susceptible to developing neuropsychiatric disorders after TBI than males (Scholten et al., 2016). In rodent models, TBI induced by various mechanisms induces anxiety-like behaviors. Specifically, blast-induced TBI decreased motor activity and center exploration in the open field assay up to one month after injury (Cernak et al., 2011).

Psychiatric disorders, such as depression, anxiety and PTSD, have been linked to the corticotropin-releasing factor (CRF) system. CRF and related family members mediate the stress response by activating CRF receptor subtypes 1 (CRFR1) and 2 (CRFR2) (Risbrough and Stein, 2006). CRFR1 is widely distributed throughout the forebrain, midbrain, diencephalon, brainstem and spinal cord (Henckens et al., 2016). Recent studies show that CRFR1$^{-/-}$ mice exhibit decreased anxiety-like behavior (Smith et al., 1998, Timpl et al., 1998). Single nucleotide polymorphisms in the CRFR1 gene in rhesus monkeys are associated with anxiety (Rogers et al., 2013). In patients suffering from anxiety and depression, blocking CRFR1 reduces psychiatric symptoms (Ising and Holsboer, 2007). Moreover, hyperstimulation of CRFR1 by CRF has been found in patients with stress-related disorders (Kehne, 2007), suggesting that CRFR1 activation promotes anxiety or depression. CRFR2 expression is more limited, found in the lateral septal nuclei, bed nucleus of the stria terminalis (BNST), hypothalamus, amygdala, and hippocampus (Henckens et al., 2016). Although less known and more complex, CRFR2 is believed to counter CRFR1 activation by dampening the stress response. For instance, CRFR2 deficient mice have increased anxiety-like behaviors (Bale et al., 2002) and region-specific agonist-induced activation of CRFR2 modulates anxiety-like behaviors (Todorovic et al., 2007, Zhao et al., 2007, Neufeld-Cohen et al., 2012, Alves et al., 2016).

There are inherent sex differences in the expression and function of the CRFRs. For example, male voles have higher CRFR2 mRNA expression in the BNST than female voles (Lim et al., 2005). There are also sex differences in CRFR1 signaling and trafficking. In the locus coeruleus, a region involved in the physiological stress response, unstressed female rats have greater coupling of the G_s protein to CRFR1 than male rats. In addition, stress has been shown to increase CRFR1 internalization in male, but not female rats (Bangasser et al., 2010). Genetic studies in patients with psychiatric disorders suggest that the inherent sex differences in rodents extend to humans, where a single CRFR2 nucleotide polymorphism correlates with increased risk of PTSD in women but not men (Wolf et al., 2013). These sex differences may influence stress sensitivity and the prevalence of psychiatric disorders after TBI.

The aim of the present study was to test the hypothesis that mbTBI can cause anxiety disorders through dysregulation of the CRFR signaling system. Basal and restraint-induced CRFR1 and CRFR2 gene expression were measured. Exposure to psychogenic restraint 7–10 days after mbTBI allowed for testing of the stress system in response to a later challenge. We have previously reported sex-dependent alterations in stress reactivity at this time point post injury in males and females (Russell et al., 2018).

EXPERIMENTAL PROCEDURES

Animals

Male and naturally cycling female C57BL/6J mice at 7–9 weeks of age were purchased from the Jackson Laboratory (Stock number 000664; Jackson Laboratory, Bay Harbor, ME). Animals were provided food and water *ad libitum* and pair housed in a temperature (22–25 °C), humidity (50%) and light (12:12 light:dark cycle; lights on at 0100 h) controlled facility.

All animal protocols were approved by the Institutional Animal Care and Use Committee at the Uniformed Services University of the Health Sciences, Bethesda, MD and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental design

To examine CRFR gene expression after mbTBI, animals were assigned to one of eight groups (males $n = 13$/group; females $n = 19$/group). This study was conducted as a $2 \times 2 \times 2$ factorial design examining the effect of injury (sham v. mbTBI), restraint (no restraint v. restraint) and sex (male v. female). Animals received either sham or mbTBI procedures and were allowed 7–10 days for post injury recovery prior to exposure to acute psychogenic restraint followed by tissue collection (Fig. 1; (Russell et al., 2018)).

Mild blast traumatic brain injury (mbTBI)

All procedures were conducted between 0700 and 1100 h (lights on) to test during the trough of stress reactivity. mbTBI was induced following previously established protocols (Russell et al., 2018). Animals were acclimated to the room for one hour prior to procedures. All animals were anesthetized by isoflurane inhalation (Isoflurane, USP, Baxter, Deerfield, IL). Sham animals received anesthesia only. The Advanced Blast Simulator (ORA Inc., Fredericksburg, VA) was used to induce mbTBI. An acetate/mesh seal separated the driver chamber from the transition and test chambers (3 acetate sheets, 0.010″ thick, 18″ × 30″, Industrial Division Sales-Grafix, Inc.; 2 mesh sheets, 14×10 grids/in^2, wire diameter 0.25″, New York Wire). Increased air pressure in the driver section ruptured the seal allowing the blast wave to travel to the test chamber via the transition chamber. In the test chamber, anesthetized animals were mounted in a prone position to receive the blast wave nose-on. Blast pressure was measured with a pencil probe pressure gauge (Model Number: 137B23A; PCB Piezotronics, Depew, NY) located at the level of the animal.

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