Experimental Neurology 295 (2017) 55–65

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Manipulating cognitive reserve: Pre-injury environmental conditions influence the severity of concussion symptomology, gene expression, and response to melatonin treatment in rats

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ARTICLE INFO

Article history:
Received 17 March 2017
Received in revised form 21 May 2017
Accepted 1 June 2017
Available online 1 June 2017

Keywords:
Mild traumatic brain injury
Epigenetic
Animal model
Telomere length
qRT-PCR

ABSTRACT

In an effort to understand the factors that contribute to heterogeneity in outcomes often associated with mTBI in youth, this study examined the role of premorbid differences in cognitive reserve on post-concussive symptoms (PCS), molecular markers, and treatment response. Male and female rats matured in one of three environmental conditions (Stress, Enrichment, Control), received a mTBI in adolescence, and were randomized to melatonin or placebo treatment. All animals underwent a behavioural test battery designed to examine PCS. Using prefrontal cortex and hippocampus tissue, expression of 9 genes was assessed in an effort to determine how the brain's epigenome was influenced by cognitive reserve, mTBI, and melatonin. Enrichment increased cognitive reserve (CR) and prevented lingering symptoms. Conversely, stress was associated with progressive worsening and manifestation of PCS in the longer-term. Melatonin was able to restore baseline function for control and enriched animals, but was ineffective for the stress condition. Epigenetic change in the prefrontal cortex was largely driven by the injury, while gene expression changes in the hippocampus were dependent upon cognitive reserve. The occurrence and severity of PCS is dependent upon a complex and multifaceted array of factors that modify behavioural and epigenetic responses to mTBI and its treatment.

1. Introduction

Mild traumatic brain injury (mTBI)/concussions are the most common form of head injury, with adolescents being at particularly high risk (Barlow et al., 2010). Given that the brain is still developing (Epstein, 1999; Casey et al., 2000; Andersen, 2003; Knudsen, 2004), injuries sustained during childhood and adolescence may disrupt critical processes and alter typical neurodevelopmental trajectories (Mychasiuk et al., 2015a, b, c). Although most youth recover without incident, a significant proportion suffer from post-concussion symptoms (PCS) such as sleep problems, headache, dizziness, difficulty concentrating, memory problems, depression, anxiety, and sensitivity to light or sound (Taylor et al., 2010). Of importance for adolescents, long-term outcomes associated with mTBI can include impaired social function (Ryan et al., 2016), executive dysfunction, and problems with impulsivity and sustained attention (Kurowski et al., 2011). Despite this propensity for negative consequences, little is known about prognostic factors and individual differences in risk or resiliency that may lead to the development of PCS and long-term cognitive deficits following mTBI.

Neuroplasticity, the brain's propensity to change in response to experience, is the key process involved in maintaining cognitive reserve (CR). The concept of CR is often used in clinical literature to describe factors that optimize or promote neurological resiliency (Stern et al., 1999; Ropacki and Elias, 2003; Whalley et al., 2004), while the term neuroplasticity is commonly used in animal modeling to describe mechanisms underlying the brain's ability to change and adapt in response to environmental conditions (Abraham and Bear, 1996; McEwen, 1999; Markham and Greenough, 2004; Radley and Morrison, 2005). An individual's CR or plasticity is believed to be a 'summation' of all positive and negative changes in neuroplasticity, whereby positive experiences increase reserve and negative experiences reduce the plastic abilities of the brain (Abraham and Bear, 1996). As post-concussion symptomologies may develop if the brain is not able to adequately repair and compensate for injury-induced dysfunction, premorbid differences in CR may contribute to individual heterogeneity in concussion susceptibility.

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http://dx.doi.org/10.1016/j.expneurol.2017.06.001
0014-4886/

Although limited, there is some research to support this theory. Epidemiological studies have linked premorbid cognitive ability, educational and occupational skill levels, socio economic status, and leisure activities such as cognitive, social, and physical activity to the severity and length of recovery in brain injury patients (Fay et al., 2010; Levi et al., 2013; Sumowski et al., 2013; Schneider et al., 2014; Mathias and Wheaton, 2015; Oldenburg et al., 2016). For example, premorbid alcoholism, drug abuse, or high fat diet consumption contributes to greater post injury deficits and heterogeneity (Ropacki and Elias, 2003; Mychasiuk et al., 2015a, b, c). Similarly, increased risk for neurocognitive decline has been associated with TBI, and appears to be moderated by pre-injury education, intelligence, and medical diagnoses (Moretti et al., 2012). Premorbid characteristics and variations in CR should therefore not only modify injury outcomes, but also contribute to individual differences in treatment response.

Factors such as sex, age, and genetics are well known to contribute to drug efficacy, tolerance, and safety (Xu et al., 2008), but literature regarding the effects of cognitive reserve on responsivity to therapeutics is lacking. Although melatonin has been proposed as a safe and well-tolerated intervention for many with clinical post-concussive syndrome (Barlow et al., 2014), initial trials indicate that it is not effective for all patients. Melatonin is a naturally occurring hormone that regulates sleep and wakefulness, with demonstrated anti-oxidant properties. Exogenous melatonin administration has been found to inhibit metalloproteinase-9, malondialdehyde, xanthine oxidase, and nitric oxide; reducing the production of free radicals and in turn attenuating damage from TBI (Ates et al., 2006; Alluri et al., 2016). Given our hypothesis that the specific pathophysiological response of the brain to mTBI is likely dependent upon premorbid differences associated with CR, the efficacy of therapeutic strategies may also be contingent upon these factors.

Although mechanisms driving the neurological effects of CR have not been fully elucidated, epigenetic changes in gene expression are likely involved. Epigenetic processes are essential for cellular development and differentiation, but also occur in mature cells to facilitate the response to environmental influences (Bird, 2002; Fagiolini et al., 2009). Previous studies in our laboratory have demonstrated that gene expression is altered in response to mTBI and various premorbid manipulations such as diet, and may therefore provide mechanistic knowledge regarding the pathophysiological changes identified (Hehar et al., 2015; Mychasiuk et al., 2015a, b, c; Mychasiuk et al., 2016).

Therefore, the purpose of this study was twofold: to determine if variations in pre-injury CR altered susceptibility to PCS and gene expression after mTBI, and to determine if pre-injury CR influenced response to melatonin treatment. We hypothesized that increasing CR prior to the injury would mitigate poor outcomes associated with mTBI while reducing CR would exacerbate poor outcomes. In addition, those animals with the greatest level of dysfunction would benefit the most from the treatment. To test this, male and female Sprague Dawley rats, matured in one of three environmental conditions (Stress, Enrichment, Control), received a single mTBI in adolescence using a clinically relevant model of sports-related concussion, and were randomized to receive melatonin or placebo treatment. All animals underwent a behavioural test battery designed to examine symptoms or behaviours consistent with clinical post-concussive syndrome (Mychasiuk et al., 2016) and gene expression was assessed in prefrontal cortex and hippocampus tissue. We found that positive/enriched environmental conditions do not always improve PCS outcomes, just as negative/stressful conditions do not always exacerbate deficits; rather manipulation of pre-injury reserve appeared to increase variability in symptomology, contributing to the heterogeneity observed in PCS and the brain's response to treatment.

2. Materials and methods

All experiments were approved by the University of Calgary Conjoint Faculties Research Ethics board and were carried out in accordance with the Canadian Council of Animal Care. All animals were maintained on a 12:12 h light:dark cycle in a temperature controlled (21 °C) animal housing facility. Twelve Sprague Dawley female rats were bred in-house to unique males and were then pair-housed (2 female rats/cage) until the day prior to delivery. At this point, females were separated and remained individually housed with their litters for the duration of weaning. At weaning, the 63 females and 54 males were randomly assigned to one of three experimental conditions: Enrichment (ENR), Stress (STR), or Control (CON). Animals in the ENR paradigm were housed in large cages with 4–6 other animals from the same-sex to increase social interaction. The ENR paradigm provided animals with novel toys that were exchanged every 3 days, access to running wheels, and prebiotic fiber supplementation with their food. Animals in the STR paradigm were pair housed in standard shoe-box cages but experienced social isolation on alternating days, (i.e. were individually housed for 24 h every other day). In addition, animals in the STR condition did not receive bedding/housing materials and were fed a high-fat diet (Dyet, Bethlehem, PA, USA), which resulted in 60% of total calories being obtained from fat. Finally, animals in the CON condition were housed in same-sex groups of 4 in large cages with normal housing and bedding material, in addition to standard food and water. Animals remained in their respective experimental conditions for the duration of the experiment.

At post-natal day 30 (P30), half of the animals received a mTBI using the lateral impact device and the other half received a sham injury (Mychasiuk et al., 2016). Briefly, animals are lightly anesthetized with isofluorane and placed chest down on a low-friction Teflon® board. A 100 g weight was propelled toward the rat's head at an average of 5.40 $\pm$ 0.16 m/s using regulated pneumatic pressure. The impact propels the rat into a 180° horizontal rotation that mimics that acceleration/deceleration and rotational forces typically identified in sports-related concussion (Viano et al., 2007). Following the mTBI or sham injury, lidocaine is administered to the rat's head and it is placed on its back in a clean, warm cage to recover. The time each rat took to right itself in the recovery cage was recorded as the *time-to-right*, which was used as an indirect measure of loss of consciousness. Loss of consciousness (LOC) is one of the most difficult symptoms to measure in a rodent. Although time to wake and right following the injury is not a perfect measure of LOC, the significant difference in the time required for righting between the sham and the mTBI groups, suggests that there are injury related differences in responsiveness.

At the time of injury induction, mTBI and sham animals were randomly assigned to receive Melatonin (MEL) or Placebo (PLC) treatment. MEL and PLC were added to the drinking water and animals had ad libitum access to MEL or PLC water for the duration of the experiment. For the MEL treatment, 1 g of melatonin (Sigma Chemicals) was dissolved in a minimum volume of absolute ethanol and then added to a litre of water. Based upon average daily MEL intake, a daily dose of 4.5 mg/kg/day was obtained for each rat. For the PLC treatment, the same volume of absolute ethanol was added to a litre of water. There were no significant differences in the amount of water consumed in the MEL and PLC groups (data not shown).

Following injury induction, animals were given 24 h to recover and were then subjected to behavioural test battery that spanned 14 days, designed to examine symptoms representative of PCS. The behavioural test battery, which has been described in detail elsewhere (Mychasiuk et al., 2014; Mychasiuk et al., 2016), included the Beam Walking task (post-injury day 1 (PID1)), Open Field (PID2), Elevated Plus Maze (EPM) (PID3), Novel Context Mismatch (NCM) (PID7–10) and Force Swim (PID13) (Yadid et al., 2001; Schallert et al., 2002; Whishaw and Kolb, 2005; Walf and Frye, 2007; Spanswick and Sutherland, 2010). The beam walking task measures acute balance, coordination, and motor disturbances, which are often reported in individuals that experience a mTBI and PCS (Ryan and Warden, 2003). Impairments in neuropsychological function and affect are commonly reported by individuals suffering with PCS, and were measured with 3 distinct behavioural