Neuroscience 332 (2016) 61–75

PATERNAL AGE AND DIET: THE CONTRIBUTIONS OF A FATHER'S EXPERIENCE TO SUSCEPTIBILITY FOR POST-CONCUSSION SYMPTOMOLOGY

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Abstract—In an attempt to improve current understanding of risk factors that influence individual susceptibility to poor outcomes following mild traumatic brain injury (mTBI) or concussion, this project investigated whether modifications to paternal experiences (Advanced Age (AA) or High-Fat Diet (HFD)) affected offspring susceptibility to behavioral symptomology and changes in gene expression following pediatric concussion in a rodent model. The study demonstrated that paternal treatment prior to conception altered behavioral outcomes and molecular characterization of offspring. Offspring of AA fathers demonstrated abnormal behavioral performance when compared to offspring of control fathers. Similarly, paternal HFD altered pathophysiological outcomes for offspring, contributing to the heterogeneity in post-concussion syndrome. Additionally, this study provided insight into the mechanisms that mediate non-genetic paternal inheritance. Paternal treatment and the mTBI significantly influenced expression of a majority of the genes under examination in the prefrontal cortex, hippocampus, and nucleus accumbens, with changes being dependent upon sex and the brain region examined. These epigenetic changes may have contributed to the differences in offspring susceptibility to concussion.

Key words: traumatic brain injury, development, epigenetics, High-Fat Diet, aging.

INTRODUCTION

Children under the age of 14 have the highest incidence of brain injury (Taylor et al., 2010). Although over 80% of these injuries are classified as mild, they often lead to adverse physical, cognitive, emotional, and behavioral effects, that may persist for more than three months. This lingering collection of symptoms that affect 10–15% of those with concussion is known as Post Concussive Syndrome (PCS) (Barlow et al., 2010, 2015). Approximately 16% of children experience a mild traumatic brain injury (mTBI) or concussion by the age of 10, with a significant proportion going on to suffer from PCS, which results in a substantial burden to the healthcare systems and a significant decrease in quality of life for these children (Arciniegas et al., 2005; Taylor et al., 2010). This field of research is further complicated by the variability in individual pathophysiology and the ineffectiveness of past studies to determine prognostic factors that best predict recovery (Kirkwood et al., 2008; Taylor et al., 2010). A better understanding of risk factors may help healthcare providers identify children that would benefit from early interventions and more effectively utilize limited health care resources to provide comprehensive monitoring and treatments to the children at risk for PCS (Kirkwood et al., 2008; Mychasiuk et al., 2015a,b).

Variations in developmental origins of health and disease may be a possible contributor to the heterogeneity in outcomes following mTBI. This is an emerging field of research that has the potential to elucidate mechanisms that explain why some individuals are predisposed to pathophysiology, whereas others are protected. As pure genetics have been unable to account for the inheritance of most diseases, research has shifted away from Mendelian inheritance and moved toward a Lamarckian focus on epigenetic influences on individual disease risks (Bohacek and Mansuy, 2013; Mychasiuk et al., 2015a,b). This has suggested a need to 'think outside the box' and examine mTBI risk factors and susceptibility using a different lens. Previous research in our laboratory with rodents has demonstrated that early life influences, via maternal diet and maintenance of this diet throughout early life, can influence severity of outcomes following pediatric concussion (Hehar and Mychasiuk, 2015; Hehar et al., 2015; Mychasiuk et al., 2015a,b). However, paternal influences on pathophysiological risk are an exciting but unexplored area of research that may actually serve as a better model for understanding transgenerational transmission of traits, as maternal influences throughout pregnancy have been known to confound trait inheritance (Mychasiuk et al., 2015a,b).

Although a novel field of study, evidence regarding the influence of paternal experiences on offspring neurological health is beginning to accumulate

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Abbreviations: AA, Advanced Age; EPM, Elevated Plus Maze; FS, Force Swim; HFD, High-Fat Diet; HPC, hippocampus; mTBI, mild traumatic brain injury; NAc, nucleus accumbens; NCM, Novel Context Mismatch; P65, Postnatal Day 65; PCS, Post Concussive Syndrome; PFC, prefrontal cortex.

http://dx.doi.org/10.1016/j.neuroscience.2016.06.039
0306-4522/

(Mychasiuk et al., 2013). Since the parental environment is generally similar to that of the offspring, adaptive traits gained by a parent that can be transmitted to the offspring may increase their chance of survival (Jenkins et al., 2014). Paternal environment has been shown to induce mutations in the germline and also mediate change through epigenetic processes (Malaspina et al., 2001; Bateson et al., 2004; Mychasiuk et al., 2013). As spermatogenesis is an ongoing process, it is highly susceptible to environmental manipulation, producing heritable epigenetic changes such as changes in DNA methylation or chromatin compaction (Kaati et al., 2002; Hammond et al., 2009). For example, fathers who consumed low-calorie diets had male offspring with reduced risk for cardiovascular death, whereas high-calorie diets were associated with increased mortality (Abraham and Bear, 1996; Bygren et al., 2001; Sinclair and Watkins, 2014). In addition, investigating the effects of parental diet on health of the developing offspring demonstrated that male reproduction is sensitive to dietary status, with diets rich in fatty acids significantly impairing sperm viability, inducing sperm DNA fragmentation, and consequently affecting embryo quality (Sinclair and Watkins, 2014). Similarly, advanced paternal age has been shown to reduce methylation of specific promoter regions, modifying gene expression, as well as being associated with an increased risk for various neurological disorders such as schizophrenia and autism (Kaati et al., 2007; Helmeke et al., 2009; Jenkins et al., 2014).

Building upon these studies, this project was designed to use a rodent model of pediatric concussion to determine if modifications to paternal experiences (Advanced Age (AA) or High-Fat Diet (HFD)) affect offspring risk for poor outcomes. As consumption of HFDs and advancing paternal age are increasingly observed in modern societies, an understanding of their impact on offspring neurological health is needed. Owing to the ongoing nature of spermatogenesis, and the susceptibility of the sperm epigenome to environmental influences, manipulations such as changes in dietary intake have the potential to significantly modify gene expression patterns in offspring (Hehar and Mychasiuk, 2015). Additionally, given that the average age of a first child for men had risen to 27.4 years of age, when measured in 2010, and is believed to be even higher now in first-world nations (U.S. Census Bureau, 2011), this study sought to examine whether changing paternal age from sexual maturity (~18 years of age) to middle-adulthood (~30 years of age) could influence the susceptibility of offspring to PCS. In order to examine this complex issue, our study utilized a comprehensive behavioral test battery to examine symptoms associated with PCS including: beam-walking (motor coordination and balance), open field (general locomotor activity), elevated plus maze (anxiety), novel context mismatch (short-term memory), and forced swim (emotional state), in addition to an extensive analysis of gene expression in three distinct but connected brain regions.

In an effort to understand the fundamental mechanisms associated with paternal transmission of concussion susceptibility, effects of paternal experiences on offspring brain epigenetic networks were considered. It was hypothesized that paternal factors such as HFD consumption and AA would influence concussion susceptibility of offspring through detectable changes in offspring brain gene expression. This study investigated gene expression in the Prefrontal Cortex (PFC), Hippocampus (HPC) and Nucleus Accumbens (NAc) due to their implicated roles in concussion pathophysiology such as executive and cognitive dysfunction, impaired learning and memory, and reward processing, respectively. Genes were chosen for investigation based upon the role they may play regulating paternal diet and age-dependent changes in the offspring brain, while considering relevance to recovery from mTBI. Expression of the following genes was analyzed: *DAT*, *DNMT1*, *DRD4*, *HSP70*, *PGC1-a*, and *PPAR-a*, in each of the three brain regions identified.

EXPERIMENTAL PROCEDURES

Paternal treatment and breeding procedures

All experiments were conducted in accordance with the Canadian Council on Animal Research and were approved by the University of Calgary Conjoint Faculties Research Ethics Board. Sprague Dawley rats were in-house bred and maintained in a husbandry room with 12-h light: dark cycles. Each male was bred with a control female that was 65 days old. For the HFD manipulation, male rats were maintained on HFD (Dyet, Bethleham, PA, USA) for three weeks prior to conception, which resulted in 60% of total calories being obtained from fat ($n = 5$). The HFD males were bred at Postnatal Day 65 (P65). For the AA paradigm, male rats were maintained on a standard diet but were not bred with a female until the male reached P105 ($n = 5$). The control males were fed a standard diet and were bred with a female at P65 ($n = 6$). All female dams continued to be maintained on standard diets for the duration of the experiment. The pups were weaned from their mothers at P21 and maintained on a standard diet for their lifetime. At weaning, the pups were randomized into group- and sex-matched cages of four in an effort to prevent litter effects.

Induction of mTBI

At P30, the mTBI was administered to half of the offspring using the Lateral Impact Device as described in Mychasiuk et al. (2016), while the other half received sham injuries. Briefly, each rat underwent brief anesthetization with isofluorane, and was placed chest down on a low-friction Teflon® board. For the mTBI, a 100-gram weight was propelled toward the rat's head at a speed of 5 m/s, driving the rat into a 180° horizontal rotation. The acceleration and rotational forces experienced by the rat's head during this injury are similar to the forces involved in producing a clinical concussion (Viano et al., 2007; Mychasiuk et al., 2016). The rat then received topical administration of lidocaine. Immediately after the injury, the rat was placed in a cage in a supine position