Neurobiology of Disease 73 (2015) 1–11

Contents lists available at ScienceDirect

Neurobiology of Disease

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

Diet, age, and prior injury status differentially alter behavioral outcomes following concussion in rats

Richelle Mychasiuk [a,*], Harleen Hehar [b], Linda van Waes [c], Michael J. Esser [b]

[a] Alberta Children's Hospital Research Institute, University of Calgary, Faculty of Medicine, Heritage Medical Research Building, Room 274, 3330 Hospital Drive NW, Calgary, AB T2N1N4, Canada
[b] Alberta Children's Hospital Research Institute, University of Calgary, Faculty of Medicine, Canada
[c] University of British Columbia, Faculty of Medicine, Canada

ARTICLE INFO

Article history:
Received 7 June 2014
Revised 25 August 2014
Accepted 12 September 2014
Available online 28 September 2014

Keywords:
Brain injury
Development
High fat diet
Repetitive TBI
Telomeres

ABSTRACT

Mild traumatic brain injury (mTBI) or concussion affects a large portion of the population and although many of these individuals recover completely, a small subset of people experience lingering symptomology and poor outcomes. Little is known about the factors that affect individual susceptibility or resilience to poor outcomes after mTBI and there are currently no biomarkers to delineate mTBI diagnosis or prognosis. Based upon the growing literature associated with caloric intake and altered neurological aging and the ambiguous link between repetitive mTBI and progressive neurodegeneration, the current study was designed to examine the effect of a high fat diet (HFD), developmental age, and repetitive mTBI on behavioral outcomes following a mTBI. In addition, telomere length was examined before and after experimental mTBI. Sprague Dawley rats were maintained on a HFD or standard rat chow throughout life (including the prenatal period) and then experienced an mTBI/concussion at P30, P30 and P60, or only at P60. Behavioral outcomes were examined using a test battery that was administered between P61–P80 and included; beam-walking, open field, elevated plus maze, novel context mismatch, Morris water task, and forced swim task. Animals with a P30 mTBI often demonstrated lingering symptomology that was still present during testing at P80. Injuries at P30 and P60 rarely produced cumulative effects, and in some tests (i.e. beam walking), the first injury may have protected the brain from the second injury. Exposure to the high fat diet exacerbated many of the behavioral deficits associated with concussion. Finally, telomere length was shortened following mTBI and was influenced by the animal's dietary intake. Diet, age at the time of injury, and the number of prior concussion incidents differentially contribute to behavioral deficits and may help explain individual variations in susceptibility and resilience to poor outcomes following an mTBI.

Introduction

Recent statistics released by the World Health Organization (https://apps.who.int/infobase) indicate that in industrialized countries like the United States and Canada, over 60% of the population is at risk for being overweight (as determined with a BMI ≥ 25). Although often overlooked, there is increasing evidence establishing a link between obesity and adverse neurological outcomes such as reduced cognitive functioning, stroke, and Alzheimer's disease (Luchsinger et al., 2002). While the exact mechanism of action of this association is unknown, negative environmental factors, such as excessive caloric intake, are believed to exert their action on the brain by directly modulating brain plasticity (Garrido, 2011). There is compelling evidence that excessive caloric intake negatively affects plasticity in the aging brain (Weindruch and Sohal, 1997), and recent animal studies have indicated that high fat diets are associated with impaired learning, reduced hippocampal dendritic spine density, and reduced long-term potentiation (Stranahan et al., 2008). Furthermore, high fat diets have also been associated with increased inflammation in the brain (Pistell et al., 2010) and impaired hippocampal neurogenesis (Lindqvist et al., 2006).

Studies in humans demonstrating a relationship between impaired plasticity, as measured via executive function, and obesity are not that far behind. Body mass index has been shown to be significantly related to performance on cognitive tests such as the digit span task, the trail making test, verbal interference, and the Austin Maze task, with normal weight individuals outperforming overweight/obese individuals on all tasks (Gunstad et al., 2007). Further studies have indicated that obese males exhibit deficits in working memory, verbal fluency, and visual organization, even when controlling for diabetes, high cholesterol, and cigarette smoking (Elias et al., 2003; Baugh et al., 2012). In addition to impaired cognition, excessive caloric intake has been linked to poor neurological outcomes, with higher BMI being associated with decreased brain volume (Raji et al., 2010; Gunstad et al., 2008) specifically in brain regions responsible for high-order functioning such as the

* Corresponding author.

http://dx.doi.org/10.1016/j.nbd.2014.09.003
0969-9961/

prefrontal cortex (Walther et al., 2010). What's more, obesity has been shown to impair recovery from stroke (Kalichman et al., 2007), is a major risk factor for ischemic stroke across all ethnic groups, and is associated with the greatest risk of stroke in younger individuals (Suk et al., 2003). Similarly, research indicates that obese individuals have more complications and higher mortality rates following hospitalization for traumatic brain injury (Brown et al., 2006). Extrapolating from these results, excess caloric intake may therefore be associated with enhanced vulnerability to poor outcomes following mTBI.

Concussion/mTBI is a complex neurological injury induced by traumatic mechanical forces to the brain (McCrory et al., 2013). Primary forces such as acceleration and deceleration produce biomechanical shearing and tearing of neurons, axons, and glia resulting in a complex neurochemical cascade (for review see Barkhoudarian et al., 2011; Kumar and Loane, 2012). Although there is often no overt structural brain damage identified following most mTBIs, acute and chronic molecular changes (the secondary injury) are thought to produce many of the associated neurological deficits (McIntosh et al., 1996). These reactive changes may also leave the brain vulnerable to repeat injury and cumulative impairments (Barkhoudarian et al., 2011). The majority of literature suggests that multiple brain injuries are generally associated with greater impairment, longer recovery times, and increased risk for earlier onset of age-related neurodegenerative diseases (Baugh et al., 2012; Huh et al., 2007; Smith et al., 2013). For example, when compared to single concussions, multiple mTBIs were associated with greater deficits on attentional/executive functioning tasks (Wall et al., 2006). Although these findings are robust, they tend to focus on adult populations and the mature brain. Keeping in mind that in the pediatric population the brain is still undergoing significant neurodevelopmental; an early life mTBI could disturb one of the many processes critical to healthy maturation. It is generally accepted that the cognitive development occurring in late childhood and adolescence is primarily subserved by the prefrontal cortex (PFC) and the integrated connections it forms with other higher-order systems (Sowell et al., 1999, 2001, 2003; Kolb et al., 2012; Casey et al., 2000). Thus the biomechanical forces impacted on the brain during an mTBI, have the potential to disrupt the formation of this complex network and may affect many down-stream processes. A concussion early in life may consequently have very different effects on the brain when compared to one experienced in adulthood when the brain has finished developing.

Using an animal model, the current study was designed to determine if life-long exposure (including the prenatal period) to a high calorie diet (high fat diet and high sugar) would exacerbate the behavioral effects of an mTBI and/or impair recovery following the injury. In addition, we sought to examine the effect of mTBI timing (childhood or adolescence) and whether or not the possible negative effects of a high calorie diet would be further intensified with repeat mTBIs. Finally, this study investigated the relationship between diet, injury exposure, and telomere length. Telomeres protect the ends of chromosomes from degradation, differ in length on an individual basis, are involved in normal aging and neurological deficits, and are influenced by experience (Blasco, 2007; Liu et al., 2004). As literature indicates that telomere length is affected by diet and neurodegenerative disease (Mattson, 2000), this study sought to determine if exposure to a high fat diet or an mTBI altered telomere length in rats. Dams were fed a high fat diet (HFD) (60% of caloric intake from fat, supplemented with 20% sucrose in the drinking water) or the standard rat chow (STD) for three weeks prior to mating and for the duration of pregnancy and weaning. Once pups were weaned from their mothers, they were maintained on their respective HFD or STD diets for the duration of the experiment. When pups reached postnatal day 30 (P30) they received a mTBI or a sham injury that was succeeded by a mTBI or sham injury at P60. Rats underwent a behavioral test battery which included measures of post-concussion syndrome following the injury at P60. Tissue from ear notches collected prior to the injury at P30 and at the time of sacrifice (~P80) was used to measure telomere length at baseline and following traumatic brain injury.

Materials and methods

Breeding and dietary procedure

All experiments were carried out in accordance with the Canadian Council of Animal Care and approved by the University of Calgary Conjoint Faculties Research Ethics Approval Board. All animals were maintained on a 12:12 light:dark cycle (lights on a 07:00) in a temperature controlled husbandry room (21 °C) with access to food and water ad libitum. Eight Sprague Dawley female rats were pair-housed in standard shoe-box cages (2 female rats/cage). Four female rats had access to standard food and water (STD) and the remaining 4 had access to a high fat diet (HFD), (Adjusted Calories Diet, ~60% of total calories are derived from fat; TD.06414, Harlan Laboratories — Madison, WI) supplemented with 20% sucrose in their drinking water. Previous studies utilizing a HFD similar to the one used in this study had demonstrated increased serum cholesterol, elevated fasting blood glucose levels, but no changes in serum insulin levels (Stranahan et al., 2008). This differs from the ketogenic diet which is a high-fat, low-carbohydrate neuroprotective diet that forces the body to burn fats rather than carbohydrates The ketogenic diet minimizes protein intake to the minimal daily requirement and dramatically restricts carbohydrates. Contrary to the HFD where animals maintain the same daily intake of carbohydrates and protein but increase their daily intake of calories from fat and sugar, animals on the ketogenic diet consume significantly less calories and actually burn more fat. Female rats began the HFD three weeks prior to mating to ensure that the females were adjusted to the dietary manipulation before pregnancy. Female rats were essentially maintained on their respective diets for 9 weeks; 3 weeks prior to mating, the 3 weeks of pregnancy, and throughout weaning. Pups born to mothers on the HFD were maintained on the HFD throughout the entire experiment (until ~P80). The HFD was continued throughout the experiment because children and young adults are highly influenced by parental eating habits and are likely to maintain a diet similar to that sustained in their family environment (Savage et al., 2007; Hood et al., 2000).

Each female was mated to a different male Sprague Dawley rat (male rats were all fed the standard diet) and then returned to their pair-housed living arrangement for the duration of pregnancy. The day prior to delivery, dams were separated and remained individually housed with their litters for the duration of weaning. Dams were weighed daily throughout pregnancy and weaning. The four HFD dams gave birth to 52 pups (26 M: 26 F) and the four STD dams gave birth to 49 pups (26 M: 23 F). Pups were weaned from their mothers at postnatal day 21 (P21) in a manner that ensured pups with similar dietary exposure were randomly assigned to same-sex cages of 4. Caution was used to avoid grouping litter-mates together, resulting in cages that contained 1 pup from each of the four dams. Forty-eight of the pups were used in this experiment (24HFD: 24STD), and the remaining pups were used as part of another study.

mTBI procedure

When pups reached P30, half of the animals received an mTBI using the modified weight drop technique (Kane et al., 2012; Mychasiuk et al., 2014), and the other half received a sham injury. Briefly, animals were lightly anesthetized and placed chest down on a piece of scored tinfoil suspended 10 cm above a foam collection sponge. In the modified weight drop technique, a 150 g weight is dropped through a guide tube and produces a glancing impact to the rat's closed head. The impact from the 150 g weight propels the rat through the tin foil, where it undergoes a 180° vertical rotation before landing on its back on the collection sponge. Immediately after the impact, topical lidocaine is applied to the rat's head and it is placed in a clean warm cage to recover. Animals