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Research report

The effects of a high-fat sucrose diet on functional outcome following cortical contusion injury in the rat

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ABSTRACT

Traumatic brain injury (TBI) is a major public health issue affecting 1.7 million Americans each year, of which approximately 50,000 are fatal. High-fat sucrose (HFS) diets are another public health issue which can lead to obesity, hypertension, and many other debilitating disorders. These two disorders combined can lead to more complicated issues. It has recently been shown that HFS diets can reduce levels of brain-derived neurotrophic factor (BDNF) leading to reductions in neuronal and behavioral plasticity. This reduction in BDNF is suspected of increasing the susceptibility of the brain to injury. To test the effects of a HFS diet on recovery of function post-TBI, male Sprague-Dawley rats were used in this study. Eight weeks prior to TBI, rats were placed on a special HFS diet ($n = 14$) or a standard rodent diet ($n = 14$). Following this eight-week period, rats were prepared with bilateral frontal cortical contusion injuries (CCI) or sham procedures. Beginning two days post-TBI, animals were tested on a battery of behavioral tests to assess somatosensory dysfunction and spatial memory in the Morris water maze, with a reference memory and a working memory task. Following testing, animals were sacrificed and their brains processed for lesion analysis. The HFS diet worsened performance on the bilateral tactile adhesive removal test in sham animals. Injured animals on the Standard diet had a greater improvement in somatosensory performance in the adhesive removal test and had better performance on the working memory task compared to animals on the HFS diet. The HFS diet also resulted in significantly greater loss of cortical tissue post-CCI than in the Standard diet group. This study may aid in determining how nutritional characteristics or habits interact with damage to the brain.

1. Introduction

The National Institutes of Health (NIH) has stated that TBI is one of the leading causes of acute and chronic disability in the United States. Each year, 1.7 million Americans endure a TBI and 50,000 die [2]. Brain injury in the US, therefore, warrants investigation because it is both a health concern that lacks an effective treatment and is an increasingly larger expenditure to the American public. Additionally, individuals more than 65 years of age have higher rates of hospitalization and mortality than any other age group [31]. The overall incidence for TBI per 100,000 individuals is 60.6; however, the rate for individuals more than 65 years of age is 155.9 [4]. Since there are no currently approved treatments the NIH stresses the importance of prevention as its primary defense against TBI. However, of equal importance are factors that may exacerbate the injury, such as age at the time of injury, dietary Mg^{2+} status, or other nutritional factors [12,18].

Recent research has shown that nutritionally based therapies are critically important in terms of TBI outcome. Therapeutic administration of nicotinamide (Vitamin B_3) following TBI has been shown to be effective at improving behavioral and histological outcome in various models of TBI [7,11,15–17,20,21,23,30]. Other nutrient compounds administered following injury, such as riboflavin (Vitamin B_2), pyridoxine (Vitamin B_6) and magnesium (Mg^{2+}) have also been shown to improve functional recovery in rodent models of TBI [13,14,19,24]. It has recently been shown that proper caloric intake following TBI can lead to greater survival rates among patients with TBI. In a recent clinical study, severe TBI patients that were not fed within 5–7 days following TBI had a 2–4 fold increased likelihood of death. Also, every 10 kcal/kg decrease in caloric intake was associated with a 30–40% increase in mortality rates [10]. Though it appears that proper caloric intake following TBI may lead to greater recovery rates; however, there is little data outlining the outcome of the effects of poor nutrition prior to TBI.

A recent review article has chronicled the effects of many nutrients on brain function, specifically focused on cognition and synaptic plasticity, and provided many interesting avenues for investigation [8]. Of most relevance to the current study is the role

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of a high-fat sucrose (HFS) diet on synaptic plasticity and recovery of function following TBI. Several studies have shown that a HFS diet impairs neural plasticity and learning [9,26,34] but only one study has shown that administration of a HFS diet prior to fluid percussion injury (FPI) resulted in abnormal neural plasticity and impaired cognitive outcome post-injury [33]. A recent study has shown that a 5-week HFS diet prior to 6-hydroxydopamine (6-OHDA) lesioning of the medial forebrain bundle resulted in greater neurodegeneration and reduced dopamine levels compared to animals on the control diet [27].

Given the typical American diet (high fat, high sucrose content) it should come as no surprise that many of these 1.4 million TBI recipients suffer from poor nutritional levels prior to injury. The standard diet that the typical American consumes each day can lead to many debilitating health problems, including obesity, hypercholesterolemia, and cardiovascular disease. Trends toward obesity from HFS diets continue to rise. In a study conducted from 1999–2000, about 66% of American adults were considered overweight or obese and the prevalence of being overweight rose to 15% in adolescents and children [5,28]. Thus, this potential risk factor is worthy of continued examination in TBI.

Although the effect of the HFS diet on neural plasticity and behavioral function in the uninjured rat is well known, few studies have examined this relationship in the injured brain [8,26,33,34]. Thus, the purpose of this study was to examine the effects of a pre-injury HFS diet on the behavioral outcome following traumatic brain injury. Eight weeks prior to TBI, rats were placed on either a standard rodent diet or a HFS diet. Following TBI, all rats were placed back on the standard rodent diet, tested on a battery of behavioral tests, and their brains harvested for histological analyses.

2. Methods

2.1. Subjects

Twenty-eight, 14–15-week old, male Sprague-Dawley rats were used in this experiment. The experimental procedures conducted within this study were reviewed and approved by the Institutional Animal Care and Use Committee and the study was conducted in a facility certified by the American Association for the Accreditation of Laboratory Animal Care. Rats were maintained on a standard 12 h light/dark cycle with food and water available *ad libitum*.

2.2. Dietary manipulation

Eight weeks prior to CCI, rats were placed on either a high-fat sucrose diet (#D12451, Research Diets Inc., New Brunswick, NJ) or remained on a standard laboratory rodent control diet (#D12450B, Research Diets Inc., New Brunswick, NJ). The HFS diet contained 45% fat, 70% carbohydrate, and 20% protein compared to its control diet that contained 10% fat, 35% carbohydrate, and 20% protein. All rats received food and H_2O *ad libitum* and their body weights and health were monitored daily. Following CCI, all animals were placed back on the standard rodent laboratory control diet.

2.3. Surgery

Aseptic procedures and conditions were maintained for all animals during the surgical procedure in accordance with previously detailed methods [21,22]. Animals were anesthetized using a mixture of Isoflurane (2–4%) and oxygen (0.8 L/min) without intubation and then prepared for surgery. When the rat was unresponsive (no ocular or pedal reflexes) its head was shaved and the animal was placed into a stereotaxic device. The head was then scrubbed with 70% alcohol, followed by betadine, and a midline incision was made in the skin and underlying fascia. A circular craniotomy (6.0 mm diameter) was made using a surgical drill and a specially designed drill bit that prevented damage to the meninges and cortex. The craniotomy was bilateral and centered over the frontal cortex (3.0 mm anterior to bregma). The contusion injury was created using the Benchmark™ Stereotaxic Impactor, an electromagnetic contusion device (www.myneurolab.com, St. Louis, MO) using a sterile, aluminum impactor tip (5.0 mm diameter) that was activated at a velocity of 2.75 m/s. The impactor tip was positioned above the cortex and upon activation of the piston, the impactor tip made contact with the cortex for 0.5 s, which resulted in a 2.0 mm compression of the cortex. Following the contusion, any bleeding was controlled and then the incision was closed with absorbable suture material. To maintain normal body temperature (37 °C) during surgery and recovery the rats were placed on a warm water recycling bed and pump system (EZ Anesthesia, Palmer, PA). Sham animals underwent the same procedures, including craniotomy, but did not receive CCI.

2.4. Bilateral tactile adhesive removal test

The bilateral tactile adhesive removal test is used to assess somatosensory dysfunction following TBI, in which the latency to remove a small round adhesive patch (113 mm^2) from the radial aspect of each forelimb was recorded [11,20]. Two trials per day were administered 5 min apart to minimize habituation effects. Each trial was terminated either when both patches had been removed or if 2 min had elapsed. Baseline latencies to remove the patches were recorded prior to injury. Animals were tested on days 2, 4, 6, 10, 12, 14, and 18 post-CCI. The dependent measure of interest was the total latency to remove both patches.

2.5. Cognitive assessment

The Morris water maze (MWM) has been widely utilized to assess cognitive performance following brain injury [17,25]. A blue fiberglass tank, 150 cm in diameter and 76 cm deep, was filled to a depth of 32 cm at 24 °C. An acrylic platform (10 cm × 10 cm) was submerged 2.0 cm below the surface of the water. The latency to escape was recorded by the SMART video tracking system software (San Diego Instruments, San Diego, CA). All animals were assessed on the acquisition of a reference memory task on days 11–14 post-CCI [11–13,17,19,25,30]. Each rat was tested for 4 trials each day, starting from each of the four randomly chosen release points. The inter-trial interval (ITI) was 15 min. The trial was terminated when the animal reached the submerged platform located in the center of the northeast quadrant or when 90 s had elapsed. Each rat was allowed to remain on the platform for 15 s, after which it was placed in a warm holding cage until the next trial.

Working memory performance was tested on days 15–17 post-CCI using previously established methods [11,13,14,17,22]. The platform was submerged at the center of a new randomly chosen quadrant (southwest, northwest, and southeast) each day. Each animal was given 4 trials per day, starting from one of four randomly chosen release points (ITI = 15 min). The first trial of each of these three days was considered an information trial and was not included in subsequent analyses. Each trial ended when the animal located the platform or when 90 s had elapsed.

2.6. Histology

At 21 days post-CCI, rats were anesthetized with urethane (3.0 g/kg, i.p.) and were transcardially perfused with 0.9% phosphate buffered saline (PBS), followed by 10% phosphate buffered formalin (PBF). The brains were then post-fixed in PBF following removal from the cranium. A 30% sucrose solution was used to cryopreserve the brains for 3 days prior to frozen sectioning. Serial sections (40 μm thick) were sliced using a sliding microtome and electronic freezing stage and collected into phosphate buffer (PB).

2.7. Lesion analysis

Following frozen sectioning, a series of coronal sections were brush mounted onto gelatin-subbed microscope slides, stained with cresyl violet, dehydrated and cover-slipped. The extent of the lesion was analyzed using an Olympus BX-51 microscope and DP-70 camera. Images throughout the extent of the injury were captured using the digital capturing system, and measurement of the lesioned tissue was performed using University of Texas Health Science Center San Antonio (UTHSCSA) ImageTool software. The Calvalieri method was used to calculate the volume of the remaining frontal cortex [3]. The number of sections and section thickness (40 μm) were multiplied by the mean area of the lesion cavity (calculated at four stereotaxic coordinates surrounding the lesion: 3.70, 2.70, 1.70, and 0.70 relative to bregma) [29]. The extent of cortical injury was measured by calculating the volume of remaining cortex and compared across groups [11,13,14].

2.8. Statistical analysis

Analysis of variance (ANOVA) tests were performed using procedures for general linear models (SPSS 15.0 for Windows; SPSS, Inc. Chicago, IL) with operations for repeated measures, where appropriate, for all behavioral measures. The between group factors were Injury and Diet group and the within group factor was day of testing. This resulted in the following experimental groups: CCI-HFS diet [$n = 7$], CCI-standard diet [$n = 7$], sham-HFS diet [$n = 7$], and sham-standard diet [$n = 7$]. Huynh-Feldt probabilities (HFP) were used to correct for Type-1 error associated with repeated measures and post hoc means comparisons, respectively [11,13,14]. Anatomical data were analyzed with one-way ANOVA procedures and post-hoc t-tests. A significance level of $p < 0.05$ was used for all statistical analyses.