Behavioural Brain Research 306 (2016) 1–7

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Short-term exposure to a diet high in fat and sugar, or liquid sugar, selectively impairs hippocampal-dependent memory, with differential impacts on inflammation

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HIGHLIGHTS

- High fat + sugar or liquid sugar diet impaired hippocampal-dependent memory in 1 week.
- Another memory task requiring the perirhinal cortex was unaffected.
- Memory deficits were not dependent on excess energy intake or weight gain.
- Liquid sugar elevated hippocampal and white adipose tissue markers of inflammation.
- There was no evidence of inflammation in perirhinal cortex or hypothalamus.

ARTICLE INFO

Article history:
Received 15 January 2016
Received in revised form 10 February 2016
Accepted 6 March 2016
Available online 10 March 2016

Keywords:
Diet
Fat
Sugar
Memory
Hippocampus
Inflammation

ABSTRACT

Chronic high-energy diets are known to induce obesity and impair memory; these changes have been associated with inflammation in brain areas crucial for memory. In this study, we investigated whether inflammation could also be related to diet-induced memory deficits, prior to obesity. We exposed rats to chow, chow supplemented with a 10% sucrose solution (Sugar) or a diet high in fat and sugar (Caf + Sugar) and assessed hippocampal-dependent and perirhinal-dependent memory at 1 week. Both high-energy diet groups displayed similar, selective hippocampal-dependent memory deficits despite the Caf + Sugar rats consuming 4–5 times more energy, and weighing significantly more than the other groups. Extreme weight gain and excessive energy intake are therefore not necessary for deficits in memory. Weight gain across the diet period however, was correlated with the memory deficits, even in the Chow rats. The Sugar rats had elevated expression of a number of inflammatory genes in the hippocampus and WAT compared to Chow and Caf + Sugar rats but not in the perirhinal cortex or hypothalamus. Blood glucose concentrations were also elevated in the Sugar rats, and were correlated with the hippocampal inflammatory markers. Together, these results indicate that liquid sugar can rapidly elevate markers of central and peripheral inflammation, in association with hyperglycemia, and this may be related to the memory deficits in the Sugar rats.

1. Introduction

The "Western diet" is highly palatable and energy-dense, containing high levels of saturated fats and processed sugars, which promote obesity. The chronic consumption of this diet is associated with various medical conditions including cardiovascular disease, gastrointestinal and respiratory difficulties, hypertension, stroke, diabetes mellitus and many types of cancers [1,2].

A rapidly expanding human literature shows that diets high in saturated fat and sugar can impair cognition in all age groups. In school-aged children this diet has been associated with impaired non-verbal intelligence (e.g., spatial perception) [3], visual-spatial learning and memory [4] and self-reported difficulties in mathematics [5]. Other studies have reported similar results in university students. For example, Francis and Stevenson [6] found that healthy students with high self-reported fat and sugar intake were impaired on memory tasks that required the hippocampus but not on other general cognition tasks or memory tasks which required the frontal

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http://dx.doi.org/10.1016/j.bbr.2016.03.018
0166-4328/

cortex. Finally, in middle-aged and older participants, diets high in saturated fat [7–10] and sugar [11] have been shown to accelerate normal age-related cognitive decline and increase the subjects' risk of developing neurological diseases including dementia.

What is more concerning is that even short-term exposure to these diets is able to induce measureable impairments in cognition [for review see Ref. [12]]. Holloway and colleagues [13] showed in healthy, young male subjects that consuming 75% of their energy from fat was sufficient to impair speed of retrieval, attention, and depress mood after only 5 days exposure. A single high glycemic meal can also impair memory performance in children and healthy young adults [14,15]. The rapidity of these impairments suggests that the effects of fat and sugar on memory can occur independently of any effects on body weight or general health.

Experiments using rodent models provide further support for these rapid diet-induced memory deficits and in addition, they indicate that hippocampal-dependent memory may be pwarticularly susceptible. Kanoski and Davidson [16] for example, reported spatial impairments on the Radial Arm Maze (RAM) after only 3 days of a high-energy diet but over 30 days exposure was necessary for stable impairments on the non-spatial version of this task. We have also previously shown selective hippocampal-dependent memory deficits following 1 month of a high-energy diet and these deficits were associated with elevated hippocampal expression of a number of pro-inflammatory and oxidative stress markers [17]. At the same time-point, other laboratories have shown a relationship between diet-induced memory deficits and reductions in hippocampal markers of neurogenesis including brain derived neurotrophic factor (BDNF) [18].

Long term high-energy diets are known to impair memory and have been inconsistently associated with inflammation and neurogenesis in brain areas crucial for memory. In the current study, we aimed to extend and clarify these findings by controlling for two potential confounds: obesity and associated metabolic comorbidities induced by the length of the diet, and the differential roles of fat and sugar. To do this, we compared how 1 week exposure to a diet high in fat and sugar (Caf + Sugar) or high in sugar (Sugar) would affect perirhinal-dependent object, and hippocampal-dependent place, recognition memory [19,20]. Reverse transcription polymerase chain reaction (RT-PCR) was used to quantify the mRNA expression of a number of inflammatory and neurogenesis markers in several brain regions (hippocampus, perirhinal cortex and hypothalamus). In addition, to examine whether a similar response was present in the periphery, we examined pro-inflammatory gene expression in white adipose tissue (WAT). Finally, we examined other markers known to be affected by longer exposure to high-energy including feeding peptides [21] and dopamine receptors [22,23].

2. Material and methods

2.1. Rats

Male Sprague-Dawley rats (192–222 g; Animal Resource Centre, Perth, WA, Australia) were housed 3 per polypropylene cage (47 cm × 29 cm × 15 cm) in a temperature controlled (18–22 °C) colony room on a 12 h light/dark cycle (lights on at 07:00 h). Rats were weight matched across diet groups and home cages (n = 12 per group; Chow 206 ± 3 g; Sugar 207 ± 2 g; Caf + Sugar 207 ± 4 g). All rats were handled daily for 9 days prior to the diet manipulations and were maintained *ad libitum* on standard rat chow (Gordon's Premium Rat and Mouse Breeder diet, NSW, Australia) and tap water. All experimental procedures were approved by the UNSW Animal Care and Ethics Committee (#13/122B).

2.2. Diet manipulations

The Chow rats had access to standard rat chow and 2 water bottles in each home cage. The Sugar rats received standard rat chow, 1 water bottle and 1 bottle of 10% sucrose solution. The Caf + Sugar rats had access to standard rat chow, 1 water bottle and 1 bottle of 10% sucrose solution, which was supplemented with a selection of cakes (e.g., chocolate mud cake, jam roll, lamingtons), biscuits (e.g., chocolate chip, monte carlo, scotch fingers) and a protein source (e.g., party pie, dim sims, dog roll). All food and liquid was available *ad libitum* and replaced daily. Body weight and food and liquid intake was measured after 1, 5 and 8 days on the diets. Items were weighed before presentation to the rats and then reweighed after 24 h. The amount eaten and drunk per home cage was converted to kilojoules (kJ) using data provided by the manufacturers and average intake per rat was calculated. Based on these measurements, macronutrient profiles were 65% carbohydrates, 22% protein, and 13% fat (Chow), 74% carbohydrates, 16% protein and 10% fat (Sugar) and 54% carbohydrates, 5% protein and 41% fat (Caf + Sugar).

2.3. Memory tasks—object and place recognition

These tasks were run as per Beilharz et al., [17]. Briefly, a video camera was positioned above a black acrylic open field arena (60 cm × 60 cm × 60 cm). The novel objects were commercial products that varied in size and texture (e.g., tuna tins, tomato sauce). Rats were acclimatized to the empty arena on days 6 and 7 and tested on days 8 and 9. Rats were tested at the same time each day and the testing order was counterbalanced across diet groups. Objects, locations and their configurations were also counterbalanced across groups and days. In the familiarisation phase, rats explored 2 identical objects for 5 min and then were removed for a 5 min retention phase. The arena and objects were cleaned. For the object test phase, objects were in the same locations but 1 object was novel and the other was identical to the familiarisation objects. In the place test phase, the objects were the same as during familiarisation but 1 was moved to a corner of the arena. The test phase lasted 3 min.

Exploration was defined as the rat's head within 2 cm of the object with the neck extended and vibrissae moving around the object. Chewing, standing or sitting on the object was not counted as exploration. Data were scored using Macropod ODlog® software by an observer blind to the diet group and purpose of the experiment, and are reported as the Exploration Ratio. That is, the time the rat spent exploring the novel object or place divided by the time they spent exploring the novel and familiar object or place ($t_{novel}/t_{novel} + t_{familiar}$). Ratios above 0.50 indicate a novelty preference whilst 0.50 indicates equal time exploring the novel and familiar objects/places.

2.4. Tissue collection

Rats were culled on days 13–15 of the diet to minimise any carry-over effect of behavioural testing on the inflammation markers [24]. Rats were anesthetized using ketamine/xylazine (100/15 mg/kg i.p), blood was collected through cardiac puncture and glucose levels assessed (Accu-Chek, Roche Diagnostics, Sydney, NSW, Australia). The plasma was separated and stored at −80 °C for subsequent determination of insulin and triglyceride concentrations. Rats were then killed by decapitation. The liver and WAT (retroperitoneal (RP) and gonadal) were dissected and weighed. RP fat was snap frozen in liquid nitrogen and stored at −80 °C. Brains were removed and coronal incisions made at the level of the optic chiasm and the rostral border of the hypothalamus (Bregma −1.30 to −4.52 mm). The hippocampus, hypothalamus and perirhinal