Brain, Behavior, and Immunity xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Dietary-induced obesity disrupts trace fear conditioning and decreases hippocampal reelin expression

Amy C. Reichelt [a,b], Jayanthi Maniam [a], R. Frederick Westbrook [b], Margaret J. Morris [a,*]

[a] School of Medical Sciences, The University of New South Wales, Sydney, Australia
[b] School of Psychology, The University of New South Wales, Sydney, Australia

ARTICLE INFO

Article history:
Received 15 May 2014
Received in revised form 25 June 2014
Accepted 8 July 2014
Available online xxxx

Keywords:
Obesity
Memory
Hippocampus
Conditioning
Context fear
Reelin
BDNF
Neuroplasticity
Neuroinflammation

ABSTRACT

Both obesity and over-consumption of palatable high fat/high sugar "cafeteria" diets in rats has been shown to induce cognitive deficits in executive function, attention and spatial memory. Adult male Sprague–Dawley rats were fed a diet that supplemented standard lab chow with a range of palatable foods eaten by people for 8 weeks, or regular lab chow. Memory was assessed using a trace fear conditioning procedure, whereby a conditioned stimulus (CS) is presented for 10 s and then 30 s after its termination a foot shock (US) is delivered. We assessed freezing to the CS (flashing light) in a neutral context, and freezing in the context associated with footshock.

A dissociation was observed between levels of freezing in the context and to the CS associated with footshock. Cafeteria diet fed rats froze less than control chow fed rats in the context associated with footshock ($P < 0.01$), indicating that encoding of a hippocampus-dependent context representation was impaired in these rats. Conversely, cafeteria diet fed rats froze more ($P < 0.05$) to the CS than chow fed rats, suggesting that when hippocampal function was compromised the cue was the best predictor of footshock, as contextual information was not encoded.

Dorsal hippocampal mRNA expression of inflammatory and neuroplasticity markers was analysed at the end of the experiment, 10 weeks of diet. Of these, mRNA expression of reelin, which is known to be important in long term potentiation and neuronal plasticity, was significantly reduced in cafeteria diet fed rats ($P = 0.003$). This implicates reductions in hippocampal plasticity in the contextual fear memory deficits seen in the cafeteria diet fed rats.

1. Introduction

People who eat a diet that is rich in saturated fat and refined sugar are likely to become overweight, even obese, and to develop cardiovascular and metabolic disorders. They are also likely to undergo a faster rate of normal age-related cognitive decline and may be more prone to neurodegenerative disorders such as Alzheimer's disease (Moreira, 2013). Obesity and over-consumption of high fat, high energy diet have been shown to induce deficits in executive function, attention, learning and memory. Obesity is associated with structural and functional changes in the brain, including hypothalamic inflammation and gliosis, reduced regional blood flow and hippocampal volume (Fotuhi et al., 2012; Shefer et al., 2013; Thaler et al., 2012). In mice, intake of a diet high in saturated fat and refined sugar has also been found to impact on hippocampal TNF-α expression and activate microglia, indicative of inflammation (Jeon et al., 2012) and also markers of cell death and atrophy (Cherbuin et al., 2012). Furthermore, dietary factors that promote excessive food intake and weight gain have been proposed to interfere with hippocampal dependent learning and memory processes (Davidson et al., 2007, 2005; Kanoski et al., 2007). These memory processes may contribute to food intake in the absence of homeostatic requirements through associative learning mechanisms (Davidson et al., 2009, 2013, 2007; Henderson et al., 2013).

Existing literature suggests that the hippocampus is particularly vulnerable to the effects of high fat and high sugar diets (e.g. Kanoski and Davidson, 2011). Studies have indicated that dietary obese rats fed saturated fat and refined sugars were impaired in the acquisition and retention of hippocampus-dependent spatial memory tasks such as Morris water maze and radial arm maze (Granholm et al., 2008; Greenwood and Winocur, 1990; Kanoski and Davidson, 2010; Molteni et al., 2002) and a non-spatial serial feature negative discrimination task (Davidson et al., 2012).

* Corresponding author. Address: School of Medical Sciences, The University of New South Wales, Sydney, NSW 2052, Australia.
E-mail address: m.morris@unsw.edu.au (M.J. Morris).

http://dx.doi.org/10.1016/j.bbi.2014.07.005
0889-1591/

Furthermore, dietary obese mice showed impairments in both cued and contextual fear conditioning (Yamada-Goto et al., 2012). The hippocampus is important in contextual fear conditioning, allowing a long lasting trace to be encoded and retrieved (Maren and Holt, 2000).

These findings suggest dysfunction of the hippocampus in obese animals, but these deficits may also extend to deficits in amygdala function due to impairments in freezing to a cue predictive of an aversive outcome, which is typically unaffected by hippocampal damage (Maren et al., 1997; Marschner et al., 2008; Phillips and LeDoux, 1992), or poor expression of freezing behaviour in these obese animals.

Studies of aversive Pavlovian conditioning have elucidated separate neurobiological mechanisms support trace and delay conditioning (Raybuck and Lattal, 2011). Trace conditioning is a form of Pavlovian conditioning in which the offset of the conditioned stimulus (CS) and the aversive footshock (unconditioned stimulus – US) are separated by an interval of time that is typically seconds long. The trace interval between the CS and US requires the animal to remember information about the CS during the time gap, and requires an intact hippocampus to maintain this representation (Clark et al., 2001; McEchron and Disterhoft, 1999). In particular, the dorsal hippocampus has been implicated in trace fear conditioning (Pang et al., 2010; Raybuck and Lattal, 2011). During conditioning animals learn an association not just between the CS and US, but between the environment and the US (Marlin and Miller, 1981). Another theory of trace conditioning is that the associations between the context and CS and context and US mediates the association between the CS and US during trace conditioning, thus the duration of the trace interval influences the CS and context information encoded (Odling-Smee, 1975a,b).

In this study we assessed whether access to a high fat, high sugar "cafeteria" diet impacted on the ability to form an association between a CS and US when required to maintain information across a trace interval, and also form a representation of the context in which aversive conditioning occurred. Additionally, we sought to establish molecular markers associated with diet-induced impairments in cognition and neuroplasticity. In particular, we analysed mRNA expression of reelin and BDNF, which are critically important for neuronal survival and plasticity, whose activity may underlie the mechanisms of synaptic plasticity and learning and memory. We also examined the hippocampal mRNA expression of the inflammatory marker tumor necrosis factor (TNF-α), the oxidative stress marker nuclear respiratory factor 1 (NRF1), transcription factor cAMP response element-binding protein (CREB) and glucocorticoid receptor (GR).

2. Methods

2.1. Subjects

Adult male Sprague–Dawley rats ($N = 32$) aged 6 weeks obtained from Animal Resources Centre (Perth, Western Australia) were used as subjects. Rats weighed 230–270 g on arrival and were housed in groups of four in plastic cages (23 cm depth × 21 cm width × 23 cm height) on woodchip bedding, located in a temperature and humidity controlled holding room (Mean temperature 20 ± 2 °C, humidity 50 ± 5%) on a 12-h light:12-h dark cycle (lights on at 07:00 am). Testing was carried out during the light phase of the cycle, between 8:00 am and 1:00 pm. Food and water was available *ad lib* throughout testing. All experimental procedures were approved by the Animal Care and Ethics Committee at the University of New South Wales and in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (revised 1996).

2.2. Diet

Rats were allowed to acclimatise to housing for one week, during which standard lab chow and water was provided and rats were handled daily. Following this acclimatisation, rats were randomly allocated to either standard lab chow (chow diet) or a cafeteria diet (cafeteria diet) condition ($N = 16$ per group). Standard chow provided 11 kJ/g, 12% energy as fat, 23% protein and 65% carbohydrate (Gordon's Specialty Stockfeeds, NSW, Australia).

The cafeteria diet consisted of lab chow supplemented with four solid foods, modeling the palatable high-fat, high sugar, nutrient-poor junk food diet that has had a dramatic impact on obesity rates world-wide in the past decade (Hansen et al., 2004; Martire et al., 2013). Foods included meat pies, assorted cakes and biscuits (e.g., chocolate chip cookies, Oreos, jam roll) changed daily. This diet provided an average of 13.6 kJ/g, 32% energy as fat, 10% protein and 57% carbohydrate, in addition to that provided by the standard laboratory chow. The cafeteria diet was presented daily, at 1:00 pm. The amount consumed was the difference between the weight of the food allocated to a cage and that remaining 24 h later, allowing the calculation of energy intake using the known energy content of each food (kJ/g). Energy intake and body weight were measured three times a week. Rats were exposed to the cafeteria diet for 8 weeks prior to conditioning and testing. Rats began behavioural testing when that had been on the diet for 8 weeks. The trace conditioning procedure took 4 days in total and was carried out in week 9. Rats were sacrificed 1 week following behavioural testing (week 10 of diet) to minimize any physiological effects that the aversive footshock conditioning may have had on the rats consumption.

2.3. Behavioural methods

2.3.1. Apparatus

The experiment was conducted in two sets of four chambers, which differed in dimensions, odour and location. One set of chambers (Context A) served as the conditioning context, measuring 33 cm high × 31 cm long × 26 cm wide. The sidewalls and ceiling of these chambers were made of aluminium and the back and front walls were made of clear plastic. The floor consisted of 5 mm diameter stainless steel rods, spaced 10 mm apart. A tray below the floor contained bedding material (sawdust). The chambers were located in separate compartments of a wooden sound-attenuating cabinet. To give these chambers a distinctive odour, 1 ml lemon essence was added to the cabinet in a glass petri dish.

The second set of conditioning chambers (Context B) served as a neutral context. These chambers differed in dimensions (30 cm high × 30 cm length × 30 cm wide), floor grid (2 mm diameter stainless steel rods, spaced 13 mm apart), material (clear Perspex) and odour (1 ml peppermint essence in a glass petri dish). A tray below the floor contained bedding material (sawdust). The chambers were placed in separate compartments of a sound-attenuating wooden cabinet, identical to that described above.

A light conditioned stimulus (CS; ~57 lux measured at the centre of the chamber) flashing at a rate of 3.5 Hz was presented from a white fluorescent tube on the back wall of each cabinet. A custom-built shock generator, (capable of delivering unscrambled alternating current 50 Hz shock to the floor of each chamber), was used for the presentation of a 0.5 s duration shock at 0.8 mA intensity. The floors of the chambers were cleaned with water after removal of each rat at the end of each session. Infrared cameras mounted on the back wall of each cabinet were used to record behaviour. Cameras were connected to a monitor and a DVD recorder located outside the test room. A computer controlled stimulus presentations via software (MatLab, Mathworks, Natick, MA, USA).

Please cite this article in press as: Reichelt, A.C., et al. Dietary-induced obesity disrupts trace fear conditioning and decreases hippocampal reelin expression. Brain Behav. Immun. (2014), http://dx.doi.org/10.1016/j.bbi.2014.07.005