



## Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin?



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### ARTICLE INFO

#### Article history:

Received 15 March 2013

Revised 31 May 2013

Accepted 21 June 2013

Available online 29 June 2013

#### Keywords:

High-fat diet

Spatial memory

Dendritic spine density

Leptin resistance

### ABSTRACT

Recent evidence has established that consumption of high-fat diets (HFD) is associated with deficits in hippocampus-dependent memory. Adolescence is an important period for shaping learning and memory acquisition that could be particularly sensitive to the detrimental effects of HFD. In the current study we have administered this kind of diets to both adolescent (5-week old) and young adult (8-week old) male C57BL mice during 8 weeks and we have evaluated its effect on (i) spatial memory performance in the novel location recognition (NLR) paradigm, and (ii) spine density and neural cell adhesion molecule (NCAM) expression in hippocampal CA1 pyramidal neurons. In order to characterize the eventual involvement of central leptin receptors we have also investigated the functionality of leptin receptors within the hippocampus. Here we report that animals that started to consume HFD during the adolescence were less efficient than their control counterparts in performing spatial memory tasks. In contrast to that, mice that were submitted to HFD during the young adult period displayed intact performance in the NLR test. In mice receiving HFD from the adolescence, the behavioral impairment was accompanied by an increase of dendritic spine density in CA1 pyramidal neurons that correlated with the up-regulation of neural cell adhesion molecule (NCAM) in this area. Deficits in spatial memory occurred concomitantly with a desensitization of the protein kinase B (Akt) pathway coupled to hippocampal leptin receptors. In contrast, the STAT3 pathway remained unaffected by HFD. All effects of HFD were long-lasting because they remained intact even after 5 weeks of food restriction. Our results provide further evidence of the susceptibility of the hippocampus to HFD in adolescent individuals and suggest that leptin signaling integrity in this brain area is pivotal for memory performance.

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### 1. Introduction

The problem of childhood and adolescent obesity has become a major challenge for public health in westernized societies and some epidemiological studies indicate that obese child/adolescents have increased chances (until 80%) of becoming obese adults (Shield & Summerbell, 2008). In addition to a well-defined risk of cardiometabolic disease, osteoarthritis and different types of cancer (Ma, Ko, & Chan, 2008), obesity has been suggested to cause deficits in learning and memory process (Nilson & Nilson, 2009; Stanek, 2011). Several studies have evidenced that nutritional imbalance accounts for cognitive deficits in humans and rodents (Fanjiang & Kleinman, 2007; Kanoski, Meisel, Mullins, & Davidson,

2007). Recent research has evidenced that high-fat diets (HFD) are deleterious for hippocampus structure and function (Greenwood & Winocur, 2005) and also for synaptic plasticity and neurogenesis (Lindqvist et al., 2006). Previous studies carried out in our laboratory and others have demonstrated that HFD impair hippocampal-dependent learning and memory processes (Boitard et al., 2012; Valladolid-Acebes et al., 2011), together with the impairment of hippocampal glutamatergic neurotransmission (Valladolid-Acebes et al., 2012).

An interesting issue related with the long-term effects of HFD on learning and memory deals with the life period when obesity starts. Thus, exposure to maternal HFD has been shown to program offspring for increased risk of obesity (Zito, Vickers, & Roberts, 1985) and learning deficits during the adulthood (Rodriguez et al., 2012). The molecular mechanisms that account for these disorders remain to be elucidated, but recent research in this field strongly suggests that both perinatal and neonatal overnutrition trigger inflammatory responses within the central nervous system.

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This event has been demonstrated to be integral to obesity pathophysiology (Rother et al., 2012; Sharpe, Pilotte, Mitchell, & De Souza, 1991; Tapia-Gonzalez et al., 2011) and might account for learning deficits.

The link between obesity and cognitive dysfunction in humans remains poorly characterized and comprehensive epidemiological studies are mandatory to better identify the eventual co-morbidity between obesity and learning deficits (Sellbom & Gunstad, 2012). Otherwise, basic aspects concerning (i) the neural mechanisms altered by obesogenic diets and leading to learning/memory impairment, and (ii) the age at which learning circuits are more sensitive to this kind of diets remain unexplored.

To address this issue we have investigated the influence of HFD, given either to adolescent (5-week old) or young adult (8-week old) mice, on learning performance and hippocampus morphology. In order to explore eventual changes of synaptic connectivity, we have analyzed the morphology of CA1 neurons by quantifying dendritic spine density in these cells as well as the density of neural cell adhesion molecule (NCAM). Because the most prominent endocrine change triggered by short-term HFD is hyperleptinemia, and leptin has been shown to drive synaptic plasticity within the hippocampus (Irving, Wallace, Durakoglugil, & Harvey, 2006), we have also investigated the effect of these diets on leptin receptor responsiveness in this brain area.

## 2. Material and methods

### 2.1. Dietary treatment

#### 2.1.1. Experiment 1

Five-week old C57BL/6J male mice (CRIFA, Barcelona, Spain) weighing 16–18 g were housed under 12-h light/12-h dark cycle, in a temperature-controlled room (22 °C). The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by European Communities Council Directive (86/609/EEC) and it was approved by the Ethics Committee of the Universidad CEU-San Pablo (SAF2011-25300). Animals were divided in two groups ( $n = 12$  per group) with similar average body weight (BW), housed four per cage, and assigned (free access) either to a standard chow or to a high-fat diet (HFD, D12451, 45% kcal from fat, 35% kcal from carbohydrates, and 20% kcal protein; 4.73 kcal/g; Test Diet Limited BCM IPS Ltd, UK) for 8 weeks. BW and food intake were monitored once a week. These groups will be referred as *Control ad libitum* 5 + 8 (*Control-al<sub>5+8</sub>*) and *HFD ad libitum* 5 + 8 (*HFD-al<sub>5+8</sub>*), respectively.

#### 2.1.2. Experiment 2

In this assay, 5-week old animals received the same treatment than mice in experiment 1 but, after 8-week HFD, mice were food-restricted (FR) during five additional weeks. During food restriction, mice ( $n = 12$  per group) were maintained with the same diet but only received 70% chow consumed during the last free-feeding day ( $3.10 \pm 0.02$  g/day for control animals and  $2.62 \pm 0.02$  g/day for HFD animals) and were maintained under these conditions until the final of the study. BW of FR mice was stabilized around 90% BW raised after 8-week dietary treatment. These groups will be referred as *Control-FR<sub>5+8+5</sub>* and *HFD-FR<sub>5+8+5</sub>*. This assay was designed in order to analyze the influence of caloric content vs. diet composition in our experimental paradigm.

#### 2.1.3. Experiment 3

In this case, 8-week old mice (8-week old;  $n = 15$  per group) were given free-access to HFD during 8 weeks. These groups will be referred as *Control-al<sub>8+8</sub>* and *HFD-al<sub>8+8</sub>*.

### 2.2. Novel object location recognition protocol

Experiments were carried out in an adapted version for mice of the novel location recognition (NLR) task used by Barker, Bird, Alexander, and Warburton (2007). The hippocampus-dependent object-location memory task exploits the observation that mice prefer to explore an object when its relative position in a box is changed vs. a previous experience (Save, Poucet, Foreman, & Buhot, 1992). The NLR test was chosen because it is specifically hippocampal-dependent (Barker et al., 2007) and also because this test has been shown to be less stressful than other experimental paradigms used to evaluate learning performance (Lucas, Chen, & Richter-Levin, 2013). Assays were carried out in a black open-field box made of wood (25 cm long  $\times$  25 cm wide  $\times$  25 cm high). The stimuli presented were identical copies of objects composed of Lego pieces (Lego UK, Slough, UK) and heavy enough to avoid displacement during testing. Animals did not receive any external cues during testing. The test was organized in three sessions: (i) During the “exploration session” animals were allowed (10 min) to freely explore the box that contained two objects, each placed 5 cm from top left and right corners. One of them was presented always in the same position and the other one was presented in changing positions. These objects will be referred from now as familiar (F) and novel (N) objects, respectively. Animals were always introduced in the box with one's back to the object and were returned to their home cage after the session. (ii) Two “retention sessions” were performed 1- and 24-h after the “exploration session”. In this case animals were allowed to re-explore the cage during 5 min. During the first retention session (1 h after exploration session), object F remained in identical position than in the “exploration session” but object N was presented 5 cm from the bottom right corner. (iii) In the second retention session (24 h after the first retention session), animals were allowed to explore the box during a 5-min period, but N was now presented 5 cm from the bottom left corner.

Mice activity was registered and analyzed by a video tracking system (Smart 2.5.21 Polivalent VTS, Panlab Harvard Apparatus, USA). In all sessions, the time interacting with objects (IT) and the number of interactions (IN) with each object were quantified. A single interaction was counted when the animal approached at least 2 cm to the object. Discrimination ratios, ITDr and INDr, were calculated as  $ITDr = IT_N / IT_{N+F}$  and  $INDr = IN_N / IN_{N+F}$ , respectively. We considered that a spatial discrimination was consistent when both ITDr and INDr were  $>0.5$  in the retention sessions. Animals showing preference for any location during the exploration session were eliminated. The final number of animals per group was  $n = 12$  (*Control-al<sub>5+8</sub>*, *HFD-al<sub>5+8</sub>* and *HFD-FR<sub>5+8+5</sub>* groups),  $n = 8$  (*Control-FR<sub>5+8+5</sub>*),  $n = 10$  (*Control-al<sub>8+8</sub>*) and  $n = 15$  (*HFD-al<sub>8+8</sub>*).

### 2.3. Plasma biochemistry

Plasma biochemistry was analyzed to assess the influence of the dietary treatment on plasma leptin and to calculate HOMA indexes. Groups of animals undergoing identical dietary treatment than the above mentioned were decapitated at 12 pm, after 4 h fasting, and their plasmas assayed for glucose (Glucose Trinder method; Roche, Barcelona, Spain), insulin (EIA, Mercodia, Uppsala, Sweden) and leptin (RIA, Linco Research, St. Charles, MO).  $N = 8$  (*Control-al<sub>5+8</sub>* and *HFD-al<sub>5+8</sub>* groups),  $n = 12$  (*Control-FR<sub>5+8+5</sub>* and *HFD-FR<sub>5+8+5</sub>* groups),  $n = 5$  (*Control-al<sub>8+8</sub>*), and  $n = 7$  (*HFD-al<sub>8+8</sub>*).

### 2.4. Dendritic spine histochemistry

After behavior assays, four animals of each experimental group (chosen randomly) were perfused transcardially with 4% paraformaldehyde and then hippocampi dissected and prepared for silver staining (Arellano, Benavides-Piccione, De Felipe, & Yuste, 2007;

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