Neuroscience 297 (2015) 170–181

NEUROCHEMICAL AND ELECTROPHYSIOLOGICAL DEFICITS IN THE VENTRAL HIPPOCAMPUS AND SELECTIVE BEHAVIORAL ALTERATIONS CAUSED BY HIGH-FAT DIET IN FEMALE C57BL/6 MICE

S. KRISHNA,[a] M. M. KERALAPURATH,[a] Z. LIN,[a] J. J. WAGNER,[a] C. B. DE LA SERRE,[b] D. A. HARN[c] AND N. M. FILIPOV[a]*

[a] *Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA*

[b] *Department of Foods and Nutrition, College of Family and Consumer Sciences, University of Georgia, Athens, GA 30602, USA*

[c] *Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA*

Abstract—Mounting experimental evidence, predominantly from male rodents, demonstrates that high-fat diet (HFD) consumption and ensuing obesity are detrimental to the brain. To shed additional light on the neurological consequences of HFD consumption in female rodents and to determine the relatively early impact of HFD in the likely continuum of neurological dysfunction in the context of chronic HFD intake, this study investigated effects of HFD feeding for up to 12 weeks on selected behavioral, neurochemical, and electrophysiological parameters in adult female C57BL/6 mice; particular focus was placed on the ventral hippocampus (vHIP). Selected locomotor, emotional and cognitive functions were evaluated using behavioral tests after 5 weeks on HFD or control (low-fat diet) diets. One week later, mice were sacrificed and brain regional neurochemical (monoamine) analysis was performed. Behaviorally naïve mice were maintained on their respective diets for an additional 5–6 weeks at which time synaptic plasticity was determined in ex vivo slices from the vHIP. HFD-fed female mice exhibited increased: (i) locomotor activity in the open field testing, (ii) mean turn time on the pole test, (iii) swimming time in the forced swim test, and (iv) number of marbles buried in the marble burying test. In contrast, the novel object recognition memory was unaffected. Mice on HFD also had decreased norepinephrine and dopamine turnover, respectively, in the prefrontal cortex and the vHIP. HFD consumption for a total of 11–12 weeks altered vHIP synaptic plasticity, evidenced by significant reductions in the paired-pulse ratio and long-term potentiation (LTP) magnitude. In summary, in female mice, HFD intake for several weeks induced multiple behavioral alterations of mainly anxiety-like nature and impaired monoamine pathways in a brain region-specific manner, suggesting that in the female, certain behavioral domains (anxiety) and associated brain regions, i.e., the vHIP, are preferentially targeted by HFD.

Key words: high-fat diet (HFD), anxiety, dopamine, ventral hippocampus (vHIP), long-term potentiation (LTP).

INTRODUCTION

Overweight and obesity affect all societal segments, irrespective of age, gender and ethnicity (WHO, 2000; Wang and Beydoun, 2007). Besides genetics, which play an important, but a minor role, several environmental factors, with high-fat consumption being a major one, drive the obesity epidemic (Swinburn et al., 2004). Worldwide prevalence of overweight and obesity in adults is increasing in both genders (Stevens et al., 2012). Importantly, obesity prevalence in adult American females from certain ethnic groups, i.e., African and Mexican Americans, is higher than in their male counterparts (Terrell, 2002; Kanter and Caballero, 2012).

Epidemiological and animal data have demonstrated emotional disturbances, such as anxiety and/or depression, associated with high-fat consumption and subsequent weight gain/obesity (Petry et al., 2008; Gadalla, 2009; Mizunoya et al., 2013). As it is the case with research in the biomedical field in general, including in neuroscience (Beery and Zucker, 2011), male subjects in obesity-related laboratory animal research are overrepresented, i.e., (Del Rosario et al., 2012; Cone et al., 2013); information on adverse neurological effects of high-fat diet (HFD) consumption and HFD-induced obesity in females is available, i.e., (Sato et al., 2010; Balasubramanian et al., 2012), but, compared to available data in males, is rather limited. Besides using mostly males, the majority of animal studies investigating the neurological effects of HFD have done so in advanced obese phenotypes induced by long-term (6–12 months)

*Corresponding author. Address: Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, 501 D.W. Brooks Drive, Athens, GA 30602, USA. Tel: +1-706-542-3014; fax: +1-706-542-3015.
E-mail address: filipov@uga.edu (N. M. Filipov).

Abbreviations: 3-MT, 3-methoxytyramine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; DA, dopamine; dHIP, dorsal hippocampus; DOPAC, 3,4-dihydroxyphenylacetic acid; EPM, elevated plus maze; fEPSPs, field excitatory post-synaptic potentials; FST, forced swim test; HFD, high-fat diet; HFS, High-frequency tetanic stimulation; HVA, homovanillic acid; LFD, low-fat diet; LTP, long-term potentiation; MBT, marble burying test; MHPG, 3-methoxy-4-hydroxyphenylglycol; N, Newton; NAc, nucleus accumbens; NE, norepinephrine; NPIs, novelty preference indices; NOR, novel object recognition test; PFC, prefrontal cortex; PPR, paired-pulse ratio; vHIP, ventral hippocampus.

http://dx.doi.org/10.1016/j.neuroscience.2015.03.068

HFD consumption (Hwang et al., 2010; Heyward et al., 2012; Karimi et al., 2013); data regarding the relatively early impact of HFD in the likely continuum of neurological deficits/dysfunctions in the face of continued HFD feeding are scarce.

HFD consumption alters brain neurochemistry in a region-specific manner (Molteni et al., 2004; Sharma et al., 2013) and this alteration might be partially responsible for the HFD-induced behavioral impairments. For example, chronic (3 months) HFD intake alters striatal and mesolimbic dopamine (DA) signaling in rodents (Davis et al., 2008; Sharma and Fulton, 2013). Besides DA, the use of selective serotonin (5-HT) and norepinephrine (NE) reuptake inhibitors as anti-obese/anti-depressant agents demonstrates their important roles in regulating obesity and associated behavioral deficits (Chudasama and Bhatt, 2009).

HFD and other palatable foods also affect the hypothalamus, the prefrontal cortex (PFC) and the hippocampus (Heyward et al., 2012; Rojo et al., 2013), with hypothalamic DA playing a key role in regulating food intake (Meguid et al., 2000). Several animal studies have reported HFD-induced hypothalamic DA dysfunction, characterized by decreased DA turnover (Levin et al., 1986), increased D4 receptor mRNA expression (Huang et al., 2005) and upregulation of genes involved in the synthesis and release of DA (Lee et al., 2010). While HFD's effects on the hypothalamus are mainly related to food intake regulation, HFD consumption also targets the hippocampus and the PFC and it affects emotional and cognitive functions. Thus, in male rodents chronically fed HFD, compromised hippocampal synaptic plasticity and cognitive deficits are observed (Stranahan et al., 2008; Valladolid-Acebes et al., 2012; Karimi et al., 2013); however, female data are limited. Besides cognition, the hippocampus also modulates anxiety and preferential roles for the dorsal hippocampus (dHIP) in spatial memory and the ventral hippocampus (vHIP) in anxiety-related behaviors have been suggested (Bannerman et al., 2004; Fanselow and Dong, 2010).

In this study, we aimed to investigate the early neurological effects caused by relatively short-term HFD intake in female C57BL/6 mice using behavioral, neurochemical and electrophysiological measures. The C57BL/6 mouse strain is the most widely used strain in neuroscience research (Kalueff and Nguyen, 2014). Importantly, compared to other strains of mice, such as SWR/J, C57BL/6 is a good model for studying human obesity as it simulates the human metabolic abnormalities (hyperinsulinemia, hyperglycemia, and hypertension) when fed *ad libitum* with a HFD, while not showing metabolic irregularities when fed a low-fat diet (LFD; Collins et al., 2004). Behavioral tests were conducted to evaluate the effects of HFD on: (i) motor function (open field, pole and grip strength), (ii) emotional disturbances, such as anxiety or depression (marble burying and forced swim) and (iii) cognitive changes (novel object recognition (NOR)). To investigate the HFD-induced neurochemical alterations underlying the behavioral changes (if any), levels of DA, 5-HT, NE and their metabolites were determined in multiple brain regions, namely PFC, nucleus accumbens (NAc), striatum, dHIP and vHIP that are implicated in the regulation of locomotor, emotional and/or cognitive functions. The two hippocampal zones (dorsal and ventral) were analyzed separately due to their distinct roles in memory and anxiety (Bannerman et al., 2004; Fanselow and Dong, 2010). Additionally, based on the neurochemical and behavioral data gathered after 5–6 weeks on HFD, synaptic function was evaluated in the vHIP after additional 5–6 weeks of HFD consumption.

EXPERIMENTAL PROCEDURES

Animals

Young adult female C57BL/6 mice (6–7 weeks old, Harlan, Indianapolis, IN, USA) were housed (4–5 per cage) in an environmentally controlled room (22–24 °C) with food and water available *ad libitum* on a 12-h light/dark cycle in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility throughout the study. All procedures were in accord with the latest National Institutes of Health (NIH) guidelines and were approved in advance by the Institutional Animal Care and Use Committee (IACUC) of the University of Georgia.

Animal treatment

Mice weighing 16.0 ± 0.20 g (mean ± SEM) were randomly divided into two groups (n = 13–14 per group) and placed on either a low-fat diet (LFD; 10% kcal from fat, D12450J, Research Diets, Inc., New Brunswick, NJ, USA) or a high-fat diet (HFD; 60% kcal from fat, D12492, Research Diets) that are micronutrients and simple sugar (sucrose) balanced for the entire duration of the study. Body weight was recorded weekly; food and water intakes were recorded twice weekly. Subset of the mice (n = 8 per treatment group) were subjected to behavioral tests after 5 weeks on LFD/HFD; a week later (6 weeks on diets, 48 h after the last behavioral test), the estrus cycle stage of these mice was determined prior to sacrifice, brains were harvested, weighed, and quickly frozen at −80 °C for neurochemical analysis. The remaining behaviorally naïve mice (n = 5–6 per treatment group) were maintained on their respective diets for 11–12 weeks; at that time, after an estrus cycle staging, brains were harvested and ex vivo hippocampal slices prepared for electrophysiology. The experimental design is depicted in Fig. 1.

Behavioral tests

Behavioral tests were performed in succession over 3 days (Fig. 1a) as we have reported before (Lin et al., 2014). Animals were naïve to the testing ambience prior to testing initiation and all tests were performed by a treatment-blinded experimenter in a specially equipped behavioral testing room separate from the one where the mice were housed.

Open field. Mouse activity was monitored for a period of 30 min in an open field arena (25 cm × 25 cm × 40 cm;