Neuroscience 326 (2016) 170–179

REMOVAL OF HIGH-FAT DIET AFTER CHRONIC EXPOSURE DRIVES BINGE BEHAVIOR AND DOPAMINERGIC DYSREGULATION IN FEMALE MICE

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Abstract—A significant contributor to the obesity epidemic is the overconsumption of highly palatable, energy dense foods. Chronic intake of palatable foods is associated with neuroadaptations within the mesocorticolimbic dopamine system adaptations which may lead to behavioral changes, such as overconsumption or bingeing. We examined behavioral and molecular outcomes in mice that were given chronic exposure to a high-fat diet (HFD; 12 weeks), with the onset of the diet either in adolescence or adulthood. To examine whether observed effects could be reversed upon removal of the HFD, animals were also studied 4 weeks after a return to chow feeding. Most notably, female mice, particularly those exposed to HFD starting in adolescence, demonstrated the emergence of binge-like behavior when given restricted access to a palatable food. Further, changes in dopamine-related gene expression and dopamine content in the prefrontal cortex were observed. Some of these HFD-driven phenotypes reversed upon removal of the diet, whereas others were initiated by removal of the diet. These findings have implications for obesity management and interventions, as both pharmacological and behavioral therapies are often combined with dietary interventions (e.g., reduction in calorie dense foods).

Key words: dopamine, high-fat diet, sex difference, reward, binge, adolescence.

INTRODUCTION

Multiple factors interact to drive obesity risk, including physical activity, genetics, stress and food consumption, specifically the overconsumption of readily available, energy dense, palatable foods (Marcus and Wildes, 2014). Palatable foods are rewarding and acute consumption leads to the release of the neurotransmitter dopamine (DA) (Geiger et al., 2009). Animal models of diet-induced obesity (DIO) involving the chronic consumption of palatable foods have documented DA adaptations in response to chronic consumption of a high-fat diet (HFD). Unlimited access to HFD causes lower basal extracellular dopamine levels in the nucleus accumbens (NAc) and the ventral tegmental area (VTA) (Geiger et al., 2007; Geiger et al., 2009; Cone et al., 2010; Rada et al., 2010). DIO models also demonstrate lower DA turnover (Davis et al., 2008), decreased DA release (York et al. 2010), and reduced DA clearance (Speed et al., 2011) in the NAc. Dopamine-related molecules, including tyrosine hydroxylase, the DA transporter, and the DA receptors DR1 and DR2, are all decreased in DIO animal models at the level of mRNA (Huang et al., 2005; Alsiö et al., 2010; Carlin et al., 2013) and protein (Huang et al., 2006; South and Huang, 2008; Johnson and Kenny, 2010). However, nearly all of the published rodent studies have been conducted exclusively in adult male rodents, leaving open the question of whether these effects extend more broadly to females and/or other developmental ages. This is significant as women exhibit higher rates of obesity, including as adolescents (Wang and Beydoun, 2007), highlighting the importance of studying females' DA response to palatable foods.

Important developmental differences in response to HFD consumption have also been observed. The majority of DIO rodent studies begin exposure to the diet in adulthood (Baladi and France, 2009; Kanoski and Davidson, 2010; Lassiter et al., 2010), and exposures during earlier developmental times are relatively understudied. During critical periods of development, such as lactation through adolescence, the brain is particularly sensitive to environmental changes (King, 2006). For example, high fat feeding was sufficient to disrupt prepulse inhibition (PPI) when given during the peripubertal period, yet high fat feeding during adulthood failed to affect PPI (Labouesse et al., 2013). Similarly, HFD consumption during adolescence, but not in adulthood, disrupts hippocampal neurogenesis and memory (Boitard

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Abbreviations: BED, binge-eating disorder; DIO, diet-induced obesity; EDTA, ethylenediaminetetraacetic acid; HFD, high-fat diet; NAc, nucleus accumbens; PPI, prepulse inhibition; VTA, ventral tegmental area.

http://dx.doi.org/10.1016/j.neuroscience.2016.04.002
0306-4522/

et al., 2012), a pattern which is also observed in response to high-fructose corn syrup consumption (e.g., memory impairment and hippocampal inflammation with adolescent exposure, but not adult exposure, (Hsu et al., 2014)). However, the impact of adolescent HFD exposure on the DA system is unknown.

Reduced levels of DA in the synapse or reduced DA signaling, as suggested by the current literature, could subsequently reduce the sensitivity of the animal to natural rewards. Alterations in dopaminergic circuitry after high fat intake have been associated with decreases in sucrose preference (Vucetic et al., 2012; Carlin et al., 2013), a decrease in operant responding for a sucrose pellet (Davis et al., 2008), and a decrease in response to food reward (Cottone et al., 2008; Johnson and Kenny, 2010; Corwin et al., 2011). These adaptations can lead to an increase in compulsive behaviors, including the overconsumption of food or drugs of abuse (Volkow and Li, 2004; Verbeken et al., 2012). Binge eating involves uncontrolled food intake, typically of highly palatable food, and is a component of eating disorders such as bulimia nervosa and binge-eating disorder. Binge eating affects both sexes but is more common in women (Hudson et al., 2007), a finding replicated in animal models, with binge-eating behavior seen more frequently in female rats than in males across a number of different experimental paradigms (Klump et al., 2013). Consistent with its role in hedonic feeding, dopamine dysregulation has been linked to binge eating. For example, dopamine neurons in the VTA were shown to be activated by binge-like intake of HFD in mice (Valdivia et al., 2015), while in human patients with binge-eating disorder (BED), food image-evoked dopamine levels in the caudate were shown to be increased only in BED patients, and correlated with binge-eating scores (Wang et al., 2011). Additional studies in both rodents and humans have been reviewed (Broft et al., 2011; van Gestel et al., 2014; Naef et al., 2015). Therefore, we examined whether the age of onset of the HFD consumption or sex could influence the development of binge behavior.

A final important question is whether these dopamine-related neuroadaptations persist upon the removal of the HFD. Diet reversal studies involve a return to normal chow after an extended period of HFD consumption. Following relatively short periods of diet reversal (1–2 weeks), reports have found a persistence of HFD-driven neuroadaptations (South and Huang, 2008; Alsiö et al., 2010; Johnson and Kenny 2010). However, a number of recent reports have found a normalization of diverse HFD-driven phenotypes, including hypothalamic inflammation (Berkseth et al., 2014), memory impairments and IL-1ß levels in the hippocampus (Sobesky et al., 2014), and dopamine-related changes (Carlin et al., 2013) using a longer reversal period (4 weeks). The following experiments were designed to test the hypothesis that beginning a HFD earlier in life would result in greater and more lasting changes in the dopamine reward system. Understanding which neurobiological changes reverse or persist after HFD removal may inform the high failure rates of human dieting and relapse to unhealthy eating habits.

EXPERIMENTAL PROCEDURES

Animals and experimental model

C57BL/6J females were bred with DBA/2J males (The Jackson Laboratory, Bar Harbor, ME, USA) and were fed control diet (Test Diet, Richmond, IN, USA #5755; 18.5% protein, 12% fat, 69.5% carbohydrate) throughout pregnancy and lactation. Offspring were weaned at 3 weeks of age and a third of the animals were placed on HFD (Test Diet, #58G9; 18% protein, 60% fat, and 20.5% carbohydrate). Another third of the animals were placed on HFD at 6 weeks of age. The final third were kept on standard chow. Animals remained on the HFD for 12 weeks. In an additional cohort of animals, after 12-week *ad lib* access to the HFD, the diet was returned to control chow for 4 weeks. Separate cohorts of animals were used for the behavioral studies and the molecular studies, and animals were group housed (5 mice/cage), except where noted for behavioral experiments. Body weights were recorded weekly, and both male (n = 5–10/experiment) and female (n = 5–10/experiment) mice were used for the behavioral experiments. Given that binge behavior was only observed in females, experiments to measure dopamine and dopamine-related gene expression changes were conducted in female mice. The Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania approved all procedures.

Fat pad weights

Animals (n = 5–10/group) were euthanized with an overdose of carbon dioxide, followed by cervical dislocation; a method recommended by the Panel on Euthanasia of the American Veterinary Medical Association. Body weights were taken and abdominal, gonadal, and inguinal fat pads were removed and weighed separately. Fat pad mass was normalized to body weight.

Sucrose preference

After 12-week exposure to HFD or HFD + 4-week chow, mice were individually housed in standard cages for 3 days with *ad lib* access to food. In a two-bottle choice task, one bottle was filled with 200 ml of 4% sucrose solution (w/v), another with 200 ml of tap water. Sucrose (ml), water (ml), and food consumption (g), were measured daily. Preference was calculated using the measurements from the last 2 days only as follows: preference % = [(sucrose consumption/sucrose + water consumption) × 100] (n = 8–10/group).

One-hour palatable food intake

After 12–14 weeks on the HFD or control diet, mice were individually housed (n = 11–12/group) in standard cages for eight days and maintained on their respective *ad libitum* diets (chow for all groups except the HFD-fed animals). Each day, home cage food intake and body weight were measured. Animals were given access to a