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Research report

Extended exposure to a palatable cafeteria diet alters gene expression in brain regions implicated in reward, and withdrawal from this diet alters gene expression in brain regions associated with stress

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HIGHLIGHTS

- A cafeteria diet reduced μ-opioid and cannabinoid-1 receptor mRNA in the VTA.
- A cafeteria diet elevated amygdala and hypothalamic marker of stress.
- Hippocampal BDNF mRNA was suppressed early in the development of obesity.
- Switching obese rats to chow for 48 h resulted in activation of the HPA axis.
- Chronically chow-fed rats switched to cafeteria had reduced amygdala GR expression.

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ABSTRACT

Like people, rodents exposed to energy-rich foods over-eat and become overweight. Removal of this diet activates stress systems, which may explain why people have difficulty dieting. We exposed rats to energy-rich foods in order to identify changes in the brain induced by that diet and by its removal. Sprague Dawley rats were fed lab-chow or an energy-rich cafeteria diet (plus chow). Following 6 or 15 weeks, half of each group was switched to the opposing diet. Rats were culled 48-h later. We measured fat mass, plasma hormones, and assessed brains for mRNA expression of several genes. Cafeteria-fed rats consumed more kilojoules, weighed more and had elevated leptin (plus reduced CORT at 15 weeks) relative to chow-fed rats. Fifteen weeks of cafeteria diet suppressed μ-opioid and CB1 receptor mRNA in the VTA, but elevated amygdala GR, and 6 weeks of cafeteria diet reduced BDNF, compared to chow-fed rats. Rats switched to the cafeteria diet ate similar amounts as rats maintained on the diet, and switching to cafeteria diet after 15 weeks reduced amygdala GR expression. Rats switched to chow ate less than rats maintained on chow, and switching to chow following 15 weeks of cafeteria diet increased hypothalamic CRH mRNA. Therefore, 15 weeks of cafeteria diet produced changes in brain regions implicated in reward processes. Switching these rats to chow activated the HPA axis, while switching chow-fed rats to the cafeteria diet decreased GR expression in the amygdala, a region associated with stress. These findings have implications for dieting in humans.

1. Introduction

The modern diet contains many foods that are rich in sugar and fat. This diet is one of the factors that has contributed to the rapid increase in obesity over the past thirty years [1,2]. Energy-rich foods are consumed not only because of their nutrient value but also because they are hedonically pleasant [3]. Such foods therefore provoke ingestion by engaging reward processes in the limbic system that override the control over ingestion normally exerted by metabolic processes [4–7]. These reward processes involve dopamine, opioids and cannabinoids [3,8–11]. For example, palatable food intake causes dopamine release from cells that originate in the ventral tegmental area (VTA) [12], and systemic injection of a μ-opioid antagonist prevents the stimulatory effect

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of palatable food on dopamine release in the nucleus accumbens (NAc) shell [13]. Moreover, μ-opioid agonists stimulate feeding when injected into the VTA of rats [14], an effect that is blocked in animals pre-treated with opioid antagonists such as naltrexone [15]. With respect to cannabinoids, evidence suggests that the cannabinoid-1 (CB1) receptor has a role in the rewarding aspects of eating [10,11]. Peripheral injection of a CB1 antagonist reduces intake of palatable sugar fat whip (canned cream) in rats [16]. Further, CB1 agonist-induced feeding can be blocked by a CB1 or general opioid antagonist, suggesting that stimulation of CB1 receptors may facilitate activation of opioid pathways [17].

The hedonic and/or rewarding aspects of eating may explain why people find it difficult to control their intake of energy-rich foods when trying to lose weight [6,18–20]. In fact, bariatric surgery apart, there is currently no known weight-loss treatment that is effective in the long term [21]. One reason that weight-loss treatments are seldom successful in the long-term is because refraining from eating energy-rich foods by dieting results in negative mood and activation of stress systems [22,23]. Extended intermittent (i.e., restricted) access to palatable foods (e.g., high-fat chow; sucrose; chocolate) in rodents is associated with increased anxiety and activation of stress circuitry, including the hypothalamo-pituitary-adrenal (HPA) axis [24–29]. Critically, brain mediators of stress, such as corticotrophin-releasing hormone (CRH) and glucocorticoids, are up-regulated (i.e., increased receptor expression) during palatable diet withdrawal in regions such as the hypothalamus, amygdala and hippocampus [23,24,30–32]. Therefore, the 'pleasure' produced by eating energy-rich foods together with the stress produced by refraining from eating them when dieting may in part explain why weight loss treatments are often unsuccessful. Brain-derived neurotrophic factor (BDNF) has also been linked to stress. For example, hippocampal BDNF mRNA levels were increased following 60 min of immobilization stress but decreased following 180 min [33]. However, whether BDNF regulates the effects of palatable diet withdrawal remains unknown.

In the present study, we provided rats with many of the energy rich foods eaten by people. Rats eat these foods (e.g., meat pies, cakes) to excess and become overweight, even obese [34,35]. Rats in one cohort were exposed to this cafeteria style diet or standard chow for 6 weeks and those in a second cohort were exposed to the cafeteria diet or chow for 15 weeks. After 6 or 15 weeks, half of the rats in the cafeteria diet conditions remained on this diet, while the remaining rats were switched to chow for 48 h in order to mimic the withdrawal of energy-dense foods in people trying to lose weight. Similarly, after 6 or 15 weeks, half of the rats in the chow condition remained on this diet while the remaining rats were switched to the cafeteria diet for 48 h to determine the acute effects of this diet. We measured the impact of the switch following 6 or 15 weeks on fat mass, plasma hormones and expression of several genes of interest. In light of evidence implicating reward processes in the overconsumption of palatable foods, we measured dopamine D1 and D2 receptors, μ-opioid receptors, and CB1 receptors in the VTA. Moreover, given the known stress-inducing effects of diet withdrawal [30,31], we examined CRH expression in the dorsal hypothalamus (DH) and amygdala, as well as glucocorticoid receptor (GR) expression in the amygdala and hippocampus. Hippocampal BDNF was also measured as it plays a role in mediating stress, and is regulated by diet [33,36,37].

2. Methods

2.1. Subjects

The subjects were 72 experimentally naïve male Sprague Dawley rats, obtained from the Animal Resource Centre (Perth, Australia), aged 6–8 weeks and weighing 240-280 g upon arrival. They were housed in plastic boxes (22 cm height × 65 cm length × 40 cm width) in a climate controlled room (22 °C) on a 12 h (7.00 a.m.–7.00 p.m.) light/dark cycle. The 15 weeks (n = 24) cohort was housed two rats per cage; the 6 weeks (n = 48) was housed four rats per cage until the beginning of week 6 (due to space limitations in the colony room), and two per cage for the remainder of the experiment. Experiments were approved by the Animal Care and Ethics Committee of the University of New South Wales and were in accordance with the guidelines provided by the Australian National Health and Medical Research Council.

2.2. Diet

After a week's acclimatization, where standard lab chow and water were provided and rats were handled daily, rats were randomly allocated to either standard lab chow or a cafeteria diet. Standard chow provided 11 kJ/g, 12% energy as fat, 20% protein and 65% carbohydrate (Gordon's Specialty Stockfeeds, NSW, Australia). The cafeteria diet consisted in 'high-fat' chow [i.e., standard chow (crushed) supplemented with pork lard and condensed milk] as well as standard chow, plus ready to eat foods obtained from a local supermarket. These commercial foods were high in protein and/or carbohydrates [meat pies, meat wrapped in rice paper (dim sims), oats] or high in fat/sugar (cakes and biscuits). Two foods high in protein/carbohydrates and two high in fat/sugar were always available and changed daily. This cafeteria style diet provided an average of 15.3 kJ/g, 32% energy as fat, 14% protein and 60% carbohydrate. Food was placed inside the home cages, cafeteria diet being presented fresh daily, at 5.00 p.m. Energy intake and body weight were measured once per week. The same five foods were used at each of the weekly energy measurements. These foods were measured at 5.00 p.m. and the amount remaining again measured 24 h later. Energy intake was calculated using the known energy content of each food (kJ/g).

2.3. Diet switch

Rats were placed on standard chow or cafeteria diet for 15 weeks (n = 12 per condition). At the start of week 16, half the rats on the cafeteria diet were switched to chow (Caf-Ch) and half remained on the cafeteria diet (Caf-Caf). Likewise, half the rats on the chow diet (Ch) were switched to the cafeteria (Caf) diet (Ch-Caf) and half remained on chow (Ch-Ch). A separate cohort of rats were placed on standard chow or cafeteria diet for 6 weeks (n = 24), and at the start of week 7, half of the rats in each condition were switched to the other diet while the remaining rats continued on the original diet. Energy intake and body weight were recorded 24 and 48 h after diet switch. Following 48 h, all rats were fasted overnight for 12 h and were then culled. The rats were fasted prior to cull in order to avoid the variations in energy intake that would have occurred if they had remained on their respective cafeteria and chow diets.

2.4. Tissue collection and molecular analysis

Rats were anesthetized [ketamine (150 mg/kg)/xylazine (20 mg/kg), i.p.] and decapitated. Blood was collected in the 15 weeks cohort via the cardiac ventricle prior to decapitation, and collected in the 6 weeks cohort via a tail nick. Blood was centrifuged (12,000 rpm, 8 min) and plasma was stored at −20 °C for subsequent radioimmunoassay for plasma leptin, insulin (Linco, Billerica, MA, USA) and corticosterone (MP Biomedical, Solon, OH, USA). Blood glucose was measured using a glucometer (Accu-Chek). White adipose tissue (gonadal and retroperitoneal; WAT), and brown adipose tissue (BAT) were dissected and weighed.