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Dietary-induced binge eating increases prefrontal cortex neural activation to restraint stress and increases binge food consumption following chronic guanfacine

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ABSTRACT

Binge eating is a prominent feature of bulimia nervosa and binge eating disorder. Stress or perceived stress is an often-cited reason for binge eating. One notion is that the neural pathways that overlap with stress reactivity and feeding behavior are altered by recurrent binge eating. Using young adult female rats in a dietary-induced binge eating model (30 min access to binge food with or without 24-h calorie restriction, twice a week, for 6 weeks) we measured the neural activation by c-Fos immunoreactivity to the binge food (vegetable shortening mixed with 10% sucrose) in bingeing and non-bingeing animals under acute stress (immobilization; 1 h) or no stress conditions. There was an increase in the number of immunopositive cells in the dorsal medial prefrontal cortex (mPFC) in stressed animals previously exposed to the binge eating feeding schedules. Because attention deficit hyperactive disorder (ADHD) medications target the mPFC and have some efficacy at reducing binge eating in clinical populations, we examined whether chronic (2 weeks; via IP osmotic mini-pumps) treatment with a selective alpha-2A adrenergic agonist (0.5 mg/kg/day), guanfacine, would reduce binge-like eating. In the binge group with only scheduled access to binge food (30 min; twice a week; 8 weeks), guanfacine increased total calories consumed during the 30-min access period from the 2-week pre-treatment baseline and increased binge food consumption compared with saline-treated animals. These experiments suggest that mPFC is differentially activated in response to an immobilization stress in animals under different dietary conditions and chronic guanfacine, at the dose tested, was ineffective at reducing binge-like eating.

1. Introduction

Bulimia nervosa (BN) and binge eating (BED) have recurrent binge eating as a cardinal feature. Binge eating not only involves eating a large amount of food in a short period of time, but is also accompanied by a sense of a loss of control over eating (American Psychiatric Association, 2013). Binge eating episodes are often associated with negative affect and are triggered by emotional stressors (Goldschmidt et al., 2012; Haedt-Matt and Keel, 2011; Hilbert and Tuschen-Caffier, 2007). Stress reactivity, attention, and mood are modulated, in part, by brain norepinephrine (NE) (Hamon and Blier, 2013). Along these lines, lower NE levels in the cerebrospinal fluid of individuals with BN have been reported (George et al., 1990; Kaye et al., 1990a; Kaye et al., 1990b), and medications that selectively inhibit NE transporters have efficacy at reducing binge eating in BN and BED (El-Giamal et al., 2000; Fassino et al., 2004; McElroy et al., 2007). Studies have also indicated that BN and BED subjects have blunted autonomic nervous system and neuroendocrine response to stress (Koo-Loeb et al., 1998; Rosenberg et al., 2013). Taken together, these studies suggest that NE-modulated pathways that control stress and feeding could be involved in the sustaining nature of binge eating.

Attention deficit hyperactivity disorder (ADHD) medications, which have varying actions on brain NE, have been successful to some extent at treating BN and BED. These findings, however, have been limited to case studies (Ioannidis et al., 2014; Keshen and Ivanova, 2013) or small clinical populations (McElroy et al., 2007). One reason for the effectiveness of these medications is that ADHD is comorbid with BN and BED. Data from the World Health Organization (WHO) estimates 15% and 10% of individuals with a lifetime diagnosis of BN and BED, respectively, have a history of ADHD (Kessler et al., 2013). In addition, there was a 5.2 times greater risk for developing BN in girls with ADHD than in girls without an ADHD diagnosis (Biederman et al., 2010). ADHD symptoms have been associated with greater severity of

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bingeing in BN and BED (Fernandez-Aranda et al., 2013; Nazar et al., in press), suggesting an overlap in the neurobiological basis of these disorders.

Guanfacine (Intuniv; [N-(diaminomethylidene)-2-(2,6-dichlorophenyl) acetamide]), a nonstimulant selective alpha-2A adrenergic agonist, was approved by the FDA in 2009 for the treatment of ADHD. Guanfacine acts on post-synaptic alpha-2A receptors on prefrontal cortical neurons to enhance cortical networks to reduce impulsive behaviors and improve attention (Biederman et al., 2006). Human imaging data suggests that guanfacine plays a role in inhibiting the amygdala-dependent emotional modulation of cognitive control (Schulz et al., 2014). While there is no data available on the use of guanfacine in eating disorders, a recent clinical study using 29 treatment-seeking cocaine-dependent subjects demonstrated that guanfacine treatment (approximately 4 weeks) reduced behavioral indices of craving, stress, and anxiety (Fox et al., 2012). Based on the NE agonist action of guanfacine and the putative role NE on binge eating, we examined whether guanfacine would be effective at reducing binge-like eating in our rodent model of dietary-induced binge eating.

Because binge eating is typically associated with defined eating behaviors, such as repeated consumption of highly palatable or preferred foods, eating a large amount of food in a short period of time, and intermittent access, we can reliably model some aspects of binge eating in rodents (Corwin et al., 2011). Previous findings from our laboratory have demonstrated that male rats exposed to a dietary-induced binge eating protocol had an attenuated feeding response to a selective NE reuptake inhibitor, reduced responses to a restraint stress, and decreased evoked locus coeruleus (LC)-NE activity (Bello et al., 2014). While these findings suggest that medications that increase NE signaling, such as guanfacine, could potentially reduce binge-like eating, it is unclear if exposure to dietary-induced binge eating differentially engages neural structures involved in the control of stress and feeding. The present study sought to expand our previous findings by examining the neural activation, by c-Fos immunohistochemistry, in response to binge food in female rats with different diet histories exposed to acute conditions of either restraint stress or no stress. In addition, we examined if chronic treatment with guanfacine would alter binge-like eating and stress reactivity.

2. Materials and methods

2.1. Animals

Adult female Sprague Dawley rats (7–8 weeks of age) acquired from Harlan Laboratories (Frederick, MD) were individually housed and placed on a 12/12 h light dark schedule (lights off at 1700 h). Rats were fed standard chow (Purina Rat Diet 5012, 13% fat, 27% protein, 3.1 Kcal/g), unless otherwise noted, and water was available at all times during the experiments. All procedures were approved by the Institutional Animal Care and Use Committee of Rutgers University and were in accordance with NIH guidelines.

2.2. Feeding schedules and experimental groups

The binge-like food used in these experiments was "sweetened fat" and consisted of vegetable shortening (Crisco®, J.M. Smucker) and 10% sucrose; 8.6 Kcal/g. All animals received a 24-h pre-exposure to the sweetened fat 7 days before being assigned to their respective feeding protocols. The pre-exposure was used to determine if the rats had initial differences in their preference to the sweetened fat and to reduce the avoidance typically associated with a novel food. The rats were divided into four groups with an initial statistically similar body weight and sweetened fat preference, which were designated as Restrict Binge, Binge, Restrict, or Naive groups. The *Restrict Binge* group (n = 10) had repeated cycles of intermittent 24-h caloric deprivation (beginning 1 h prior to lights off) followed by refeeding with standard chow and an optional 30-min access to sweetened fat. The exposure to intermittent calorie deprivation occurred on days 2 and 5, while refeeding with standard chow and the 30-min access to the sweetened fat on days 3 and 6 of the 7-day feeding schedule. In this fashion, the Restrict Binge group was exposed to a repeated cycle that consisted of three no-restriction days (days 1, 4, and 7), two weekly episodes of calorie restriction (days 2 and 5), and two weekly episodes of scheduled re-feeding starting with 30-min access to an optional palatable food (days 3 and 6). The second group, the Binge group (n = 10) had ad libitum standard chow, but had a 30-min access to the sweetened fat (days 3 and 6) at the same time and frequency as the Restrict Binge group (Corwin, 2004; Corwin et al., 1998). A third group, *Restrict* group (n = 10), had an identical pattern of calorie deprivation with standard chow (days 2 and 5) as the Restrict Binge group, but did not have repeated access to the optional sweetened fat upon refeeding on days 3 and 6. A *Naive* group (n = 10) had ad libitum standard chow with no access to the sweetened fat. These feeding protocols are from a previously published procedure for a rat model of dietary-induced binge eating (Bello et al., 2014). Cumulative caloric intakes were recorded weekly and cumulative caloric intakes were determined once a week at the end of day 1 of the feeding schedules. Food intakes and body weights were measured to the nearest 0.1 g.

2.3. Vaginal cytology

The stage of estrous was assessed by vaginal cytology. This was done twice a week 2 h before feeding on days 3 and 6 of the weekly schedule (i.e., re-feeding days or "binge days"). Vaginal cytology was also performed immediately before the restraint stress procedure. Briefly, the vaginal cavities of rats were lavaged with sterile saline (0.9%), and the cells were characterized by vaginal epithelial cell morphology. Proestrus–estrus was classified by the presence of nucleated epithelial and cornified cells. Diestrus was classified by the presence of leukocytes.

2.4. c-Fos immunohistochemistry response to "binge food" with or without exposure to restraint stress

After 6 weeks of feeding, rats were placed on standard chow for 5 days. Because each groups had a history of different feeding schedules, we exposed all animals to a 24-h food deprivation to ensure they had similar acute feeding and metabolic conditions. Following the 24-h food deprivation at 1200 h, each of the 4 groups was separated in half and acutely exposed to either a stress or no-stress trial. The restraint stress involved transporting the rat to a separate room to be immobilized by placement into a well-ventilated plexiglass flat-bottom restrainer (Braintree Scientific, Braintree, MA) for 60 min. The no stress test involved leaving the rat undisturbed for 60 min inside their home cage. For those animals undergoing the stress condition, prior to (baseline), during (0.5 h), and at the completion of the 1-h restraint stress approximately 50 μl of blood was collected from a tail nick. Each blood sample was maintained on ice until centrifugation at 3000 rpm for 10 min at 4 °C, and the plasma was stored at −80 °C. A standard radioimmunoassay kit was used to determine plasma corticosterone (sensitivity: 25 ng/ml; MP Biomedicals, Santa Ana, CA) levels. After the 60 min stress or no stress test, all rats were given access to a standardized meal of 2 g of sweetened fat for 5 min in their home cage. Ninety minutes after given access, the rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, IP) and given a cardiac perfusion, first with 0.9% NaCl and then with 4% paraformaldehyde in PBS. The brains were collected and stored in 4% paraformaldehyde with 25% (wt/vol) sucrose. The brains were stored overnight and sliced at 40 μm using a cryostat. The sections cut included the dorsal medial frontal cortex (mPFC; 3.72 mm Bregma), medial shell of the nucleus accumbens (1.92 mm Bregma), anterior bed nucleus of the stria terminalis (BNST; 0.12 mm Bregma), paraventricular nucleus (PVN; −1.75 mm Bregma) and arcuate nucleus (−2.64 mm Bregma) of the hypothalamus, and the nucleus of the solitary tract (NTS; −13.68 mm Bregma) (Paxinos and Watson, 2007).