Published in final edited form as:

Physiol Behav. 2015 December 01; 152(Pt A): 249–256. doi:10.1016/j.physbeh.2015.10.012.

Differential mesocorticolimbic responses to palatable food in binge eating prone and binge eating resistant female rats

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Abstract

Binge eating is a key symptom of many eating disorders (e.g. binge eating disorder, bulimia nervosa, anorexia nervosa binge/purge type), yet the neurobiological underpinnings of binge eating are poorly understood. The mesocorticolimbic reward circuit, including the nucleus accumbens and the medial prefrontal cortex, is likely involved because this circuit mediates the hedonic value and incentive salience of palatable foods (PF). Here we tested the hypothesis that higher propensity for binge eating is associated with a heightened response (i.e., Fos induction) of the nucleus accumbens and medial prefrontal cortex to PF, using an animal model that identifies binge eating prone (BEP) and binge eating resistant (BER) rats. Forty adult female Sprague–Dawley rats were given intermittent access to PF (high fat pellets) 3×/week for 3 weeks. Based on a pattern of either consistently high or consistently low PF consumption across these feeding tests, 8 rats met criteria for categorization as BEP, and 11 rats met criteria for categorization as BER. One week after the final feeding test, BEP and BER rats were either exposed to PF in their home cages or were given no PF in their home cages for 1 h prior to perfusion, leading to three experimental groups for the Fos analysis: BEPs given PF, BERs given PF, and a No PF control group. The total number of Fos-immunoreactive (Fos-ir) cells in the nucleus accumbens core and shell, and the cingulate, prelimbic, and infralimbic regions of the medial prefrontal cortex was estimated by stereological analysis. PF induced higher Fos expression in the nucleus accumbens shell and core and in the prelimbic and infralimbic cortex of BEP rats compared to No PF controls. Throughout the nucleus accumbens and medial pre-frontal cortex, PF induced higher Fos expression in BEP than in BER rats, even after adjusting for differences in PF intake. Differences in the neural activation pattern between BEP and BER rats were more robust in prefrontal cortex than in nucleus accumbens. These data confirm that PF activates brain regions responsible for

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encoding the incentive salience and hedonic properties of PF, and suggest that binge eating proneness is associated with enhanced responses to PF in brain regions that exert executive control over food reward.

Keywords

Binge eating; Eating disorders; Palatable food; Reward; Nucleus accumbens; Medial prefrontal cortex

1. Introduction

Binge eating involves the consumption of a large amount of food in a short period of time and a loss of control during the binge episode [1]. Binge eating is a common, core feature cutting across all of the major subtypes of eating disorders included in the DSM-5 (e.g., bulimia nervosa, binge eating disorder, anorexia-nervosa binge/purge type) [1]. Individuals who binge eat suffer from significant psychological distress, including elevated depression scores, reduced quality of life, and a general decline in psychological function [2]. Scientific inquiry into the etiology of binge eating has traditionally focused on psychosocial variables, although a growing body of evidence also points to biological underpinnings [3–5]. Nonetheless, the neurobiology underlying binge eating remains poorly understood. The present study aimed to address this gap by 1) using an animal model of binge eating that allows investigation of neurobiological variables without the confound of psychosocial variables, and 2) focusing on the involvement of the mesocorticolimbic reward circuit in binge eating behaviors, since this circuit mediates food reward [6]. Specifically, we asked whether enhanced mesocorticolimbic neural responsiveness to palatable food (PF) is associated with a higher propensity to binge eat.

Several components of the mesocorticolimbic reward circuit, including the nucleus accumbens (NA) and the medial prefrontal cortex (mPFC), are activated in conjunction with PF intake [6]. In animal studies, neural activation is commonly assessed by examining expression of Fos, the protein product of the immediate early gene *c-fos*, in brain regions of interest. Fos expression increases within 60–90 min in neurons in response to depolarizing stimuli; therefore microscopic quantification of Fos immunoreactivity can be used as a proxy for neural activation after stimulus exposure [7]. Fos expression within the mesocorticolimbic reward circuit is increased after intake of PF, and the Fos response to PF is greater than that elicited by standard lab chow [8,9]. However, no studies to date have examined PF-induced activation of the mesocorticolimbic circuit in an animal model of individual differences in binge eating, e.g., in high versus low binge eaters. This is an important consideration for the human condition, because access to PF is virtually ubiquitous, yet only a small proportion of humans binge eat, indicating wide-ranging individual differences in binge eating proneness [10–12]. Here we test the hypothesis that mesocorticolimbic responsiveness to PF positively correlates with the propensity to binge eat, using an animal model that identifies naturally occurring within-group differences in binge eating, specifically the binge eating prone (BEP) and binge eating resistant (BER) rat model.

The BEP/BER rat model has high face and construct validity for studying the biological underpinnings associated with binge eating, largely due to its ability to model a continuum of binge eating behaviors, to identify extreme binge eating phenotypes (i.e., BEP and BER rats), and to identify natural, individual variation in binge eating of PF [11]. PF exposure in this model is intermittent (3 d/week) and brief (4 h/day), similar to binge episodes in the human condition that are also intermittent and discrete (i.e., a few hours) [1,10–12]. BEP rats binge only on PF and not on standard rat chow, suggesting that they are not general over-eaters, and BEP rats do not gain excessive weight throughout the testing period as compared to their BER counterparts [10–14]. Finally, similar to humans [15], sex differences are apparent in the BEP/BER paradigm, such that female rats are more likely to display binge eating proneness as compared to male rats [13].

Given the robust behavioral differences between BEP and BER rats in their propensity to binge on PF, we hypothesized that PF is a more salient reward in BEP rats than in BER rats. To test this hypothesis, we compared the Fos responses to PF in the NA and mPFC in three different groups: 1) BEP rats exposed to PF, 2) BER rats exposed to PF, and 3) a group of control rats not exposed to PF. Our hypothesis predicted that the Fos response to PF within the NA and mPFC would be higher in BEP rats as compared to both control rats and BER rats exposed to PF. Our study results support these predictions, and provide preliminary evidence consistent with the hypothesis that binge eating proneness is associated with enhanced responsiveness to PF, particularly higher order executive processing of PF reward.

2. Methods

2.1. Subjects

Forty adult (postnatal day 60; P60) female Sprague–Dawley rats were obtained from Harlan Laboratories (Madison, Wisconsin) and individually housed upon arrival in clear Plexiglas cages (45 × 23 × 21 cm) with ad libitum access to chow (Harlan Teklad Global Diets: 8640, Madison, Wisconsin) and water. Rats were maintained on a 12:12 h light:dark cycle (lights on at 0200 h and off at 1400 h) at 72 ± 4 °F and were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All protocols were approved by the Michigan State University Institutional Animal Care and Use Committee.

2.2. Feeding tests and BEP/BER classification

2.2.1. Acclimation—One week prior to the start of feeding tests, all rats were acclimated to daily handling and body weight measurements. Specifically, rats were picked up from their home cages and held for ~2 min (i.e., the approximate amount of time required for cage bedding searches on feeding test days, see below) and were weighed in a covered container on a standard electronic balance.

2.2.2. Feeding tests—The BEP/BER paradigm used in this study began on P67 and was an adapted form of the protocol outlined in [10] and that has been used previously in our lab [11–14]. This paradigm consists of feeding test days, during which PF is provided in addition to standard chow, and non-feeding test days during which only chow is provided. We conducted the BEP/BER paradigm over a period of three weeks. On feeding test days,

which occurred three days per week (MWF), body weights and 24-hour chow intake were recorded in the mornings before lights out. Approximately 10 min prior to lights out, rats were given pre-measured PF (~15–17 g; High Fat Diet pellets, #D12451, Research Diets Inc., New Brunswick, NJ) placed in ceramic dishes on the floor of home cages. These ceramic dishes stayed in each rat's home cage throughout the entire testing period, including days when rats were not exposed to PF. Rats were also allowed unlimited access to standard chow during the period of PF access on these feeding test days. After 4 h of PF access, all remaining PF and chow were measured using standard electronic balances and PF was removed from each rat's cage. Cage bedding was searched to ensure that all chow and PF pellets were included in the 4-hour measurements. The 4-hour consumption values were later used to identify BEP and BER rats, as this short, discreet amount of time models binge eating in humans (usually 1–2 h) and has been used previously to elicit binge eating in rats [10–14]. On non-feeding test days (i.e., all other days of the week), both body weights and total 24-hour chow intake were recorded in the mornings before lights out.

2.2.3. BEP/BER classification—Using methods outlined in [11–14], we identified BEP and BER rats using a tertile approach based on 4-hour PF consumption data across eight of the nine feeding tests (3 feeding tests per week; 3 weeks total of testing). Data from the first feeding test were omitted from the final analysis due to inadvertent inaccurate measurement of PF consumption in a subset of rats. Prior to BEP/BER classification, all 4-hour PF intake values on each testing day were standardized by body weights using the formula: intake (g) / body weight (g)^{2/3} [13,16]. Standardization removes any confound of normal body weight variation on PF intake values that could unduly influence tertile calculations and BEP/BER classifications. Mean standardized PF intake values from each feeding test day were then divided into top, middle, and bottom tertiles, such that each rat scored within one of the three tertiles on each feeding test day. Rats were categorized as BEP if they scored within the highest tertile on at least 4 of the 8 feeding tests (i.e., 50% of feeding test days) and never within the lowest tertile. Rats were categorized as BER if they scored within the lowest tertile on at least 4 of the 8 feeding tests (i.e., 50% of feeding test days) and never within the highest tertile. Past studies in our lab using the BEP/BER model have analyzed a wide range of tertile criteria for identifying BEP and BER rats (i.e., requiring 50% to 83% of feeding test days to be high/low for BER/BEP groups) [11–14]. We chose to use criteria at the lower end of the range in order to maximize our sample size while still identifying rats that were consistently eating the highest (BEP) and the lowest (BER) amounts of PF [13]. Overall, this method of classifying rats as BEP or BER takes into account both the frequency and the consistency of either high or low amounts of PF consumption across the testing period. This approach closely follows the methods used for identifying binge eaters and non-binge eaters in the human condition, whereby individuals who binge eat at once per week are considered binge eaters, while those who seldom binge eat are considered non-binge eaters [1].

2.3. Induction of c-Fos and quantification of Fos-immunoreactivity

2.3.1. Induction of c-Fos expression—The rats identified as BEP (8/40, 20% of the sample) and BER (11/40, 28% of the sample) from the feeding test protocol were used to compare mesocorticolimbic activation patterns in response to PF in extreme binge eating

phenotypes. After the last feeding test on P87, rats were handled daily for one week without any further exposure to PF. During this period of time, ceramic food dishes remained in each rat's home cage, and all rats were carted daily to the room where final testing would occur prior to sacrifice on P94. On the day of Fos induction, rats were randomly assigned to receive either PF (15–17 g in ceramic dishes) in their home cages ($n = 5$ BEP; $n = 7$ BER, referred to as “BEP w/PF” or “BER w/PF”) or no PF in their home cages ($n = 3$ BEP; $n = 4$ BER, combined into one group and referred to as “No PF”)¹ for a total of 1 h beginning just after lights out. During this exposure period, chow remained in the cages of all PF-exposed and No PF animals, and ceramic food dishes remained in the cages of all PF-exposed and No PF animals. PF and ceramic dishes were removed from all cages after the one-hour exposure period, and thirty minutes later (i.e., 90 min after the introduction of PF or the empty dish), rats were given a lethal dose of Fatal Plus® i.p. (sodium pentobarbital diluted to 50 mg/mL in sterile saline; 150 mg/kg). This 90-minute period between initial stimulus introduction and the induction of euthanasia has been shown to be an optimal period of time for peak Fos expression to occur in response to food rewards [8,17]. Rats were then intracardially perfused with 300 mL of 0.1 M buffered saline rinse followed by 300 mL of 4% paraformaldehyde. Brains were removed and placed in 4% paraformaldehyde overnight and then stored in 20% sucrose in 0.1 M buffered saline. Brains were cryostat-sectioned at 40 μ m into four series through the mPFC and the NA, and sections were stored in cryoprotectant at -20°C until further processing.

2.3.2. Immunohistochemistry for Fos immunoreactivity (Fos-ir)—One set of sections from the 1 in 4 series from each rat was processed for Fos immunohistochemistry. Sections were rinsed in Tris Buffered Saline (TBS, 3×5 min) and then were immersed in 0.1% sodium borohydride in 0.5 M TBS for 15 min, followed by 10 min in 1% hydrogen peroxide in TBS. Sections were rinsed in TBS (3×5 min) and then incubated for 30 min in a blocking solution of 20% normal goat serum, (NGS; Pel Freez Biologicals, Rogers, AR) in 0.3% Triton X-100 in TBS. Blocking was followed by a 48-hr incubation at 4°C in a 1:10,000 dilution of rabbit anti-Fos primary antisera (Santa Cruz Biotech; Santa Cruz, CA) in TBS with 2% NGS and 0.3% Triton X-100. Sections were rinsed in TBS (3×5 min) and then incubated for 60 min in a 1:500 dilution of goat anti-rabbit biotinylated secondary antisera (Vector Laboratories, Burlingame, CA) in TBS with 2% NGS and 0.3% Triton X-100. Sections were then rinsed again in TBS (3×5 min), after which they were incubated for 60 min in avidin–biotin complex (Vector Laboratories, Burlingame, CA). Diaminobenzidine tetrahydrochloride (10 mg tablets; Sigma-Aldrich, St. Louis, MO) was dissolved in TBS and 30% hydrogen peroxide to visualize brown-black, Fos-immunoreactive (Fos-ir) nuclei. Sections were mounted onto slides, counterstained with Toluidine blue, dehydrated using a graded alcohol series, cleared in xylene, and coverslipped. Due to poor tissue quality after immunohistochemical processing, one BER from the BER w/PF group and one BER from the No PF group were excluded from the Fos expression analysis. This resulted in a final sample size of 8 total BEP ($n = 5$ in BEP w/PF

¹The number of Fos-ir cells did not differ significantly between BEP and BER “No PF” rats within any of the analyzed brain regions (p values 0.29–0.91). Therefore, data from these rats were combined into a single “No PF” group for all statistical analyses to maximize sample size and minimize the number of statistical comparisons.

and $n = 3$ in the No PF group) and 9 total BER rats ($n = 6$ in BER w/PF and $n = 3$ in the No PF group) for the Fos analysis.

2.3.3. Quantification of Fos-ir in the mesocorticolimbic circuit—Total number of Fos-ir cells in each region of interest was estimated using unbiased stereological methods. The optical fractionator method in Stereoinvestigator (Microbrightfield Biosciences, Willingston, VT) was used to estimate Fos-ir cells in each region of interest within the NA and mPFC, and all tracings and cell counts were performed on an Olympus BX51 microscope and Q-Imaging Color 12 bit camera. An experimenter blind to the behavioral phenotype and exposure status of the animals quantified Fos-ir cells in each region of interest. Analyzed sections in the medial prefrontal cortex (mPFC) corresponded to plates 9–12 (+3.72 mm to +2.76 mm from Bregma) of the Paxinos and Watson [18] rat brain atlas (Fig. 1A). The prelimbic cortex (PrL), infralimbic cortex (IL), and cingulate area 1 (Cg1) were all traced relative to the forceps minor (fmi) and in accordance with prefrontal cortex traces used in Chisholm and Juraska [19]. Tracings of the mPFC were performed under a 4× (NA 0.13) air objective, and counts were performed with a 40× (NA 0.85) air objective. For stereological estimates of the number of Fos-ir cells in the mPFC, the counting frame was set to 125 μm^2 within a grid size of 400 μm^2 , and the optical dissector height was set to 6 μm with top and bottom guard zones of 2 μm . Analyzed sections in the nucleus accumbens (NA) corresponded to plates 16–23 (+2.04 mm to +1.2 mm from Bregma) of the Paxinos and Watson (2005) rat brain atlas (Fig. 1B). The NA core (NAC) and NA shell (NAS) were traced using a 4× (NA 0.13) air objective, and cell counts within each contour were conducted using a 40× (NA 0.85) air objective. For stereological estimates of the number of Fos-ir cells in the NA, the counting frame was set to 125 μm^2 within a grid size of 350 μm^2 , and the optical dissector height was set to 6 μm with top and bottom guard zones of 2 μm .

2.4. Statistical analyses

2.4.1. Preliminary analyses of feeding test data—Mixed design ANOVA models were used to examine mean differences in 4-hour PF consumption, 4-hour chow consumption, 24-hour chow consumption, and body weights (measured at 24 h only) between BEP and BER groups across the three-week feeding test period. The within-group variables were 4-hour PF consumption, 4-hour chow consumption, 24-hour chow consumption, or body weights, while the between subjects factor was BEP/BER phenotype. Given prior research (e.g., [10–14]), these analyses were necessary to confirm that BEP rats consumed significantly more PF than BER rats and to verify that BEP and BER rats did not differ in average body weights across the study.

2.4.2. Fos-ir in the NA and mPFC—Although ANOVA with *post-hoc* t-tests could have been used to test all pairwise group comparisons on mean Fos-ir cells in the NA and mPFC, we had a priori hypotheses that focused on two specific planned comparisons. Specifically, a priori hypotheses predicted that the BEP w/PF group would show the highest number of Fos-ir cells in each brain region (NA core, NA shell, Cg1, PrL, and IL mPFC) as compared to the other two experimental groups (No PF and BER w/PF rats). We therefore focused the analyses on specific planned comparisons only. Our examination of only two comparisons is

also consistent with recommendations for planned contrasts (i.e., conducting one less contrast than the number of groups: $k - 1$).

First, we conducted t-tests comparing mean estimated number of Fos-ir cells in each brain region (NA core, NA shell, Cg1, PrL, and IL mPFC) between BEP w/PF and No PF rats. Higher mean Fos-ir cells in the BEP w/PF group, relative to the No PF group, would provide support for the hypothesis that BEP rats experience heightened neural response in the NA and mPFC, following PF exposure. Second, we compared mean Fos-ir cells in each brain region between the BEP w/PF versus BER w/PF groups using ANCOVAs; ANCOVAs were used for this set of planned comparisons to control for PF consumption during the one hour access period prior to sacrifice. Notably, statistically controlling for between-rat variation in total 1-hour PF intake (via ANCOVA models) was an important consideration since the BEP w/PF group consumed more PF than the BER w/PF group during the pre-sacrifice testing period (PF in grams: BEP $M(SD) = 8.46 (1.47)$, BER $M(SD) = 6.60 (1.78)$, Hedges' $g = 1.02$; PF in grams standardized by body weight: BEP $M(SD) = 0.24 (0.04)$; BER $M(SD) = 0.19 (0.05)$, Hedges' $g = 0.98$). ANCOVA models therefore ensured that higher total Fos-ir cells in the BEP w/PF group, relative to the BER w/PF group, could not be accounted for merely by between-rat variation in absolute levels of 1-hour PF consumption prior to sacrifice; instead, between-group differences would be indicative of differences in the neural responsiveness to PF exposure.

All planned comparisons used one-tailed p -values given our unidirectional a priori hypothesis that the BEP w/PF group would show more Fos-ir cells than each of the other two experimental groups. Given the smaller sample sizes in this study, Hedges' g effect sizes, which is a variation of Cohen's d that corrects for biases when data consist of small sample sizes [20], were also computed to provide a standardized measure of the magnitude of mean differences between groups (small, $g = .20$; medium, $g = .50$; large, $g = .80$).

3. Results

3.1. Preliminary analyses of feeding test data

Results from the mixed design ANOVAs for PF and chow consumption during the eight feeding tests are shown in Table 1 and Fig. 2. BEP rats ate significantly more PF than BER rats throughout the entire testing period, yet BEP rats ate significantly less chow than BER rats on both feeding test days and non-feeding test days (Table 1 & Fig. 2). BEP rats also ate less chow than BER rats during the 4-hour period on feeding test days, though this difference did not reach statistical significance. BEP and BER rats showed non-significant, but medium effect size, differences in body weight (see Table 1 & Fig. 2). However, consistent with prior research [10], mean body weights for both the BEP and BER groups were within the normal weight range for age (see Growth Curve data for Sprague Dawley rats: www.Harlan.com; Harlan Laboratories, Inc.), indicating the BEP rats did not gain excessive weight during the feeding test paradigm.

3.2. Fos expression

3.2.1. Fos expression in the NA and mPFC

3.2.1.1. BEP w/PF versus No PF: Fos-ir cell count comparisons between BEP w/PF and No PF rats largely supported hypotheses. The BEP w/PF group had significantly higher Fos expression in the NAC and PrL and a statistical trend towards higher Fos expression in the NAS and IL, as compared to No PF rats (see Table 2 and Fig. 3a), with effect sizes large in magnitude (NAC: Hedge's $g = 1.25$; NAS: Hedge's $g = 0.89$; PrL: Hedges' $g = 1.15$; IL: Hedges' $g = .97$). In the Cg1 region, we found medium-to-large effect sizes for higher mean Fos-ir cell counts in BEP w/PF than No PF rats (Hedges' $g = .62$), even though the statistical test failed to reach significance (see Table 2).²

3.2.1.2. BEP w/PF versus BER w/PF: Consistent with hypotheses, Fos-ir cell counts were significantly higher in BEP w/PF rats versus BER w/PF rats in the PrL, IL, and Cg1 and approached significance in the NAC and NAS, after adjusting for total PF consumption prior to sacrifice (see Table 3 and Fig. 3b). Effect sizes were large in magnitude for all neural regions (see Table 3): NAC (Hedge's $g = 1.02$), NAS (Hedge's $g = 0.82$), PrL (Hedges' $g = 2.59$), IL (Hedges' $g = 1.55$), and Cg1 (Hedges' $g = 1.64$).³ These findings highlight that Fos expression differences between the BEP w/PF and BER w/PF groups cannot be accounted for by greater PF intake in BEP rats, since pre-sacrifice PF consumption differences were statistically controlled for, and instead reflect enhanced neural responsiveness to PF in BEP rats.

4. Discussion

This study is the first to provide preliminary evidence that an enhanced mesocorticolimbic response to PF is associated with a higher propensity to binge eat. First, we demonstrated that PF activates the NA core and shell and prelimbic and infralimbic mPFC in BEP rats, as evidenced by a greater number of Fos-expressing cells in these regions in BEP rats exposed to PF compared with that in rats not exposed to PF. The large effect sizes for all of these comparisons indicate biological significance. The NA shell is home to a hedonic hot spot for PF reward, regulating the affective response to PF [6,21], whereas NA core is necessary for instrumental learning associated with food reward [22] and the execution of motor behaviors to obtain food reward [23]. Moreover, the PrL and IL cortices respectively project to the NA core and shell [24], encode the palatability of different tastes [25], and are activated by PF (e.g., high fat or chocolate pellets) and chow consumption [8,17,26]. Thus, compared to chow alone (i.e., No PF group), PF consumption by BEP rats more strongly activates brain regions involved in the perception of the hedonic properties of PF and the motivation to attain and consume PF. Second, we found greater PF-induced neural activation of the NA

²As previously noted, BEP and BER rats in the No PF group did not significantly differ on the number of Fos-ir cells within any of the analyzed brain regions, and thus, were combined into one group for analyses. It is unlikely that our results were unduly affected by this decision; in general, BEP w/PF showed substantially higher mean Fos-ir cells as compared to the BEP rats in the No PF group (all neural regions: Hedges' $g = .83$ – 1.29 , mean $g = 1.03$) and the BER rats in the No PF group (with exception of Cg1, all other neural regions: Hedges' $g = .72$ – $.98$, mean $g = .85$).

³ANCOVA models controlled for between-rat differences in 'absolute levels (in grams)' of 1-hour PF consumption prior to sacrifice, but notably, similar results were obtained when 'body-weight adjusted' 1-hour PF consumption values were used as the covariate (i.e., large effect sizes in all neural regions; data available upon request).

core and shell and all regions of the mPFC in BEP than in BER rats, even after statistically controlling for the total amount of PF consumed prior to sacrifice. Effect sizes for all of these comparisons were large in magnitude, but in mPFC, the group means were statistically significant and effect sizes were ~1.5–2 times greater than those in the NA, indicating that differences in the neural activation pattern between BEP and BER rats are more robust in the mPFC than in the NA. Thus, while binge eating proneness is associated with enhanced activity in both prefrontal cortical and subcortical reward regions during PF intake, our results suggest that binge eating proneness may be more strongly linked to dysregulated executive control of PF consumption than to food reward.

The neurotransmitter systems in NA and mPFC that are affected by PF activation of the reward circuit remain to be identified. Two candidate neurotransmitters within NA are dopamine and endogenous opiates (EOP), which have both been linked to the coding of hedonic properties and incentive salience of PF [6,27]. Thus, the Fos response to PF in NA may reflect dopamine and EOP release and activation of their receptors on medium spiny NA neurons. If so, then enhanced neurotransmission, either in the amount of neurotransmitter released or receptor signaling, could underlie the more extensive activation of NA neurons in BEP than in BER rats. Within the mPFC, complex interactions between GABAergic interneurons and glutamatergic projection neurons mediate executive control of goal-directed behaviors, including the amount and microstructure of chow and PF consumption [8,28–32]. In mice, PF induces differential Fos expression in inhibitory and excitatory mPFC neurons; the vast majority of excitatory projection neurons are activated by PF, whereas only a small subset of inhibitory interneurons is activated by PF [8]. If PF also differentially activates excitatory and inhibitory mPFC neurons in rats, then binge eating proneness may be related to differences in the particular pattern or proportion excitatory and inhibitory prefrontal neurons activated by PF in BEP and BER rats. This possibility could be empirically probed by determining the phenotype of Fos-expressing mPFC neurons (i.e., glutamatergic versus GABAergic) in BEP and BER rats.

Our results are consistent with some recent human neuroimaging data that has linked aberrant activity in the mesocorticolimbic circuit to binge eating and the associated eating disorders. Filbey et al. demonstrated that mesocorticolimbic activity during the consumption of a high calorie liquid (e.g., Pepsi, chocolate milk, cream soda) increased linearly with scores from a self-reported measure of binge eating [33]. Similarly, individuals with bulimia nervosa and binge eating disorder have higher prefrontal cortical activity in response to pictures of high calorie foods (e.g., French fries, ice cream, cake, chips), versus neutral pictures, as compared to healthy controls [34]. On the other hand, Bohon and Stice demonstrated that individuals with bulimia nervosa have reduced insular and prefrontal cortical activity during consumption of a chocolate milkshake as compared to healthy controls [35]. These variations in neuroimaging findings highlight the complexity of neural functioning in eating disorders in the human condition, and further point to the need for a combined effort employing both human and animal research to uncover the neurobiology underlying these conditions. Nonetheless, the overlap in findings between our animal data and some aspects of human neuroimaging data does suggest that the BEP/BER model is a useful and appropriate tool for future studies that aim to conduct translational research on the neurobiological mechanisms underlying binge eating.

Despite the strengths of our current study, there are some important limitations that deserve comment. First, sample sizes in this study are small, due to 1) the number of BEP and BER rats that are identified by the BEP/BER criteria, and 2) the necessity to further separate BEP and BER groups into PF exposure and No PF groups. However, in both sets of comparisons (BEP w/PF vs. No PF and BEP w/PF vs. BER w/PF), we found medium to large effect sizes for pairwise comparisons in all regions, even when they did not reach statistical significance. Effect sizes indicated that group differences are substantial and biologically meaningful, yet future replications warrant the use of larger sample sizes. Second, although we statistically controlled for total PF intake by the use of ANCOVA models in this study, we cannot rule out the possibility that behaviors associated with higher PF intake (motor behaviors, licking behaviors) affected Fos expression. Future studies employing the Fos induction protocol used here could aim to equate the amount of PF available for BEP and BER rats to avoid this potential confound.

Third, in contrast to prior studies using the BEP/BER model in which chow consumption did not differ between the two phenotypes (e.g., [10–12,14]), in the present study, BEP rats consumed significantly less chow than BER rats on both feeding test days and non-feeding test days. The phenotypic differences in chow consumption in the present study could be related to the type of PF used, which here had a higher fat content than the higher sugar content of the PF (vanilla frosting) previously used in our lab. Because a high fat PF source is more energy dense than a PF source higher in sugar, it is possible that the reduction in chow consumption in BEP rats was a direct consequence of consuming more fat on feeding test days. We also note that the proportions of BEP and BER rats found in this study are slightly less than what has been found previously in our lab using the BEP/BER protocol [11–14]. Again, the higher fat content of the PF used in this study may have contributed to this outcome, as rats may be less likely to consistently consume high amounts of a PF source (i.e., be classified as BEP) that is higher in fat versus one that is higher in sugar. Future studies should, therefore, consider the possibility that different types of PFs could induce different levels of binge eating proneness in addition to qualitative (i.e., regional specificity) or quantitative (i.e., magnitude of Fos expression) differences in neural activity in the mesocorticolimbic circuit.

Finally, we did not monitor estrous cycles in the present study, which could be an important factor driving PF consumption given the contribution of estradiol and progesterone to food intake and energy homeostasis [36]. However, it is unlikely that estrous cycles, alone, contributed to the consistent behavioral differences between the BEP and BER phenotypes since animals in both groups were likely in various stages of the estrous cycle across the course of the feeding test period. In the future, it may be beneficial to either monitor estrous cycles throughout the study or experimentally control ovarian hormone exposure, as doing so could ensure that circulating hormone concentrations are equal between all rats throughout the feeding test period and on the day of the Fos induction protocol. Moreover, future work should investigate the role of ovarian hormones in contributing to BEP/BER differences in neural responsiveness to PF within the mesocorticolimbic circuit, in light of the fact that ovarian hormones exert significant control over mesocorticolimbic responsiveness to other “synthetic” rewards, such as drugs of abuse and alcohol [37–39]. Future studies would also benefit from similar analyses of neural correlates for binge eating

prone to binge eating using male rats to investigate whether there are sex-specific and/or hormone-dependent mechanisms underlying binge eating risk.

In conclusion, results from this study largely support our initial hypothesis that binge eating prone individuals are associated with higher mesocorticolimbic responsiveness to PF. Greater activation of NA in BEP rats suggests that binge eating prone and binge eating resistant phenotypes differ in their perception of food reward and/or motivation to consume PF. However, differences between BEP and BER rats in PF-induced neural activation were more robust in the mPFC than in NA, suggesting that variation in executive control of PF consumption may be more strongly associated with binge eating risk. These findings prompt additional investigation of the relative contributions of reward and executive function to PF consumption in binge eating prone individuals.

Acknowledgments

The authors thank Jane Venier and Ray Figueira for their invaluable technical assistance, and Britny Hildebrandt, Margaret Mohr, and Jenny Kim for helpful comments on earlier drafts of the manuscript. This work was supported by faculty set-up funds for Cheryl L. Sisk and Kelly L. Klump from Michigan State University, a Provost Undergraduate Research Initiative Award (DRG), NIH grant DA030444 (KAR), and the Hilda and Preston Davis Foundation (KMC).

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HIGHLIGHTS

- We identified binge eating prone (BEP) and binge eating resistant (BER) rats.
- We induced Fos expression via pre-sacrifice palatable food (PF) exposure.
- BEP rats had higher Fos than BER rats in the nucleus accumbens and prefrontal cortex.
- Comparisons between BEP and BER rats were stronger in the prefrontal cortex.
- Binge eating proneness may be linked to altered executive control over PF intake.

Fig. 1.

Atlas images (Paxinos and Watson, 2005) depicting the contours used for trading the mPFC (A) and NA (B). Adjacent to atlas images are representative images of Fos-ir cells in the mPFC and NA from a “No PF” rat and a rat exposed to PF prior to sacrifice. Images were taken from regions identified by the black boxes in the diagrams. “No PF” and “PF” images were both taken from a BEP rat in each condition. Images were taken using a 40 $\times$ objective. Scale bar represents 50 μ m.

Fig. 2. BEP/BER differences in (a) PF and chow intake on feeding test days, (b) chow intake on non-feeding test days, and (c) body weights. All values represent average measurements across the three week testing period. Standardized intake: intake (g)/BW (g)^{2/3}; error bars represent one standard error. *** $p < .001$; † $p < .10$.

Fig. 3.

Mean comparisons of Fos expression in the nucleus accumbens (NA) and medial prefrontal cortex (mPFC) in BEP rats exposed to PF (BEP w/PF) versus No PF rats (A), and BEP rats exposed to PF (BEP w/PF) versus BER rats exposed to PF (BER w/PF), controlling for total 1-hour PF consumption at sacrifice (B). Groups were compared using planned pairwise comparisons and one-tailed p values. Error bars represent one standard error. ** $p < .01$; * $p < .05$; † $p < .10$.

Table 1

Mean comparisons between BEP and BER rats on PF, chow, and body weight from the eight feeding tests.

BEP vs. BER group	Mean (S.E.)	BEP vs. BER mean comparisons		
		Statistics		Effect size hedges' <i>g</i>
		<i>F</i> (1,17)	<i>p</i> -Value	
Body weight (g) (24 hr measurement)				
BEP	212.72 (3.47)	1.24	.28	0.48
BER	207.63 (2.96)			
<i>Feeding test days</i>				
Palatable food (4 hr intake)				
BEP	0.39 (0.009)	128.42	<.001	4.63
BER	0.26 (0.008)			
Chow (4 hr intake)				
BEP	0.03 (0.007)	3.78	.07	1.02
BER	0.05 (0.006)			
Chow (24 h intake)				
BEP	0.04 (0.01)	57.65	<.001	3.79
BER	0.16 (0.01)			
<i>Non-feeding test days</i>				
Chow (24 h intake)				
BEP	0.35 (0.005)	49.09	<.001	2.75
BER	0.39 (0.004)			

Note: Mean values for palatable food and chow are standardized scores, adjusted for body weight (i.e., intake (g)/body weight (g)^{2/3}). Sample sizes: BEP, *n* = 8 and BER, *n* = 11. Estimates were calculated across 8 feeding tests, from P67 to P85. Two-tailed *p*-values are presented. Hedges' *g* values reflect effect sizes, and thus, provide a standardized measure of the magnitude of mean differences between groups (effect size interpretation: small, *g* = .20; medium, *g* = .50; large, *g* = .80).

Table 2

Mean comparisons on Fos expression between BEP w/PF and No PF groups.

Experimental group	Mean (S.E.)	Planned pairwise comparisons: BEP w/PF No PF		
		Statistics		Effect size
		<i>t</i> (1, 9)	<i>p</i> -Value	Hedges' <i>g</i>
<i>Nucleus accumbens</i>				
NA core (NAC)				
No PF	5791.42 (951.87)	−2.14	.04	1.25
BEP w/PF	11,424.82 (2460.34)			
NA shell (NAS)				
No PF	6925.79 (1186.59)	−1.62	.07	0.89
BEP w/PF	10,428.15 (1896.81)			
<i>Prefrontal cortex</i>				
Prelimbic (PrL)				
No PF	22,611.74 (2204.77)	−1.99	<.05	1.15
BEP w/PF	32,368.52 (4380.44)			
Infralimbic (IL)				
No PF	11,573.19 (1523.60)	−1.78	.05	0.97
BEP w/PF	15,737.90 (1803.93)			
Cingulate (Cg1)				
No PF	16,711.54 (2507.99)	−1.13	.14	0.62
BEP w/PF	21,018.31 (2888.68)			

Note: BEP w/PF = binge eating prone rats exposed to PF; BEP w/PF = binge eating resistant rats exposed to palatable food; No PF = binge eating prone and binge eating resistant rats that were in the control condition, i.e., no exposure to PF. Sample sizes: No PF, *n* = 6, BEP w/PF, *n* = 5, and BEP w/PF, *n* = 6. Since a priori hypotheses predicted unidirectional mean differences between groups (i.e., BEP w/PF > No PF), planned comparisons were performed using one-tailed *p*-values. Hedges' *g* values reflect effect sizes, and thus, provide a standardized measure of the magnitude of mean differences between groups (effect size interpretation: small, *g* = .20; medium, *g* = .50; large, *g* = .80).

Table 3

Mean comparisons on Fos expression between BEP with PF and BER with PF groups, controlling for palatable food consumption.

Experimental group	Raw descriptive mean (S.D.)	Planned pairwise comparisons, controlling for pf consumption: BEP w/PF vs BER w/PF		
		Statistics	Effect size	Hedges' <i>g</i>
		<i>F</i> (1, 8)	<i>p</i> -Value	
<i>Nucleus accumbens</i>				
NA Core (NAC)				
BER w/PF	8346.18 (1672.46)	3.00	.06	1.02
BEP w/PF	11424.82 (5501.49)			
NA shell (NAS)				
BER w/PF	9414.59 (1722.21)	1.91	.10	0.82
BEP w/PF	10428.15 (4241.39)			
<i>Prefrontal cortex</i>				
Prelimbic (PrL)				
BER w/PF	20425.51 (8668.29)	19.26	.001	2.59
BEP w/PF	32368.52 (9794.97)			
Infralimbic (IL)				
BER w/PF	10740.47 (3804.17)	6.94	<.02	1.55
BEP w/PF	15737.90 (4033.71)			
Cingulate (Cg1)				
BER w/PF	16314.52 (6811.50)	7.75	.01	1.64
BEP w/PF	21018.31 (6459.28)			

Note: ANCOVA models covaried 1-hour palatable food consumption at sacrifice. Raw descriptive mean = mean value for Fos expression that was not adjusted for palatable food consumption; ANCOVA Adjusted Mean = mean value of Fos expression that controls for 1-hour palatable food consumption. BEP w/PF = binge eating prone rats exposed to palatable food; BER w/PF = binge eating resistant rats exposed to palatable food. Sample sizes: BEP w/PF, *n* = 5 and BER_PF, *n* = 6. Since a priori hypotheses predicted unidirectional mean differences between the two groups (i.e., BEP w/PF > BER w/PF), one-tailed *p*-values are reported. Hedges' *g* values reflect effect sizes, and thus, provide a standardized measure of the magnitude of mean differences between groups (effect size interpretation: small, *g* = .20; medium, *g* = .50; large, *g* = .80).