EXPLORING REWARD SYSTEM RESPONSIVITY IN THE NUCLEUS ACCUMBENS ACROSS CHRONICITY OF BINGE EATING IN FEMALE RATS

By

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ABSTRACT

EXPLORING REWARD SYSTEM RESPONSIVITY IN THE NUCLEUS ACCUMBENS ACROSS CHRONICITY OF BINGE EATING IN FEMALE RATS

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Objective: Binge eating is characterized by an overconsumption of palatable food, a natural reinforcer. Therefore, there is increased interest in the role of reward-based processes in binge eating. To date, results have been mixed across studies examining reward system responsivity and binge eating, showing both increased and decreased activation in the nucleus accumbens and other brain structures within reward pathways. One contributing factor to differences in results might be chronicity of binge eating (i.e., early vs. chronic), where the reward system is initially hyper-responsive to binge eating, but over time, the system becomes hypo-responsive to binge eating. As a result, in later stages of binge eating, more frequent or more severe levels of binge eating might be needed to achieve the same level of responsivity. Despite chronicity of illness being a plausible mechanism to explain differences in reward-related responsivity, no studies have examined duration of binge eating as a potential factor contributing to differences in responsivity over time. The current study used an animal model of binge eating to directly examine differences in brain activation in response to palatable food in the nucleus accumbens across chronicity of binge eating. **Methods:** 120 Sprague-Dawley female rats were exposed to intermittent, palatable food feeding tests. Binge eating prone (BEP) and binge eating resistant (BER) rats were identified using established methods, and randomly assigned to the early stage (i.e., six feeding tests) or chronic stage (i.e., 24 feeding tests) group. Fos expression, a measure of neural activation, was quantified in the nucleus accumbens and compared across the BER and BEP groups. **Results:** While there were no changes in palatable food intake (i.e., behavioral

responsivity) found over time in BEP rats, there were changes in neural responsivity over time in BEP rats. Specifically, BEP rats had higher levels of c-Fos expression in the nucleus accumbens core and shell at the early stage of binge eating, compared to BER rats, suggesting an initial hyper-responsivity to palatable food in BEP rats. At the chronic stage, BEP rats showed significantly lower levels of c-Fos in the nucleus accumbens core and shell, suggesting a downregulation in responsivity to palatable food over time. This change was specific to BEP rats, suggesting that the downregulation is in response to long-term, consistent, high-levels of palatable food intake found in BEP rats (rather than lower levels of consistent palatable food intake found in BER rats). **Discussion:** These data strongly suggest that duration of binge eating leads to differences in neural function of the reward system. Furthermore, findings point to duration of binge eating as a factor contributing to inconsistent findings in past studies examining reward system functioning and binge eating. Results from the current study strongly suggest a need to control of stage of binge eating in future studies by including duration of illness as a covariate or examining different stages of binge eating as independent groups. Work should also focus on other structures (e.g., prefrontal cortex) and mechanisms (e.g., incentive motivation) in the reward system in order to understand if similar or other processes exhibit the same downregulation over time.

This dissertation is dedicated to my parents,
for their immeasurable support and encouragement.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Eating disorders (i.e., anorexia nervosa, bulimia nervosa, binge eating disorder) are serious mental health conditions that are etiologically complex. Among these disorders, binge eating is a core eating disorder symptom that is present across nearly all eating disorder diagnoses (e.g., bulimia nervosa, binge eating disorder, eating disorders otherwise specified; American Psychiatric Association, 2013). Binge eating involves the consumption of objectively large amounts of food in a short period of time; and those who binge eat feel they cannot control what and/or how much they are eating during a binge episode (APA, 2013). Critically, binge eating is associated with elevated rates of obesity (Spitzer et al., 1993; Stice, Cameron, Killen, Hayward, & Taylor, 1999; Stice, Presnell, & Spangler, 2002), significant medical consequences (e.g., type II diabetes; Herpertz et al., 1998), and poor psychosocial outcomes (Telch & Stice, 1998). Despite the notable negative impact of binge eating on both physiological and psychological health, the etiology of binge eating is still relatively unknown.

Given that binge eating episodes are characterized by an overconsumption of a palatable food (i.e., high in sugar and fat, low in nutritional value), a natural reinforcer (Berridge, 1996; Kelley & Berridge, 2002), there is high interest in neurobiological factors related to reward based processing in binge eating. Notably, previous work has shown a robust connection between palatable food intake and the reward system (e.g., Berridge, 1996). In general, neurotransmission of dopamine and opioids is activated, or increased, during receipt of a palatable food reward in animals as well as humans (Hernandez & Hoebel, 1988; Zhang, Gosnell, & Kelley, 1998). More specifically, research studies have repeatedly shown that dopamine and opioid neurons within the nucleus accumbens, a key structure underlying reward based processes (Carlezon & Thomas, 2009), are activated in response to palatable food intake in animals and humans (Alsiö et al.,

2010; Peciña & Berridge, 2000, 2005; Shin, Pistell, Phifer, & Berthoud, 2010; Wyvell & Berridge, 2000).

Importantly, studies in humans investigating reward system responsivity more broadly (i.e., beyond the nucleus accumbens) in binge eating and binge-related disorders (e.g., bulimia nervosa (BN), binge eating disorder (BED)) have shown increased activation (i.e., hyper-responsivity) in brain structures associated with reward in response to palatable food using neuroimaging techniques. For example, after viewing photos of palatable food, women with BN showed increased responsivity in the insula and anterior cingulate cortex compared to healthy control women, and increased activation in the medial prefrontal cortex was found in women with BED compared to healthy controls (Schienle, Schäfer, Hermann, & Vaitl, 2009). Furthermore, amplification of dopamine signaling, using a dopamine reuptake inhibitor, resulted in increased dopamine release in the caudate of BED individuals in response to palatable food (i.e., viewing, smelling, and tasting highly palatable foods; Wang et al., 2012). Taken together, while intake of palatable substances has been shown to lead to an increase in reward system responsivity generally, individuals that engage in binge eating appear to have hyper-responsivity of the reward system in response to palatable food intake.

However, other studies have found a *decrease* in reward-related activity in response to palatable food intake. For example, neuroimaging studies have reported that women with BN showed less activation in response to palatable food in structures associated with reward (e.g., insula) compared to control women (Bohon & Stice, 2011). Moreover, opioid responsivity, as measured by opioid receptor binding, has been observed to be lower in women with BN compared to healthy controls (Bencherif et al., 2005). Women recovered from BN for at least one year have also shown decreased dopamine responsivity in a PET scan in response to a

palatable sweet liquid compared to controls (Frank et al., 2006). Overall, these results suggest that there might actually be hypo-responsivity present in response to palatable food in women who binge eat.

Interestingly, previous investigators have hypothesized that this hypo-responsivity might be due to the amount of time participants engaged in binge eating, where longer-term binge eating might lead to decreased activation of the reward system (Bohon & Stice, 2011). Initially, individuals more prone to binge eating might have heightened or hyper-responsivity of the reward system in response to palatable food. This initial intense response might lead those individuals to engage in binge eating and increase binge eating frequency in order to achieve the highly rewarding response. However, over time, more chronic binge eating could lead to a down-regulation of the reward system (i.e., hypo-responsivity), which could, in turn, result in the need to engage in more frequent and/or more severe levels of binge eating in order to achieve the same rewarding response. This pattern could contribute to the discrepant results described above, with some studies showing hyper-responsivity/sensitivity (i.e., those in early stage binge eating) and others showing hypo-responsivity/sensitivity (i.e., those in the late/chronic stages of binge eating).

Unfortunately, the theory of differences in reward system responsivity *across* chronicity of binge eating has never been directly examined, nor has length of binge eating typically been reported or included as an important covariate in analyses. Furthermore, most work has compared clinical populations to healthy or obese controls (e.g., Bencherif et al., 2005; Bohon & Stice, 2011) rather than within one group (e.g., BN, BED) across chronicity of binge eating. However, some indirect evidence of differences across stages of binge eating have been found, such that normal weight children of obese parents (i.e., at risk group for development of binge

eating) exhibit hyper-responsivity of the reward system compared to individuals at low-risk for obesity in response to palatable food, thus suggesting hyper-responsivity prior to over-consumption (Stice, Yokum, Burger, Epstein, & Small, 2011). Moreover, lower dopamine responsivity has been found in the nucleus accumbens of rats after long-term/chronic exposure to palatable food (suggesting hypo-responsivity at chronic stages, Alsiö et al., 2010). While there are limited findings related to binge eating chronicity, differences in reward system responsivity across chronicity (i.e., hyper- at early, hypo- at chronic) have been observed for artificially rewarding substances (e.g., drugs, alcohol) that target the same reward-related brain structures and systems (e.g., nucleus accumbens) as palatable food (see Boileau et al., 2003; Dawe, Gullo, & Loxton, 2004; Nestler, 2005; Willner, James, & Morgan, 2005). Thus, it is possible that the same pattern of differential responsiveness in brain reward pathways is present across stage of binge eating in response to a natural reinforcer like palatable food. One fruitful way to explore this possibility is to use animal models to directly compare brain responsiveness across chronicity of binge eating. Animal models allow for the ability to directly examine brain structures associated with the reward system (e.g., nucleus accumbens), and activation in these areas can be pinpointed at clearly defined stages of binge eating (e.g., early stage, chronic stage).

One particularly strong model of binge eating in animals is the well-established Binge Eating Resistant/Binge Eating Prone animal model of binge eating (see Boggiano et al., 2007). The BER/BEP model identifies binge eating prone (BEP) and binge eating resistant (BER) rats based on amount of palatable food consistently consumed during the first four hours of intermittent (i.e., approximately every other day) 24-hour feeding tests. Four-hour intakes have been shown to be a reliable time frame to observe differences in palatable food intake (e.g., Boggiano et al., 2007; Klump, Suisman, Culbert, Kashy, & Sisk, 2011; Oswald, Murdaugh,

King, & Boggiano, 2011; Sinclair et al., 2015), similar to binge eating patterns observed in humans, which occur over a short period of time (APA, 2013). Notably, prior work has only found differences in palatable food intake after *intermittent* exposure of palatable food, not continuous access, suggesting that BEP rats demonstrate binge eating rather than just a preference for palatable food (Boggiano et al., 2007). Additionally, the intermittent presentation of palatable food feeding tests differentiates the BER/BEP model from other models of food intake (e.g., diet induced obesity; see Hariri & Thibault, 2010 for review) where animals are allowed continuous access to palatable food, often leading to significant increases in body weight rather than binge-like eating. Animals classified as BEP consistently consume high amounts of palatable food during feeding tests (see Methods for classification methods). Importantly, BEP rats binge eat on palatable food, but they do not over-consume chow (Boggiano et al., 2007; Boggiano, Dorsey, Thomas, & Murdaugh, 2009; Klump, Suisman, Culbert, Kashy, Keel, et al., 2011; Klump, Suisman, Culbert, Kashy, & Sisk, 2011; Oswald et al., 2011). The preference for palatable food, rather than chow, during binge eating episodes in BEP rats is similar to the binge eating that is present in humans where food consumed during binge eating episodes is typically highly palatable food (Hagan, Chandler, Wauford, Rybak, & Oswald, 2003). BEP animals do not differ in body weight when compared to BER animals (Boggiano et al., 2007). This is similar to data showing that women with BN typically are not overweight but are of average weight (Boggiano et al., 2007; Oswald et al., 2011). Finally, the model also examines palatable food intake on a continuum, from low to high palatable food intake where, similar to humans, all animals consume some amount of palatable food, but might not fall into the extreme BER or BEP groups. This feature makes the BER/BEP model particularly attractive for examining chronicity, as it allows for examination of general differences in reward system responsivity (i.e.,

in response to palatable food intake generally in all rats), as well as in more extreme binge eating phenotypes (i.e., BER and BEP groups).

Notably, previous work using the BER/BEP model has shown increased activation in the nucleus accumbens core and shell of BEP rats as compared to BER rats, after only 8 feeding tests (Sinclair et al., 2015). This pattern of effects suggests that there is overall hyper-responsivity in both areas of the nucleus accumbens in response to palatable food at the early stages of binge eating. This study did not examine animals in more chronic stages of binge eating (e.g., 9 tests or more) and thus, it is unknown whether hypo-responsivity would be observed in later stages of binge eating.

Therefore, the current study aimed to be the first to examine whether responsivity to palatable food changes across chronicity of binge eating using the BER/BEP model. The proposed study examined responsivity in two ways – by examining changes in palatable food intake and changes in neural activation in the nucleus accumbens at early versus chronic stages of binge eating. The focus on both behavioral and neural indices of responsivity comes from studies in substance abuse showing an increase in the amount of a substance used/consumed over time in order to compensate for a downregulation of the reward system (e.g., Boileau et al., 2003; Dawe et al., 2004; Nestler, 2005; Willner et al., 2005).

The current study focused on neural responsivity in the nucleus accumbens core and shell given previous work showing that the nucleus accumbens is activated in response to both natural (e.g., food) and artificial (e.g., alcohol) substances of abuse. The core and shell are involved in the hedonic (i.e., pleasure) properties of natural and artificial rewards, as well as the motivation (i.e., implicit drive) to consume rewards. Notably, the nucleus accumbens is also activated in both animals and humans that binge eat (e.g., Alsiö et al., 2010; Peciña & Berridge, 2000, 2005;

Shin et al., 2010; Wyvell & Berridge, 2000), and recent work has targeted the nucleus accumbens as a region of interest in treatment of binge eating using deep brain stimulation techniques (Doucette, Khokhar, & Green, 2015; Halpern et al., 2013). Taken together, evidence suggests that the nucleus accumbens plays a key role in binge eating behaviors.

In the current study, there were several hypotheses across both the neural and behavioral indices of responsivity. It was anticipated that at the early stages of binge eating, BEP animals would have greater activation in the nucleus accumbens (i.e., increased responsivity) in response to palatable food as compared to BER rats (replicating previous work, Sinclair et al., 2015). However, at the chronic stage, it was anticipated that the pattern would be reversed such that chronic stage BEP animals would have lower responsivity compared to early stage BEP rats and chronic stage BER rats. Second, it was also expected that BEP animals would consume higher amounts of palatable food compared to BER rats at the early stage of binge eating. It was further anticipated that this BER/BEP difference would increase in the chronic stage of binge eating, as BEP rats would increase their palatable food intake over time to compensate for a downregulation of the reward system. These patterns would suggest an overall downregulation in responsivity of the nucleus accumbens that is specific BEP animals after chronic, long-term high consumption of palatable food.

METHODS

Animals

A sample of 120 adult Sprague-Dawley female rats was obtained from Harlan (Madison, Wisconsin) on approximately postnatal day 70 (P70). Rats were individually housed in clear Plexiglas cages (45 x 23 x 21 cm) that were outfitted with a wire cage lid. Animals were given ad

libitum access to both standard chow (Rodent diet 8640; Harlan Teklad Global Diets, Madison, Wisconsin) and water. Temperature was held at 21 ± 2°C, and the room was on a light cycle allowing for 12 hours of light and 12 hours of dark (on at 2400h, off at 1200h). Animals were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals; and all protocols were approved by the Michigan State University Institutional Animal Care and Use Committee.

Experimental Design

The study followed the well-established binge eating resistant/binge eating prone (BER/BEP) animal model of binge eating (Boggiano et al., 2007), with modifications to feeding test frequency and duration (see Feeding Tests below). Similar to previous work using the BER/BEP model, feeding tests were administered three times per week (Monday, Wednesday, and Friday) (e.g., Klump, Racine, Hildebrandt, & Sisk, 2013; Klump, Suisman, Culbert, Kashy, & Sisk, 2011). Administration of feeding tests three times per week has produced highly similar results compared to studies using the original BER/BEP model feeding test frequency (i.e., 2x/week; Boggiano et al., 2007). Furthermore, this modification helps to generate more feeding test data for determining binge eating status.

Animals were run in two cohorts (n = 60 per cohort) to accommodate the collection of palatable food, chow, and body weight measurements under the time constraints. Each day prior to dark onset (1200hr), daily body weight and chow measurements were recorded. Chow was in pellet form to make locating spillage in the bedding easier. Any spillage detected after searching the bedding was added to the chow measurement. All body weight and chow measurements were taken to the nearest tenth of a gram using an electronic scale.

On feeding test days, rats had ad libitum access to chow, water, and palatable food for the entirety of the feeding test. Previous work using the BER/BEP model has examined palatable food and chow intake at the 1, 4, and 24-hour time points (e.g., Klump et al., 2013; Klump, Suisman, Culbert, Kashy, & Sisk, 2011). Across studies, the 4-hour time point has been consistently used to determine binge eating status (e.g., Boggiano et al., 2007; Klump et al., 2013) given that this is most reflective of binge eating episodes in humans (i.e., binge eating episodes take place over a short period of time; APA, 2013). Furthermore, previous work examining c-Fos activation in response to palatable food in the nucleus accumbens using the BER/BEP model removed the palatable food after the measurement at the 4-hour time point (Sinclair et al., 2015) and found higher activation in the nucleus accumbens of binge eating prone rats compared to binge eating resistant rats. Therefore, the current study modeled this pattern of exposure and removed the palatable food from the cages after the 4-hour measurement on feeding test days.

Feeding tests began at 1200h. Fifty to eighty grams of new chow was added to the cage, as well as 20–25g of palatable food (i.e., Betty Crocker Creamy Vanilla Frosting, General Mills Inc., Minneapolis, MN; for example, used previously in Hildebrandt, Klump, Racine, & Sisk, 2014; Klump, Suisman, Culbert, Kashy, Keel, et al., 2011). Palatable food was placed in small petri dishes and hung inside the cage via wire hook. Palatable food was left in position for the full 4-hours of the feeding tests. Both chow and palatable food were weighed at the 4-hour time point using the same weighing and rounding cutoffs described above. The feeding tests were administered identically each time following the same procedures described.

Assignment of Early and Chronic Stage and BER/BEP Phenotypes

Classification of Early versus Chronic Stage. While there are no established criteria for

defining early versus chronic stages of binge eating in rats (or in humans, for that matter), the classifications used in this study were based off of pilot data from our lab and corroborative evidence in the field. Specifically, early stage binge eating was defined as binge eating in the first 6 feeding tests only. These 6 feedings tests occur over a relatively brief period of time (i.e., 12 days/~2 weeks in the rat life cycle, which is equivalent to ~1 year in a human - see Andreollo, Santos, Araújo, & Lopes, 2012) and are similar to what has been shown to produce activation in the nucleus accumbens in BEP rats (i.e., 8 feeding tests; Sinclair et al., 2015). Consequently, animals that were randomized to the early stage condition were immediately sacrificed after the initial 6 tests (see Figure 1).

Animals randomized to the chronic stage condition completed an additional 18 feeding tests, for a total of 24 feeding tests across 60 days/9 weeks (i.e., four times longer than the early stage rats)[1]. Chronic stage animals were sacrificed after the 24th feeding test (see Figure 1). Again, given that there are no established criteria for defining chronic stage of binge eating in animals, this number of feeding tests was selected since it is at least two times longer than the typical number of tests in previous adulthood studies of the BER/BEP model (Boggiano et al., 2007; Klump et al., 2013; Sinclair et al., 2015), and therefore represents a more "chronic" type of exposure to palatable food than what has been used previously. Given the duration of time chronic stage animals are included in the study (60 days), it is important to note that the chronic stage group were still young adults at the end of the study (i.e., 19 weeks of age, P133) and thus

[1] During Cohort 1's feeding test 12, palatable food was removed from cages after 8 hours instead of the standard 4 hours. Due to this experimental error, feeding test 12 has been removed from all analyses, including phenotyping. Therefore, phenotyping of the second group of feeding tests is based on data from feeding test 7 to feeding test 11. Additionally, given that the overall aim of the current study was to investigate brain responsivity to palatable food exposure over time, Cohort 2 received the same duration of palatable food exposure during feeding test 12 (i.e., 8 hours) to ensure any observed differences in c-Fos responsivity were unrelated to the error in Cohort 1.

likely did not experience age-related declines in food intake that have been shown to present in middle and older adulthood (see Thomas, Rice, Weinstock, & Corwin, 2002). Moreover, the length of time for the feeding tests (i.e., 9 weeks) in the rats is equivalent to ~5 years in a human's life course (see Andreollo et al., 2012). Previous work has suggested that a large portion of women are fully recovered, or partially recovered, from BN within 5 years of disorder onset (Fairburn, Cooper, Doll, Norman, & O'Connor, 2000; Keski-Rahkonen et al., 2009). Therefore, the equivalent of 5 years of binge eating suggested for the chronic stage in the current study likely reflected a chronic stage of binge eating in human eating disorders.

Classification of BER/BEP Phenotypes. Using methods established by Boggiano et al. (2007) and Klump et al. (2011), animals were identified as binge eating resistant (BER) or binge eating prone (BEP) by examining the 4-hour time point of palatable food intake across the initial six feeding tests. Tertiles for determining BER/BEP groups were calculated using total palatable food intake across all animals. Animals scoring in the top tertile of 4-hour palatable food intake half of the time (i.e., 3 out of 6 feeding tests, 50%), and never scoring in the bottom tertile, were classified as BEP. Conversely, animals scoring in the bottom tertile half of the time, and never eating in the top tertile, were classified as BER. For the current study, 50% (i.e., 3 out of 6 feeding tests) was used to determine binge eating status. While criteria used to define BER and BEP classifications have varied across previous studies using the BER/BEP model, where some studies have required four out of six and/or five out of six feeding tests to determine BER/BEP status, results have been similar when using 50% as criteria, as well as at more stringent classifications (Hildebrandt et al., 2014; Klump et al., 2013). The 50% criterion also resulted in the highest number of BER and BEP animals, and is the same classification used in previous work where differences in responsivity in the nucleus accumbens were found between BER and

BEP rats (Sinclair et al., 2015).

Notably, tertiles were calculated using three different approaches. Immediately after the 6th feeding test (and before the "early binge eating" cohort was sacrificed), palatable food intake tertiles were calculated, and animals were phenotyped as BER, BEP, or neutral (i.e., did not meet criteria to be phenotyped as BER or BEP, see Table 1 – cohort specific tertiles). Because animals were run in two cohorts, these tertiles were cohort specific, but they were necessary in order to randomize animals to the early/chronic groups and ensure that there were approximately equal numbers of BER, BEP, and neutral rats in these groups (see Figure 1).

However, given that the ultimate goal was to combine across cohorts in order to increase statistical power and maximize animals available for analyses, "combined" tertiles were calculated that aggregated data across cohorts and was based on palatable food consumption during the first 6 feeding tests in the full sample of rats ($N = 120$). Critically, prior to combining cohort data, potential cohort differences in variables of interest (i.e., 4-hour palatable food intake, 4-hour feeding test chow intake) and body weight across the first 6 feeding tests were examined. Results from independent sample t-tests showed no significant differences in 4-hour palatable food or 4-hour chow intake between cohorts (see Table 2; ps = 0.16 – 0.88), but a significant difference in body weight ($p = <.001$) was found. Despite differences in body weight, there was no significant correlation between average body weight and average palatable food consumption across the first six feeding tests ($r = 0.05$, $p = 0.64$), suggesting that body weight was independent of palatable food consumed. Additionally, since all animals fell within standards for expected body weight for Sprague-Dawley female rats (see Growth Curve data for Sprague-Dawley rats: www.Harlan.com; Harlan Laboratories, Inc.), cohort differences in body weight were likely within normal expected variation. Therefore, cohort 1 and cohort 2 data were

combined, and final BER/BEP classifications were based on the combined cohort 1 and cohort 2 tertiles (see final tertiles, Table 1). A small subset of animals (BER = 2/25 (8%), BEP = 1/18 (6%) originally phenotyped as BER or BEP became "neutral" when using the combined tertile calculations. Notably, no animals switched phenotype (i.e., BER to BEP or vice versa) when tertiles we recalculated, providing further evidence for limited differences between cohorts.

Finally, given that the overall aim of the current study was to examine the neural responsivity of a group of animals that *consistently* engaged in binge eating over an extended period of time, it was important to investigate the stability of the BEP phenotype over time. If analyses included all phenotyped animals originally based on the first six feeding tests, this would not account for animals that did not continue to maintain their phenotype, and thus, were not a true chronic binge eating group. Therefore, using the combined, final cohort data, chronic stage animals were phenotyped for BER/BEP status every 6 feeding tests across tests 7 to 24 (excluding feeding test 12) using the methods described above (i.e., a total of 4 feeding test groupings: feeding tests 1-6, 7-11, 13-18, 19-24). Notably, with the exception of one chronic stage phenotyped animal (that changed from BEP in the first 6 feeding tests to BER in the last 6 feeding tests), no chronic stage animals transitioned between BER/BEP phenotypes (i.e., changed from BER to BEP or vice versa) across the study period.

However, not all animals phenotyped as BER or BEP after the first 6 feeding tests met the stringent criteria to be classified as BER or BEP at every feeding test grouping. Across the four different BER/BEP groupings, there was variability in how many times a chronic stage BER (total $n = 12$) or chronic stage BEP (total $n = 10$) rat met criteria for: a) one out of four groupings (BER = 2/12 (17%), BEP = 4/10 (30%)), b) two out of four groupings (BER = 5/12 (42%), BEP = 3/10 (30%)), c) three out of four groupings (BER = 2/12 (17%), BEP = 2/10 (20%)), d) four

out of four groupings (BER = 3/12 (25%), BEP = 1/10 (10%)), or e) changed phenotypes across the study (i.e., changed from BER to BEP or vice versa, BER = 0/12 (0%), BEP = 1/10 (10%)). In an effort to examine a more consistent group of BER and BEP animals, a "Consistent Chronic" group of BER/BEP rats was identified. The consistent rats met criteria in the first six feeding tests *and* at least one additional feeding test grouping in the chronic stage (consistent chronic BER = 10/12 (83%), consistent chronic BEP = 6/9 (67%); see Table 1). The 2 out of 4 criterion was selected to maximize chronic stage sample sizes while still examining a group of animals that more consistently exhibited their phenotype throughout the chronic stage. All behavioral analyses (i.e., palatable food intake, chow intake, body weight) and categorical analyses (see Statistical Analyses) focused on this consistent chronic group. Notably, however, results were broadly consistent when using BER/BEP phenotypes from the first 6 feeding tests only (data not shown), and all animals (even those that were not consistently BER or BEP) were included in the continuous analyses BER/BEP analyses (see Statistical Analyses below).

It should be noted that the number/proportion of chronic stage animals initially phenotyped as BEP meeting criteria at some, but not all, chronic stage feeding test groupings (i.e., feeding tests 1-6, 7-11, 13-18, 19-24) does not suggest unreliability of the BEP phenotype. Rather, it points to a similarity between humans with binge related disorders (i.e., bulimia nervosa, binge eating disorder). Previous work has shown that humans with binge related disorders have periods of recovery and relapse over time rather than remaining in a consistent binge eating state across longer durations of illness. Relapse rates in women with bulimia nervosa across nine years have been shown to be approximately one third (i.e., 35%; Keel, Dorer, Franko, Jackson, & Herzog, 2005). Similar rates of relapse (i.e., 27%) have also been found to occur within six months after discharge from treatment, where a significant predictor of

relapse was severity and frequency of binge eating/purging behaviors (Olmsted, MacDonald, McFarlane, Trottier, & Colton, 2015).

Determination of a Continuous BER/BEP Tertile Count. While the categorical approach described above allows for examination of extreme binge eating and non-binge eating groups, the majority of animals did not meet these stringent classification requirements and fell into the "neutral" category (n = 69) or the "inconsistent" chronic BER/BEP group (n = 6). In order to utilize these animals and maximize sample sizes, a continuous BEP variable approach was used that has been used extensively in the past (e.g., Hildebrandt et al., 2014; Klump, Suisman, Culbert, Kashy, & Sisk, 2011) and allows for the inclusion of all animals in the study. This approach uses a binge eating prone "count" variable that counts the number of times each rat scored in the highest tertile (i.e., highest palatable food intake) across the feeding tests (score range= 0-6 for early stage animals, 0-23 for chronic stage animals).[2] Because there was an uneven number of feeding tests between the two stages, all count variables were standardized within stage via z scores prior to analyses.

Induction of c-Fos and Quantification of c-Fos

Palatable Food Exposure for c-Fos Stimulation and Perfusions. Procedures followed methods used by previous studies for palatable food exposure and perfusions (see Sinclair et al., 2015), where prior to sacrifice, animals were exposed to palatable food for one hour in order to stimulate c-Fos expression. By exposing animals to palatable food prior to sacrifice, the current study modeled human studies examining brain responsivity to palatable food, where differences

[2]A BER tertile count variable was calculated as well, but it was highly correlated with the BEP count variable ($r = 0.89$, $p < .001$). Given the substantial overlap in variance between the BEP and BER count variables, all analyses focused on the BEP tertile counts only.

in brain responsivity have been examined across groups (e.g., BN, BED) after equal exposure to a palatable substance (e.g., Stice, Yokum, Blum, & Bohon, 2010).

All animals were sacrificed by an injection of sodium pentobarbital based on body weight (~0.7mL) 90 minutes after initial palatable food exposure. Previous findings have shown that translation of c-Fos mRNA into Fos protein peaks between 60 and 120 minutes after initial exposure to food rewards to euthanasia injection (Blancas, González-García, Rodríguez, & Escobar, 2014; Gaykema et al., 2014). Therefore, 90 minutes was selected in order to capture the highest peak of c-Fos activation in response to palatable food. Rats were then sacrificed via intracardial perfusion (i.e., process of fixing tissue for collection) with 300mL of 0.1M sodium phosphate buffered saline rinse followed by 300mL of 4% paraformaldehyde in 0.1M sodium phosphate buffered saline. Brains were removed and placed in 4% paraformaldehyde overnight, then transferred and stored in 20% sucrose in 0.1M sodium phosphate buffered saline. Brains were sectioned at 40µm into four series using a Cryostat and stored in cryoprotectant at -20°C.

Immunohistochemistry for c-Fos. A group of animals was selected for c-Fos immunohistochemistry (see Table 1). These animals included the majority of BER and BEP animals, as well as a random sample of neutral animals that had good tissue quality ratings (see below). Notably, a very small number of BER/BEP animals (N = 5 animals; N = 3 BER (2 early stage, 1 chronic state); N = 2 BEP (both early stage) were mistakenly excluded from c-Fos immunohistochemistry. Fortunately, the vast majority (i.e., 4/5) of excluded rats were early stage animals; given that early stage analyses were essentially replications of prior work (see Sinclair et al., 2015), this omission had minimal effects on analyses of the primary study aim (i.e., to examine hypo-responsivity in the chronic stages of binge eating).

Methods for immunohistochemistry followed well-established protocols used in previous work (e.g., Sinclair et al., 2015). One series of brain sections from the selected animals were placed into well-plates, with one animal per well. Sections were rinsed in 0.05M Tris-buffered saline (TBS, 4 rinses x 5 minutes), followed by 10 minutes in 1% hydrogen peroxide in TBS. Sections were rinsed in TBS (3 rinses x 5 minutes) and then tissue was immersed in a blocking solution of 20% normal goat serum (NGS; Pel Freez Biologicals, Rogers, AR) in 0.3% Triton X-100 in TBS for 30 minutes. Blocking was followed by a 48 hours incubation at 4°C in a 1:10,000 dilution of c-Fos primary antisera made in rabbit (Santa Cruz Biotech; Santa Cruz, CA) in TBS with 2% NGS and 0.3% Triton X-100. After primary incubation, tissue was rinsed in TBS (3 rinses x 10 minutes) and then tissue was exposed to 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 for 60 minutes. After secondary incubation, tissue was rinsed in TBS (3 rinses x 10 minutes) and then exposed to avidin-biotin complex (Vector Laboratories, Burlingame, CA) for 60 minutes followed by rinsing in TBS (3 rinses x 5 minutes). Diaminobenzidine tetrahydrochloride (10mg tablets; Sigma-Aldrich, St. Louis, MO) was dissolved in TBS with 30% hydrogen peroxide to visualize brown-black Fos-immunoreactive (Fos-ir) nuclei. Sections were mounted onto slides, dehydraded using a graded alcohol series, cleared in xylene, and coverslipped.

Cresyl Violet Staining and Nucleus Accumbens Tracing. A second series of nucleus accumbens sections were mounted onto slides, dehydrated with a graded alcohol series, stained with cresyl violet, and coverslipped. This series was used to identify regions of interest (i.e., nucleus accumbens core and shell). Nissl-stained sections were selected corresponding to plates 13-20 (between +2.52 mm and +1.56 mm from Bregma) of the Paxinos and Watson (2005) brain

atlas. Manual bilateral tracing of the nucleus accumbens core and shell was done under a 4x (NA 0.13) air objective using Neurolucida (version 7; Microbrightfield, Williston, VT, USA) and an Olympus BX51 microscope and Q-Imaging Color 12 bit camera.

Evaluation of Immunohistochemistry Processed Tissue. After completion of immunohistochemistry and nucleus accumbens tracing, slides were examined under a microscope to determine overall tissue quality in order to remove poor quality tissue from analyses that might negatively contribute to results. Tissue from each rat processed for c-Fos immunohistochemistry (see Table 1) was given a score of 0, 1, or 2 based on the overall quality and appearance of the tissue. The principal investigator served as the rater and was blinded to BER/BEP phenotype and early/chronic stage during rating of tissue quality. Brain tissue that had holes or cuts in the nucleus accumbens region, and/or uneven staining, was given a score of "0". A score of "1" indicated that the tissue had some holes and/or minimal staining imperfections, but overall, there were no major impediments to counting the fos cells. Finally, a score of "2" was given to tissue that was free of major flaws (e.g., holes), staining was even, and fos cells were easily identifiable. All animals with a score of "1" or "2" were included in statistical analyses described below (see Table 1), but animals with a score of "0" were excluded from analyses of c-Fos data (number excluded: early stage BER = 5/16 (31%), early stage BEP = 4/9 (44%), early stage neutrals = 0/3 (0%), consistent chronic stage BER = 1/9 (11%), consistent chronic stage BEP = 0/10 (0%), chronic stage neutrals = 0/6 (0%)). It should be noted that there was a higher percentage of early stage animals that were excluded due to poor tissue quality than chronic stage. While it is difficult to determine exactly what contributed to poorer tissue quality, it may have been due to experimenter training progress (e.g., learning how to section brain tissue, conduct perfusions, and refine immunohistochemistry skills). Notably, a majority of

chronic stage animals received good tissue quality ratings (i.e., "1" or "2"), and were able to be included in analyses examining primary study hypotheses. Additionally, all animals, regardless of tissue quality rating, could be included in behavioral analyses (i.e., palatable food intake, chow intake, and body weight) since the outcome variable was not related to c-Fos.[3]

Quantification of c-Fos Cells. Similar to tracing methods used above, all measurements of c-Fos were made using Neurolucida programming and an Olympus BX51 microscope and Q-Imaging Color 12 bit camera. Cell counts were performed at higher magnification with a 40x (NA 0.85) air objective. Four traces from the Nissl-stained sections were selected and matched to corresponding immunohistochemically treated tissue sections for cell counting. Cell counts were made within each traced section by a single experimenter who was blinded to BER/BEP phenotype. After completion of counting, Neurolucida Explorer was used to determine an average Fos density (in fos cells/mm^2) for each traced section (i.e., nucleus accumbens core and shell in left and right hemispheres).

Statistical Analyses

Data Preparation. Prior to analyses, all data was examined for outliers by examining the skewness and kurtosis of the data. Within a range of -1 to +1 was considered normally distributed, and no follow up tests to detect specific outliers was needed. No outliers were detected in the behavioral data (i.e., palatable food intake, chow intake, body weight), but c-Fos data were followed up using the outlier labeling approach (Hoaglin & Iglewicz, 1987; Hoaglin, Iglewicz, & Tukey, 1986) in which the top and bottom quartiles of c-Fos densities were calculated separately for each group (i.e., early stage BER, early stage BEP, consistent chronic

[3] Behavioral analyses were also run examining only the animals included in the categorical ANCOVA analyses (i.e., animals that received a tissue quality ratings of "1" or "2"). Results were consistent with the results reported below (data not shown).

BER, consistent chronic BEP) in order to assess group specific outliers. For each group, the bottom quartile was subtracted from the top quartile and multiplied by a standard factor "g" (i.e., 1.5). This value was then subtracted from the bottom quartile value and added to the top quartile value to provide a standard range for the data. Data points that fell above or below these thresholds were considered outliers. This process was done separately for the nucleus accumbens core and shell. A small amount of outliers were found and excluded from c-Fos analyses (see Table 1 for the final sample sizes for these analyses).

Analysis of c-Fos Activation. Analyses comparing BER and BEP rats in their c-Fos activation in the nucleus accumbens core and shell tested whether there were neural differences in responsivity in the nucleus accumbens across duration of binge eating in BER and BEP animals. Analysis of covariance (ANCOVA) was used to compare differences in c-Fos expression (in fos cells/mm^2) across stage (early versus chronic) and BER/BEP phenotype. Since there was a significant difference between BEP rats (M = 6.00, SD = 1.25) and BER rats (M = 5.09, SD = 1.17) in amount of palatable food consumed during the 1-hour exposure prior to perfusion (see Methods; t(49) = -2.62, p = .01), the 1-hour palatable food consumed measurement was included as a covariate in analyses to ensure that any observed differences in c-Fos expression were related to underlying neural differences in response to palatable food rather than the amount of palatable food consumed (Sinclair et al., 2015).[4]

ANCOVAs were run twice, separately for the nucleus accumbens core and shell.[5] The main effect of binge eating phenotype (i.e., BER, BEP) tested whether there are differences in c-

[4] ANCOVA analyses were also run without covarying amount of palatable food consumed in the 1-hour prior to sacrifice. Results were consistent with the results reported below (data not shown).

[5] Analyses examining differences in c-Fos responsivity in the nucleus accumbens core and shell across BER and BEP animals were run including body weight on sacrifice day as a covariate in

Fos expression in BER and BEP rats, regardless of chronicity, while main effects of stage (i.e., early, chronic) examined if c-Fos expression was different across stages of binge eating, independent of binge eating phenotype. The phenotype (i.e., BER, BEP) by stage (i.e., early, chronic) interaction examined if there were significant differences in c-Fos expression across different phenotypes at different stages of binge eating. Significant interaction effects were followed-up using additional ANCOVAs that examined the effects of stage (i.e., BER, BEP) and phenotype (i.e., early, chronic) in separate models. Notably, these phenotype by stage interactions were of primary interest in this study, as they tested whether c-Fos differences between BER and BEP rats differed by stage, such that 1) early stage BEP rats had significantly higher c-Fos activation than early stage BER rats; while 2) chronic stage BEP rats had significantly lower c-Fos activation as compared to chronic stage BER rats and early stage BEP rats.

In addition to categorical analyses examining BER and BEP phenotypes, linear regressions were used to examine the continuous BEP tertile counts. These analyses included all animals processed for c-Fos (see Table 1), including the neutral animals and the inconsistently chronic BER and BEP animals. The standardized BEP tertile count variable was entered as a predictor of c-Fos expression (the outcome variable), and stage of binge eating was examined as a moderator (i.e., early, chronic) of the association between BEP counts and c-Fos expression. The main effect of BEP tertile count and the BEP tertile count by stage interaction was tested. The main effect of BEP count tested whether differences in c-Fos expression varied across BEP counts regardless of stage, while main effects of stage (i.e., early, chronic) examined if c-Fos

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order to further account for possible effects of body weight. Notably, there was no significant main effect of body weight (nucleus accumbens core: $F(1,20) = 0.67$, $p = 0.42$; nucleus accumbens shell $F(1,21) = 0.14$, $p = 0.71$), and results and patterns of effects were similar to analyses not including body weight as a possible covariate (data not shown).

activation is different across stages of binge eating, regardless of BEP count. The interaction (BEP tertile count x stage) was of primary interest since it examined if the relationship between BEP tertile count and c-Fos expression was moderated by stage of binge eating. Significant interactions were followed-up using a graphical analysis to determine the direction of effects. Effects were plotted according to 1 standard deviation below the binge eating prone count mean (i.e., -1: "lower"), and 1 standard deviation above the mean (i.e., 1: "higher"). Similar to hypotheses for the categorical BER/BEP groups, it was expected that this interaction would be significant and show a positive association between BEP counts and c-Fos density in the core and shell at the early stage. At the chronic stage, a negative association was anticipated between BEP counts and c-Fos density in the core and shell.

Analyses of Palatable Food Intake. Analyses of palatable food intake were completed in order to investigate the behavioral index of potential downregulation of the reward system across stage of binge eating. In addition, chow intake and body weight were also examined in order to rule out the possibility that observed changes in PF consumption and neural responsivity were due to changes these variables over time.

Mixed linear models (MLM) were used to compare differences in 4-hour palatable food consumption, 4-hour chow consumption on feeding test days, 24-hour chow consumption on days following feeding tests, and body weights between BER and BEP groups across the study period.[6] These analyses included early stage BER and BEP animals phenotyped after the first six feeding tests and consistent chronic animals (see Table 1). In these models, the upper-level unit of analysis was the animal (i.e., the level at which observations are independent), and the lower-

[6] Notably, MLMs are able to account for the varying amounts of repeated data between the early (6 feeding tests) and chronic (23 feeding tests) stage animals (see Hektner, Schmidt, & Csikszentmihalyi, 2007).

level unit of analysis was feeding test (i.e., the level at which outcome scores are measured). Phenotype (i.e., BER, BEP), and stage of binge eating (i.e., early, chronic) were entered as predictors in the model, and an autoregressive (lag 1) error structure was used to model the residual covariance from one feeding test/measurement to the next. The main effect of binge eating phenotype (i.e., BER, BEP) tested whether there were differences in behavioral data (i.e., palatable food, chow, body weight) in BER and BEP rats, regardless of chronicity, while main effects of stage (i.e., early, chronic) examined if behavioral data was different across stages of binge eating, independent of binge eating phenotype. The phenotype (i.e., BER, BEP) by stage (i.e., early, chronic) interaction examined if there were significant differences in behavioral data across different phenotypes at different stages of binge eating. The phenotype by stage interaction was of primary interest given that it directly tested whether chronic stage BEP rats consumed significantly higher amounts of palatable food than BER rats (at all stages) and the early stage BEP rats.

RESULTS

c-Fos Responsivity

Overall, results from the ANCOVAs of categorical phenotypes and regression analyses of BEP tertile counts generally confirmed study hypotheses. BEP rats were found to have significantly higher responsivity in the nucleus accumbens at the early stage of binge eating, but they tended to show decreased responsivity in the chronic stages. Results were more robust for the core than the shell, and at times, findings were only of trend-level significance. Nonetheless, effect sizes were medium-to-large across all analyses (partial eta squared (η^2) = .06 - .49; *partial*

η^2 = .01; medium, *partial* η^2 = .06; large, *partial* η^2 = .14), suggesting that the effects are biologically significant and robust.

c-Fos responsivity in the Nucleus Accumbens Core. Categorical ANCOVA results showed a significant main effect of phenotype ($F(1,21) = 20.06$, $p < .001$), with increased responsivity in BEP as compared to BER rats, regardless of stage. Although there were no significant effects of stage ($F(1,21) = 1.28$, $p = 0.27$), there was a trend towards a phenotype x stage interaction ($F(1,21) = 3.24$, $p = 0.08$) that was of large effect size (see Table 3, $d = 2.08$). Follow-up ANCOVA analyses were used to decompose this interaction by investigating BER/BEP group differences within stage (i.e., BER vs. BEP in the early stage group; BER vs. BEP in the chronic stage group) and within phenotype (i.e., early BER vs. chronic BER; early BEP vs. chronic BEP; see Table 4). Within stage analyses revealed that at the early stage, BEP animals had significantly higher densities of c-Fos cells compared to early stage BER animals (see Figure 2). This finding supported study hypotheses and previous work (Sinclair et al., 2015), such that BEP animals in the early stage of binge eating have a stronger neural response to palatable food. By contrast, in the chronic stage, BEP animals showed no significant difference in c-Fos density as compared to BER animals, While it was expected that chronic stage BEP rats would have lower responsivity compared to chronic stage BER rats, group differences were attenuated compared to the early stage. Within phenotype analyses suggested that the attenuation of differences was due to changes in responsivity in BEP rats, as these rats showed lower c-Fos density at the chronic stage compared to the early stage. While this difference did not reach statistical significance, the effect size was large in magnitude ($d = 1.18$). Finally, there were no significant differences in c-Fos densities between early stage and chronic stage BER rats, suggesting minimal changes in responsivity in BER animals.

Results for the BEP tertile count variable were highly similar to those reported above (see Figure 3 and Table 5). Once again, there was a significant main effect of BEP count (b = .72 (.21), t(3) = 3.44, p <.001), but no significant main effect of stage (b = .12 (.28), t(3) = .42, p =.68). The BEP count by stage interaction also was of trend-level significance (b = -.50 (.29), t(3) = -1.74, p = .09) and follow-up graphical analyses showed higher BEP counts were positively associated with higher c-Fos density at the early stage, and there was a negative association at the chronic stage such that higher BEP counts were associated with lower c-Fos expression (see Figure 3). Specifically, at the early stage of binge eating, higher BEP tertile counts were associated with higher c-Fos densities in the nucleus accumbens core. By contrast, in the chronic stage group, differences in c-Fos densities across high versus low BEP tertile counts were more attenuated (see Figure 3 and Table 5). Attenuated differences seem to be partly due to decreases in responsivity in those animals with higher BEP tertile counts. However, results also suggested some movement in those with lower BEP tertile counts, as c-Fos densities seemed to increase across early to chronic groups.

c-Fos Responsivity in the Nucleus Accumbens Shell. Overall, results for the nucleus accumbens shell were similar to results in the core, although effects were slightly less strong. ANCOVA analyses again revealed a significant main effect of phenotype (F(1,22) = 7.81, p = 0.01), no significant main effect of stage (F(1,22) = 2.13, p = 0.14), and a trend-level phenotype by stage interaction (F(1,22) = 2.69, p = .10) that was of large effect size (d = 2.06). In follow-up ANCOVAs (see Table 4), BEP animals had significantly higher densities of c-Fos as compared to BER rats in the early stage (see Figure 2), while in the chronic stage, BEP and BER animals did not differ significantly differ from each other. Within phenotypes, chronic BEP rats had a trend towards lower c-Fos density as compared to early BEP animals (p = .09, see Figure 2). This

effect was large in magnitude (d = 1.29), suggesting again, an overall pattern of down-regulation in the nucleus accumbens shell in BEP rats. There were no significant differences between early stage and chronic stage BER rats.

Results for the continuous BEP tertile counts corroborated these results (see Figure 3 and Table 5). Linear regressions revealed a significant main effect of BEP count (b = .60 (.22), t(3) = 2.70, p = .01), but no significant main effect of stage (b = -.11 (.30), t(3) = -.36, p = .72). Although BEP count by stage interaction was not significant (b = 0.46 (.30), t(3) = -1.53, p = .14), there was a trend towards significance (p = .14), and the effect was large in magnitude (R^2 = .18). Follow-up graphical analyses revealed a very similar pattern of effects in which BEP tertile counts were associated with higher c-Fos densities in the early stage, but there were minimal associations between BEP tertile counts and c-Fos densities in the chronic stages of binge eating.

Analyses of Feeding Test Data

Analyses of palatable food intake were completed in order to investigate the behavioral index of potential downregulation of the reward system across stage of binge eating. In addition, chow intake and body weight were also examined in order to rule out the possibility that observed changes in PF consumption and neural responsivity were due to changes these variables over time. Results from the MLMs examining behavioral data across stage (i.e., palatable food intake, chow intake, and body weight) are presented in Figure 4 and Table 6. Analyses examining palatable food intake over time showed a main effect of phenotype such that BEP animals consumed significantly more palatable food than BER rats. This is not surprising given that it is a requirement of phenotyping that BEP animals consistently fall in the highest tertile of palatable food intake compared to BER rats. Interestingly, the main effect of stage showed a significant trend-level effect (p = .09), such that levels of palatable food intake were

higher at the chronic stage than the early stage. Notably, the phenotype by stage interaction was not significant, suggesting that, counter to hypotheses, BEP animals did not consume significantly more palatable food at the chronic stage as compared to the early stage.

Despite not finding a significant interaction for palatable food intake, there was a significant BER/BEP phenotype x stage interaction for 24-hour post-feeding test chow intake (see Table 6). Specifically, BER rats appeared to consume greater amounts of chow compared to BEP rats at both the early and chronic stage of the study. However, BEP rats increased their chow intake across the study period, such that chronic stage BEP rats consumed significantly more chow on the days after feeding tests than early stage BEP rats (see Figure 4). The opposite pattern was found in chronic stage BER rats where they consumed significantly less chow at the 24-hour post-feeding test time point than the early stage BER rats. Taken together, results suggest that BEP rats may have been attempting to compensate for a downregulation in reward system responsivity in chow intake.

Finally, there were minimal differences observed for chow intake on feeding test days and body weight across the study period. For chow intake during feeding tests, there was a significant main effect of phenotype (see Table 6) such that BER animals tended to always consume significantly more chow than BEP animals in both the early and chronic stage groups (see Figure 4). This is not unexpected given that BEP rats are expected to spend more time consuming palatable food during feeding tests than chow. Finally, the main effect of stage was significant for body weight such that all animals gained weight across the study period (see Figure 4), but there was no significant main effect of phenotype or significant phenotype by stage interaction, suggesting that changes in body weight were likely just due to natural development over time.

DISCUSSION

This study was the first to directly investigate differences in neural responsivity to palatable food across chronicity of binge eating in rats. Results generally confirmed study hypotheses such that BEP animals at the early stage had higher responsivity in the nucleus accumbens core and shell compared to BER rats. Furthermore, there was evidence to suggest that at the chronic stage, BEP animals experienced a downregulation of responsivity over time, such that chronic stage BEP rats showed a trend towards lower responsivity in the nucleus accumbens core and shell compared to early stage BEP rats. All significant and trend-level significant effects were large in magnitude, and replicated across categorical and continuous analyses, providing further support for the patterns observed in the study. Despite finding changes in the neural index of responsivity, notably, results showed no changes in palatable food intake over time in BEP animals. While it was originally anticipated that BEP animals would increase palatable food consumption over time in order to compensate for any downregulation of reward related responsivity at the chronic stage of binge eating, BEP animals showed no significant differences in palatable food intake between early and chronic stage. However, BEP rats did show an increase in 24-hour post-feeding test chow consumption over time, which indirectly suggests that BEP rats may indeed be attempting to compensate for a downregulation in responsivity to palatable food.

While previous human and animal studies have been mixed in whether the reward system is hyper- or hypo-responsive to palatable food intake in individuals who binge eat (Bencherif et al., 2005; Bohon & Stice, 2011; Frank et al., 2006; Schienle et al., 2009; Wang et al., 2012), the current study pointed to a progression of effects in the nucleus accumbens, such that initially there is a hyper-responsivity to palatable food, and a downregulation over time after long-term

engagement in binge eating. These results suggest important considerations for studies of neural substrates of binge eating. Specifically, findings from the current study suggest that past inconsistent findings in neurobiological studies may be due to not taking into account stage/duration of binge eating in study samples, rather than a lack of association between binge eating and reward system functioning. This is evident in previous human studies where duration of illness has not been reported, controlled for, or included as a covariate in analyses, and thus, there is likely a mix of patients included in study samples (i.e., early stage, chronic stage). While animal studies of binge eating and reward system functioning have been clearer about reporting duration of study (i.e., duration of time animals engage in binge eating), these studies have also been inconsistent in the duration of the study period (e.g., 1-6 weeks; Bello, Patinkin, & Moran, 2011; Giuliano, Robbins, Nathan, Bullmore, & Everitt, 2012; Johnson & Kenny, 2010). Therefore, it may be that animal studies suffer from the same limitations as human investigations, where inconsistencies across study findings are likely due to not appropriately accounting for how long animals are engaged in binge eating. Taken together, future studies examining binge eating and the reward system appropriately report and control for stage/duration of binge eating.

While a general pattern of downregulation in neural responsivity was found in the nucleus accumbens core and shell of BEP rats over time, it was surprising that there was no associated difference found in palatable food consumption over time in BEP animals. However, results did show a pattern of increased chow consumption 24-hours after feeding tests in chronic stage BEP rats compared to early stage BEP rats. It may have been that BEP rats consumed a maximum amount of palatable food during the 4-hour feeding test, but over 24-hours, they may have been able to consume more palatable food. Thus, a 24-hour feeding test timeframe might

have provided evidence of an increase in palatable food intake to compensate for a downregulation of the reward system in BEP rats. While some studies using the BER/BEP model have left palatable food in the cage and measured both a chow and palatable food consumption at 24-hours (Hildebrandt et al., 2014; Klump, Suisman, Culbert, Kashy, Keel, et al., 2011; Klump, Suisman, Culbert, Kashy, & Sisk, 2011), the current study chose to remove palatable food at the 4-hour time point to more closely model previous work examining responsivity in the nucleus accumbens (Sinclair et al., 2015). As a result, findings from the current study suggest examining palatable food intake at the 24-hour time point in future studies might provide evidence for changes in palatable food intake over time. Furthermore, it should be noted that no studies have directly examined if individuals increase the amount of food consumed during binge eating episodes. Therefore, it is important that future human studies investigate potential increases in amount consumed during binge eating episodes to provide further insight into this potential change.

Nonetheless, future studies may not find changes in palatable food intake over time in BEP animals, even with 24-hour palatable food measurements. No observed changes in palatable food intake over time might suggest changes in *sensitivity* to reward over time. Previous work has shown that after chronic exposure to artificially rewarding substances, the nucleus accumbens shows less c-Fos expression, similar to current study findings, but there is an increase in ΔfosB expression (e.g., Kelz et al., 1999; Nestler, 2005; Zachariou et al., 2006), and ΔfosB accumulation has been shown to increase the sensitivity of the reward system in response to a drug (Kelz et al., 1999). Therefore, in the current study, BEP animals at the chronic stage might be more sensitive to the effects of palatable food after chronic exposure, and do not need to eat

increased amounts to compensate for a downregulation. Future studies should examine potential changes in sensitivity (e.g., using ΔfosB as a marker) to palatable food over time in BEP rats.

In addition, future studies should attempt to identify the specific types and function of the cells that are activated within the nucleus accumbens in response to palatable food in BEP rats. The majority of neurons within the nucleus accumbens (i.e., approximately 95%) are medium spiny neurons (e.g., Yager, Garcia, Wunsch, & Ferguson, 2015; Zhang, Balmadrid, & Kelley, 2003), which contain dopamine and GABA receptors. Changes in dopamine and GABA receptor function have previously been implicated in changes in consumption of artificial (e.g., Lee et al., 2006) and natural (e.g., food) rewards (e.g., Zhang et al., 2003). While work is currently underway to investigate specific cells activated in the prefrontal cortex of BEP animals (Sinclair et al., *in preparation*), findings from other studies have demonstrated that stimulation of both dopamine and GABA receptors directly impacts food intake. Specifically, dopamine and GABA receptors have been shown to play a role in increasing motivation/palatability of food as well as motor response respectively (Zhang et al., 2003). Given this, it is possible that use of pharmacologic agents targeting dopamine and/or GABA receptors contained in medium spiny neurons in the nucleus accumbens might be appealing targets for pharmacologic treatment of bing eating. While there is some work broadly targeting binge related disorders (e.g., binge eating disorder; McElroy, Guerdjikova, Mori, & O'Melia, 2012), more studies are warranted. Future work should also account for receptor responsivity to binge eating across chronicity with the hope of developing more effective and targeted pharmacological treatments.

Beyond cellular activation in response to binge eating in BEP rats over time, future studies should also target more specific mechanisms and neurotransmitter function in the nucleus accumbens. Years of previous research have suggested that opioids and dopamine within the

nucleus accumbens are associated with "liking" and "wanting" of palatable food. Specifically, opioid neurotransmission within the nucleus accumbens shell is linked to changes in the reward system substrate "liking" (i.e., a hedonic response when consuming a natural and artificial reward; Berridge, 1996, 2009a, 2009b; Peciña & Berridge, 2005; Smith & Berridge, 2007), while dopamine in the nucleus accumbens core underlies the reward system substrate "wanting" (i.e., the motivation to seek a reward; Berridge, 1996, 2009a, 2009b; Robinson & Berridge, 1993). It may be that both opioid and dopamine neurotransmission are increased/hyper-responsive in the early stages of binge eating in response to palatable food in BEP rats, leading to increased levels of "liking" and "wanting," and increased levels of binge eating. At chronic stages, dopamine and opioid neurotransmission may be downregulated in response to palatable food leading to decreased "liking" and "wanting" of palatable food. Alternatively, no observed changes in palatable food intake might suggest that binge eating becomes more conditioned and habitual over time, such that animals become conditioned to the feeding test schedule and consume a consistent amount in response to the palatable food stimulus despite a neural change in reward system responsivity. Future work should examine neural and behavioral changes in "liking"/opioids and "wanting"/dopamine in order to understand if fluctuations in these systems mimics findings from the current study (i.e., a downregulation at the chronic stage compared to the early stage). Studies should also examine potential differences in these systems across duration of binge eating (e.g., effects are stronger in the core or shell at the early stage versus chronic stage).

While the nucleus accumbens is a key region of interest in studies examining reward system functioning, it is important to note that other structures in the reward system beyond the nucleus accumbens could play a role in these processes. For example, previous work has

suggested that the prefrontal cortex is also responsive to palatable food in BEP rats (Sinclair et al., 2015). The presence of afferent projections from the prefrontal cortex to the nucleus accumbens may suggest that a larger network of structures and pathways play important roles in the responsivity to palatable food, and may be dysregulated in BEP animals. Furthermore, numerous efferent projections extending from the nucleus accumbens to other areas of the brain (e.g., ventral tegmental area (VTA)) could also play a role in the larger network of reward system structures. Given the interplay between reward system structures, it possible that hyporesponsivity is present in these other structures OR that the timing (e.g., do changes in neurotransmitter expression happen in one region prior to another) and strength of downregulation (e.g., are effects stronger in a specific structure) may vary in important ways that contribute to binge eating.

Other reward based processes such as cue-potentiated feeding (i.e., eating in response to external cues; Johnson, 2013; Petrovich, Ross, Gallagher, & Holland, 2007; Reppucci & Petrovich, 2012) and incentive motivation (i.e., hypersensitivity to motivating effects of rewarding substances, Lutter & Nestler, 2009; Robinson & Berridge, 2008) have been cited as critical areas of research in overall reward system functioning in response to natural and artificial rewards. These different perspectives on reward system functioning in response to palatable food intake might lead to additional interpretations of findings from the current study. Previous work has shown that, after pairing external cues with palatable food, rats will over consume chow in the presence of the same cues, even when there is no palatable food available (Boggiano et al., 2009). Therefore, BEP rats might experience palatable food/feeding tests as a cue that leads them to consume palatable food (and increased chow at 24 hours) despite potentially low levels of "liking" and "wanting". Future studies should examine the role of chronicity of binge eating

across different domains of reward system responsivity. Findings could contribute to a deeper understanding about changes in different patterns of reward system functioning in neural response and food intake changes beyond just cellular responsivity to binge eating over time.

The pattern of effects observed in the nucleus accumbens of BEP rats over time may also be reflective of habit formation (i.e., the process by which behaviors become automatic). Evidence for behavioral habit formation in response to artificially rewarding substances has shown a transition from goal-directed to a habitual pattern of behavior (e.g., Zapata, Minney, & Shippenberg, 2010) after long-term operant training (i.e., 56-61 training sessions total), *not* short-term (i.e., 20-25 training sessions) using cocaine (Zapata et al., 2010). The palatable food intake data from the current study may also suggest habit formation in BEP animals after repeated, long-term presentation of palatable food. Previous work examining continuous, daily access to palatable food in the BER/BEP model has shown that BER and BEP animals become more similar to one another in palatable food in take, where ultimately no significant differences in palatable food intake are observed between BER and BEP animals with continuous access (Boggiano et al., 2007). While the current study did not provide palatable food daily, it may be that after long-term, chronic intermittent access to palatable food, BEP animals may indeed become more similar to BER animals after a habit is formed. Additionally, research has shown that over time, there is evidence of neural habit formation where habitual behavior becomes associated with higher activation in the dorsal regions of the striatum rather than ventral areas (i.e., the location of the nucleus accumbens; Everitt & Robbins, 2016; Vollstädt‐Klein et al., 2010). Therefore, given the similarity between artificial and natural substances of abuse, results from the current study might point to a similar pattern of effects such that as binge eating transitions to a habit in BEP rats, the neural activation in the nucleus accumbens becomes hypo-

responsive, and the dorsal striatum may show increased activation. It will be important that future studies use behavioral paradigms such as operant conditioning in conjunction with examination of neural activation across the ventral and dorsal striatum in order to examine habit formation in BEP animals.

Despite the strengths of the findings from the current study, it is important to consider limitations to the current work. First, sample sizes across phenotypes were small, particularly after excluding animals with poor tissue quality and inconsistent chronic stage phenotypes. While additional cohorts of animals could have been run to increase sample sizes, the timeframe of the current project did not allow for any further data collection. Fortunately, effect sizes suggested that the findings from the current study were mostly large effects, providing additional support for the findings. Furthermore, the use of the continuous BEP tertile count approach yielded similar findings to the categorical analyses. Thus, although findings from the current study need to be replicated with larger sample sizes, results across multiple analyses (i.e., ANCOVA, regression) provided further support for study findings.

Second, the current study was the first to define a "chronic" duration of binge eating using the BER/BEP animal model. The current study based the chronic stage duration (i.e., 9 weeks, 24 feeding tests) on treatment studies examining binge related disorders (i.e., bulimia nervosa, binge eating disorder). Studies generally found an average duration of illness of five years in treatment populations (Fairburn et al., 2000; Keller et al., 1989; Reas, Williamson, Martin, & Zucker, 2000), but the range varies across studies (insert a mix/max range, cites). Similar to neurobiological investigations of binge related disorders (which often recruit study samples from treatment studies), there has been variability in treatment outcome over time, and some have pointed to variability in chronicity of illness as contributing to the differences in study

findings (Quadflieg & Fichter, 2003; Wonderlich et al., 2012). Therefore, while five years of illness is likely a strong model of a chronic duration of binge eating illness, clearly more work is needed across research studies, human and animal, to more clearly define a chronic stage of binge eating. Future work using the BER/BEP model should build on findings from the current study and examine a variety of time points to compare Fos expression over time. It may be that downregulation in the reward system begins earlier than the nine weeks used for the current study, while study durations longer than 9 weeks might show more significant downregulation after longer amounts of time and observe changes in palatable food consumption at even more chronic stages.

An additional limitation of the current study was the use of a cross-sectional design of different groups of animals at the early versus chronic stages. Future studies should use longitudinal designs to investigate potential changes in brain responsivity to palatable food over time using other techniques available in both human and animal populations. For example, functional magnetic resonance imaging (fMRI) techniques are available for both human and animal populations. Therefore, future studies could use similar techniques to examine binge eating populations of humans and rats at the early stages of binge eating, and then follow-up the same samples over time. This design would allow for a more direct investigation into changes in brain responsivity to palatable food. While human studies have already used fMRI techniques to investigate binge eating populations (e.g., Bohon & Stice, 2011), there has been no work directly targeting brain reward system changes across duration of illness. Therefore, combining data gathered from human and animal studies, would provide multiple levels of inference, and allow for stronger conclusions about the effect of stage on neural responsivity.

Finally, the current study did not observe any associated changes in palatable food intake over time associated with the downregulation found in BEP rats. While the current study aimed to replicate previous work at the early stage of binge eating (Sinclair et al., 2015), and thus, selected to remove palatable food at the 4-hour time point, this may have limited the ability to see observe in palatable food intake over time. Notably, BEP rats appeared to increase their chow intake over time at the 24-hour time point, suggesting that BEP rats have the capacity to continue consuming meals across a longer, 24-hour period. As previously discussed, allowing rats to continue consuming palatable food for a longer duration might result in an increase in palatable food intake over time. Therefore, it will be important for future BER/BEP studies to examine the 24-hour time point to see if there are changes in palatable food intake at chronic stages of binge eating.

In conclusion, results from this study largely support study hypotheses, and provide evidence for a downregulation across time in responsivity of the reward system in binge eating rats. These findings strongly suggest a rethinking of how binge eating and binge eating related illnesses (i.e., BN, BED) are conceptualized over time, such that the neural functioning of individuals at the early stage is significantly different than those at the chronic stage. Notably, hyper-responsivity at the early stages of binge eating might be an important risk factor for development of more severe binge eating over time. Future studies investigating the neural effects of binge eating should make efforts to more clearly examine binge eating across chronicity to build on findings from the current study and develop a clearer understanding of binge eating.

APPENDIX

Table 1

Sample Sizes Across BER/BEP Classifications and Stage of Binge Eating

	Early Stage			**Chronic Stage**		
	Cohort 1 (N = 30 animals)	Cohort 2 (N = 30 animals)	Total Early Stage (N = 60 animals)	Cohort 1 (N = 30 animals)	Cohort 2 (N = 30 animals)	Total Chronic Stage (N = 60 animals)
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Tertile Classification Method						
Cohort Specific Tertiles						
BER	8 (27%)	6 (20%)	14 (23%)	5 (17%)	6 (20%)	11 (18%)
BEP	4 (13%)	4 (13%)	8 (13%)	6 (20%)	4 (13%)	10 (17%)
Neutral	18 (60%)	20 (67%)	38 (63%)	19 (63%)	20 (67%)	39 (65%)
Final Tertiles						
BER	12 (40%)	6 (20%)	18 (30%)	8 (13%)	4 (13%)	12 (20%)
Consistent Chronic BER	-- --	-- --	-- --	*6 (20%)*	*4 (13%)*	*10 (17%)*
BEP	5 (17%)	6 (20%)	11 (18%)	6 (20%)	4 (13%)	10 (17%)
Consistent Chronic BEP	-- --	-- --	-- --	*4 (13%)*	*2 (7%)*	*6 (10%)*
Neutral	13 (43%)	18 (60%)	31 (52%)	16 (53%)	22 (37%)	38 (63%)
c-Fos Immunohistochemistry Animals						
Animals Processed for c-Fos (total N = 53)						
BER	10 (33%)	6 (20%)	16 (27%)	6 (20%)	3 (10%)	9 (15%)

Table 1 (cont'd)

BEP	4 (13%)	5 (17%)	9 (15%)	6 (20%)	4 (13%)	10 (17%)
Neutral	2 (7%)	1 (3%)	3 (5%)	3 (10%)	3 (10%)	6 (10%)
Tissue Quality Score of "0" or "1" (total N = 43)						
BER	6 (20%)	5 (17%)	11 (18%)	6 (20%)	2 (7%)	8 (13%)
BEP	2 (7%)	3 (17%)	5 (8%)	6 (20%)	4 (13%)	10 (17%)
Neutral	2 (7%)	1 (3%)	3 (5%)	3 (10%)	3 (10%)	6 (10%)
Excluding c-Fos NAc Core Outliers (total N = 32)						
BER	5 (17%)	4 (13%)	9 (15%)	6 (20%)	2 (7%)	8 (13%)
BEP	2 (7%)	3 (17%)	5 (8%)	6 (20%)	4 (13%)	10 (17%)
Excluding c-Fos NAc Shell Outliers (total N = 33)						
BER	5 (17%)	5 (17%)	10 (17%)	6 (20%)	2 (7%)	8 (13%)
BEP	2 (7%)	3 (17%)	5 (8%)	6 (20%)	4 (13%)	10 (17%)

Note. BER = binge eating resistant, BEP = binge eating prone, Neutral = animals that did not meet criteria to be categorized as BER/BEP. NAc = nucleus accumbens. Cohort Specific Tertiles = animals were phenotyped based on tertiles calculated from their specific cohort's palatable food intake from feeding tests 1-6. Final Tertiles = data from cohort 1 and cohort 2 were combined, and tertiles were calculated based on feeding test 1-6 palatable food intake across all animals (N = 120). Consistent Chronic = animals originally identified as BER/BEP and randomized to the chronic stage condition were required to continue to meet classification for

Table 1 (cont'd)
that phenotype at least one other group of 6 feeding tests during the chronic stage of the study (i.e., feeding tests 7 – 24). Tissue Quality Score of "0" or "1"= animals that were processed for c-Fos immunohistochemistry and were evaluated to have good tissue quality for c-Fos analyses.

Table 2

Descriptive Data for Palatable Food Intake, Chow Intake, and Body Weight across Feeding Tests 1-6 in all Rats (N = 120)

	Mean (SD)		Statistics		Effect Size
Measure	Cohort 1 (n = 60)	Cohort 2 (n = 60)	t(df)	p	Cohen's d
4-Hour PF Intake (g)	7.70 (1.42)	8.06 (1.34)	-1.43 (118)	.16	0.26
4-Hour Chow Intake (g) on FT Day	2.44 (0.54)	2.63 (0.77)	-1.60 (105)	.11	0.29
24-Hour Chow Intake (g) Post FT Day	8.28 (1.57)	8.23 (1.45)	0.17 (117)	.88	0.03
Body Weight (g)	219.15 (8.98)	212.86 (7.08)	4.26 (112)	<.001	0.78

Note. PF = palatable food, FT = feeding test. Feeding test 12 was excluded from all analyses due to experimental error. Cohen's d values reflect effect sizes (i.e., standardized measure of the magnitude of mean differences between groups; effect size interpretation: small, d = .20; medium, d = .50; large, d = .80).

Table 3

ANCOVA Results for Differences in c-Fos Density in the Nucleus Accumbens Core and Shell by BER/BEP Phenotype and Stage of Binge Eating.

	M (SE)	*F* (df,df)	*p*	Partial eta squared
Nucleus Accumbens Core				
Phenotype		*F* (1,21) = 20.06	<.001	.49
BER	23.43 (2.02)			
BEP	37.29 (2.31)			
Stage		*F* (1,21) = 1.28	.27	.06
Early	32.07 (2.11)			
Chronic	28.65 (2.18)			
Phenotype x Stage		*F* (1,21) = 3.24	.08	.13
BER Early	22.35 (2.52)			
BEP Early	41.78 (3.38)			
BER Chronic	24.50 (3.15)			
BEP Chronic	32.79 (3.14)			
Nucleus Accumbens Shell				
Phenotype		*F* (1,22) = 7.81	.01	.26
BER	39.16 (3.59)			
BEP	54.70 (4.19)			
Stage		*F* (1,22) = 2.13	.14	.10
Early	51.09 (3.77)			
Chronic	42.77 (3.98)			
Phenotype x Stage		*F* (1,22) = 2.69	.10	.12
BER Early	38.57 (4.36)			
BEP Early	63.62 (6.16)			
BER Chronic	39.76 (5.76)			
BEP Chronic	45.78 (5.70)			

Note. ANCOVA models covaried 1-hour palatable food intake amounts prior to sacrifice. BER = binge eating resistant, BEP = binge eating prone. Partial eta squared (*partial* η^2) values reflect effect sizes (i.e., standardized measure of the magnitude of mean differences between groups; effect size interpretation: small, *partial* η^2 = .01; medium, *partial* η^2 = .06; large, *partial* η^2 = .14). Sample sizes for nucleus accumbens core: early stage BER n = 9, early stage BEP n = 5, chronic stage BER n = 6, chronic stage BEP n = 6. Sample sizes for nucleus accumbens shell: early stage BER n = 10, early stage BEP n = 5, chronic stage BER n = 6, chronic stage BEP n = 6.

Table 4

ANCOVA Results for Follow-Up Comparisons of c-Fos Density in the Nucleus Accumbens Core and Shell by Binge Eating Resistant and Binge Eating Prone Phenotypes and Stage of Binge Eating

Comparisons	M Difference (SE)	*F* (df, df)	*p*	Effect Size Cohen's *d*
Nucleus Accumbens Core				
Within Stage				
Early - BER vs. BEP	19.43 (4.04)	*F* (1, 11) = 23.14	<.001	2.57
Chronic - BER vs. BEP	8.04 (5.23)	*F* (1, 9) = 2.38	.16	1.08
Within BER/BEP Phenotype				
BEP - Early vs. Chronic	9.20 (6.67)	*F* (1, 8) = 1.90	.21	1.18
BER- Early vs. Chronic	1.89 (2.48)	*F* (1, 12) = 0.58	.46	0.28
Nucleus Accumbens Shell				
Within Stage				
Early - BER vs. BEP	24.47 (7.86)	*F* (1, 12) = 9.69	.01	1.82
Chronic - BER vs. BEP	0.79 (6.45)	*F* (1, 9) = 0.02	.91	0.43
Within BER/BEP Phenotype				
BEP - Early vs. Chronic	18.00 (9.37)	*F* (1, 8) = 3.69	.09	1.29
BER- Early vs. Chronic	0.97 (7.19)	*F* (1, 13) = 3.20	.90	0.09

Note. BER = binge eating resistant, BEP = binge eating prone. Mean difference is differences between two groups. Group specific means are presented in Table 3. Cohen's *d* values reflect effect sizes (i.e., standardized measure of the magnitude of mean differences between groups; effect size interpretation: small, *d* = .20; medium, *d* = .50; large, *d* = .80). Sample sizes for nucleus accumbens core: early stage BER n = 9, early stage BEP n = 5, chronic stage BER n = 6, chronic stage BEP n = 6. Sample sizes for nucleus accumbens shell: early stage BER n = 10, early stage BEP n = 5, chronic stage BER n = 6, chronic stage BEP n = 6.

Table 5

Results from Regressions Examining c-Fos Density across Stage and Binge Eating Proneness

	B (SE)	*t*	*p*
NAc Core (R^2 = 0.26)			
BEP Count	0.72 (0.21)	3.44	<.001
Stage	0.12 (0.28)	0.42	.68
BEP Count * Stage	-0.50 (0.29)	-1.74	.09
NAc Shell (R^2 = 0.18)			
BEP Count	0.60 (0.22)	2.70	.01
Stage	-0.11 (0.30)	-0.36	.72
BEP Count * Stage	-0.46 (0.30)	-1.53	.14

Note: NAc = nucleus accumbens, BEP = binge eating prone. Total N = 41.

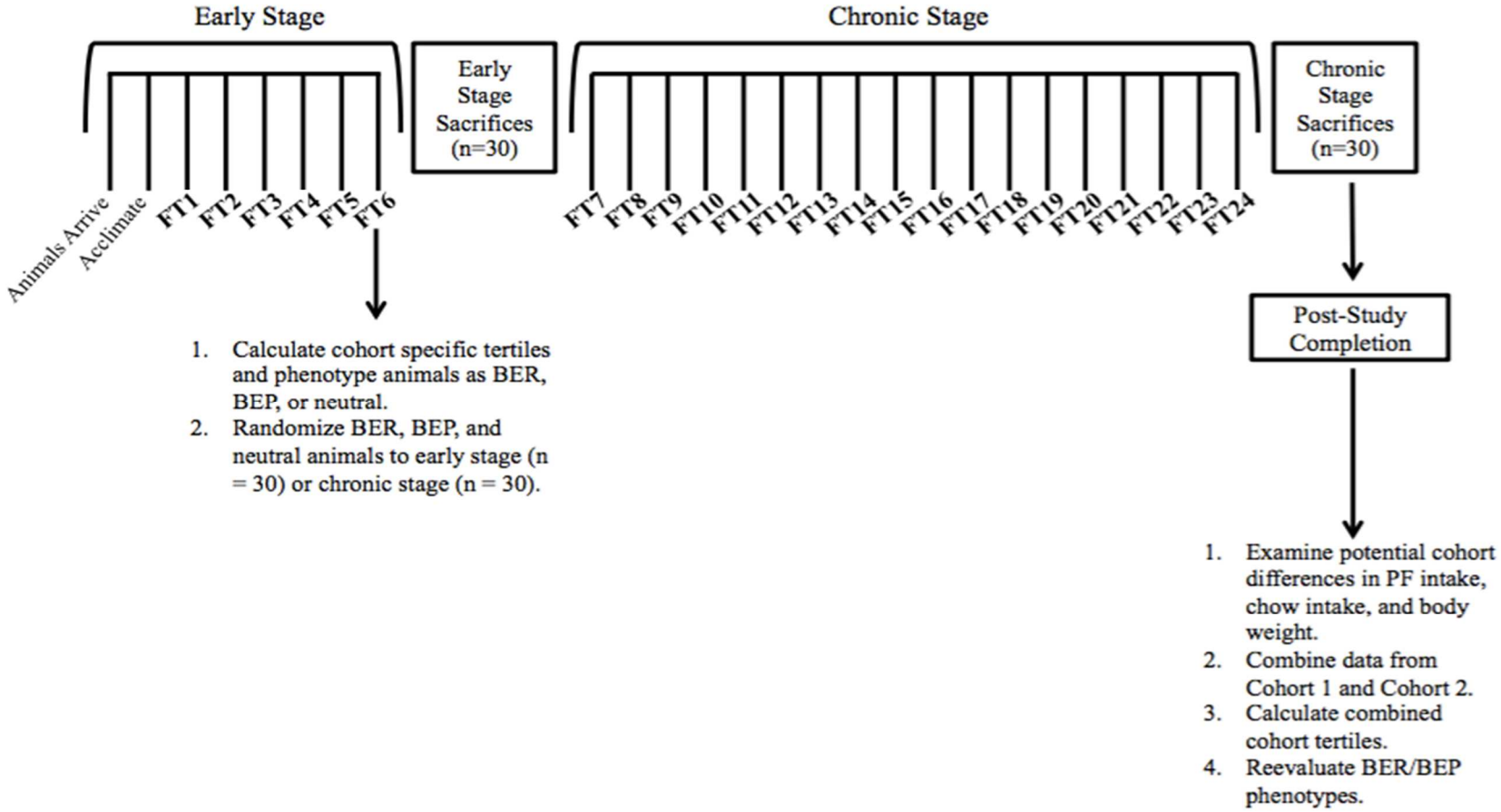
Table 6

Results from Mixed Linear Models Examining Palatable Food Intake, Chow Intake, and Body Weight across Stage and BER/BEP Phenotypes

BER/BEP Phenotype	Early Stage M(SD)	Chronic Stage M(SD)	Phenotype Main Effect	Stage Main Effect	Phenotype x Stage Interaction
4-Hour PF			$F(1, 107) = 50.97$***	$F(1, 94) = 2.86$†	$F(1, 95) = 0.23$
BER	6.22 (1.86)	6.69 (1.63)			
BEP	9.50 (2.40)	9.54 (2.00)			
24-Hour Post FT Chow			$F(1, 138) = 20.50$***	$F(1, 125) = 14.92$***	$F(1, 125) = 7.56$**
BER	8.92 (2.41)	7.61 (2.12)			
BEP	6.73 (2.15)	7.04 (2.39)			
4-Hour FT Chow			$F(1, 150) = 3.02$†	$F(1, 137) = 0.01$	$F(1, 137) = 0.70$
BER	2.71 (1.51)	2.67 (1.14)			
BEP	2.30 (1.07)	2.50 (1.31)			
Body Weight			$F(1, 51) = 0.05$	$F(1, 39) = 7.75$**	$F(1, 39) = 0.71$
BER	214.30 (8.94)	230.36 (14.59)			
BEP	213.09 (11.77)	236.78 (15.99)			

Note. BER = binge eating resistant, BEP = binge eating prone, PF = palatable food, FT = feeding test. All values presented are in grams. Feeding test 12 was excluded from all analyses due to experimental error. Sample sizes: early stage BER n = 18, early stage BEP n = 11, chronic stage BER n = 10, chronic stage BEP n = 6. ***$p < .001$; **$p \leq .01$; †$p < .10$.

Figure 1
Timeline of Study.

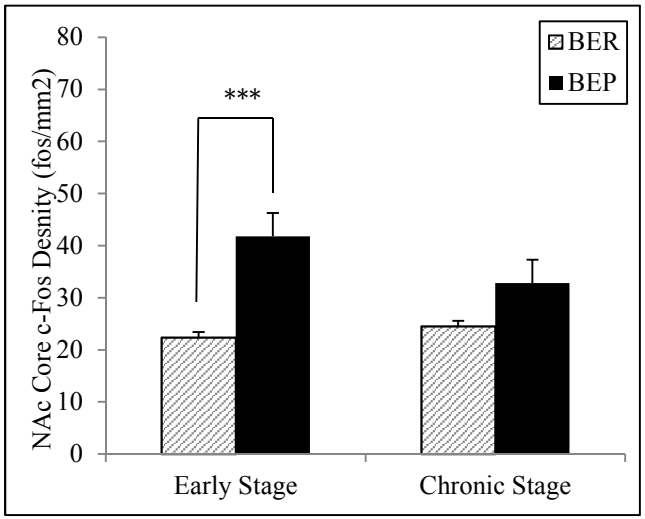


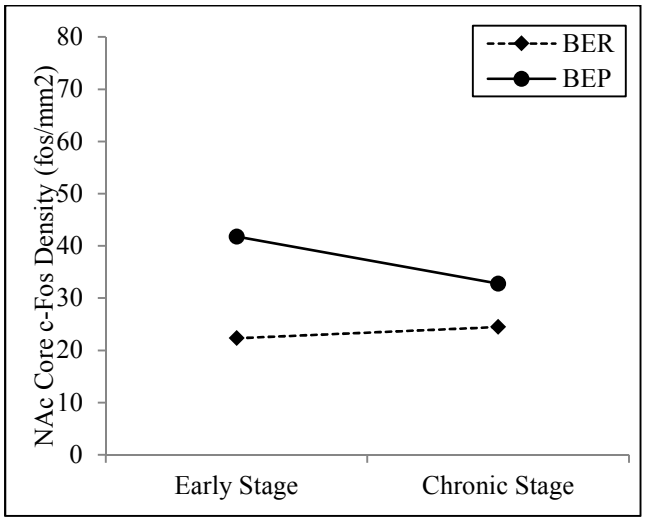
Note. Timeline of study where process was repeated twice, once for cohort 1 and once for cohort 2. FT = feeding test, BER = binge eating resistant, BEP = binge eating prone. Animal arrival and acclimation took place over a total of two days. All animals were phenotyped after the initial six feeding tests (i.e., cohort specific tertiles), then randomized to the early stage or chronic stage. Early stage perfusions took place approximately two days after the 6th feeding test. Chronic stage perfusions took place approximately two days after the 24th feeding test.

Figure 2

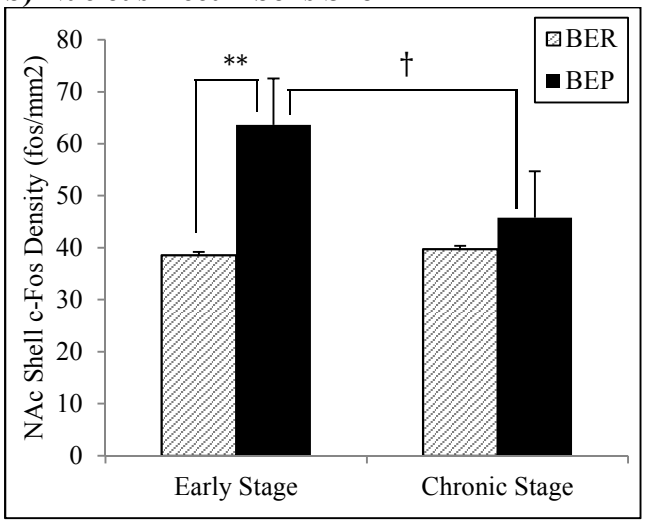
Differences in c-Fos Density Between Binge Eating Prone and Binge Eating Resistant Rats across Stage of Binge Eating in the Nucleus Accumbens Core and Shell.

a) Nucleus Accumbens Core





b) Nucleus Accumbens Shell



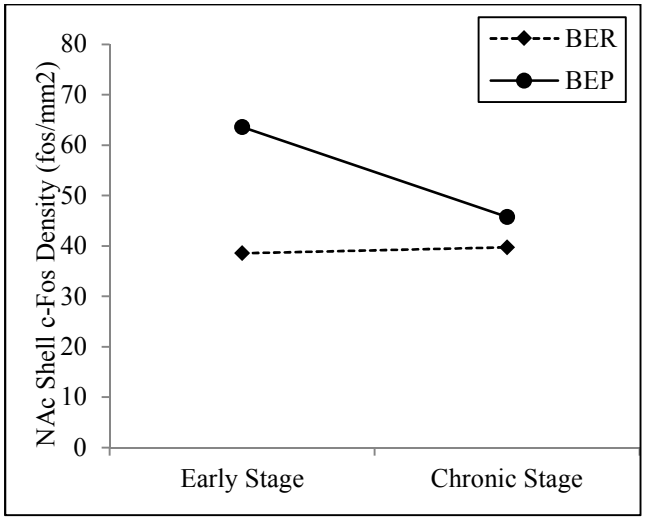
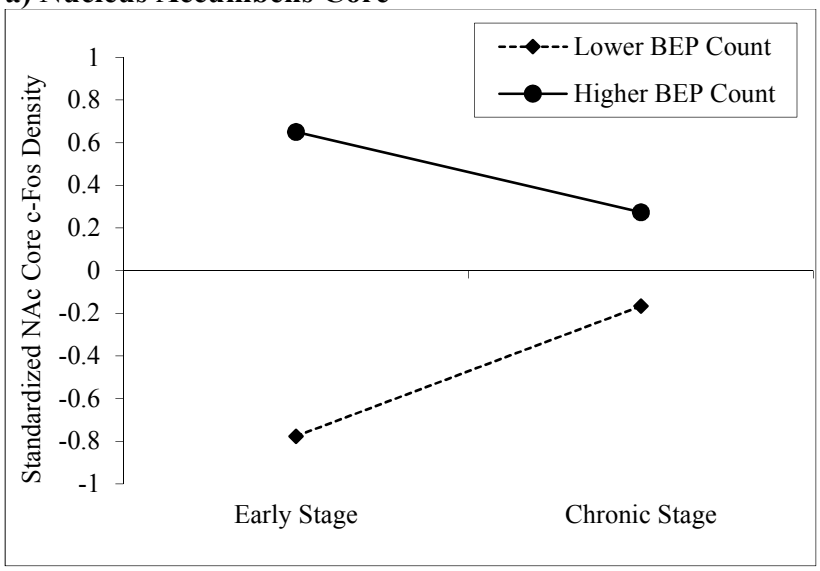


Figure 2 (cont'd)
Note. Data presented across bar graphs and line graphs is the same, but was presented in both formats to maximize interpretation. Mean comparisons of Fos density (Fos cells/mm^2) in the a) nucleus accumbens core and b) nucleus accumbens shell, controlling for 1-hour palatable food consumption prior to sacrifice. BER = binge eating resistant, BEP = binge eating prone, NAc = nucleus accumbens. Early Stage = feeding tests 1-6, Chronic Stage = feeding tests 7-24. Error bars represent one standard error. Sample sizes for nucleus accumbens core: early stage BER n = 9, early stage BEP n = 5, chronic stage BER n = 6, chronic stage BEP n = 6. Sample sizes for nucleus accumbens shell: early stage BER n = 10, early stage BEP n = 5, chronic stage BER n = 6, chronic stage BEP n = 6. ***$p < 0.001$; **$p \leq .01$; †$p < .10$

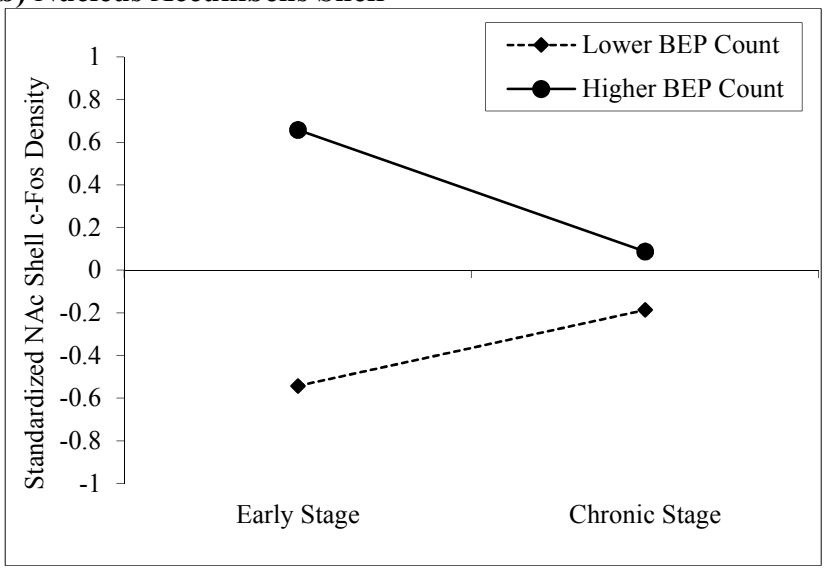
Figure 3

Associations Between Binge Eating Prone Tertile Count and Stage of Binge Eating on c-Fos Density in the Nucleus Accumbens Core and Shell.

a) Nucleus Accumbens Core



b) Nucleus Accumbens Shell

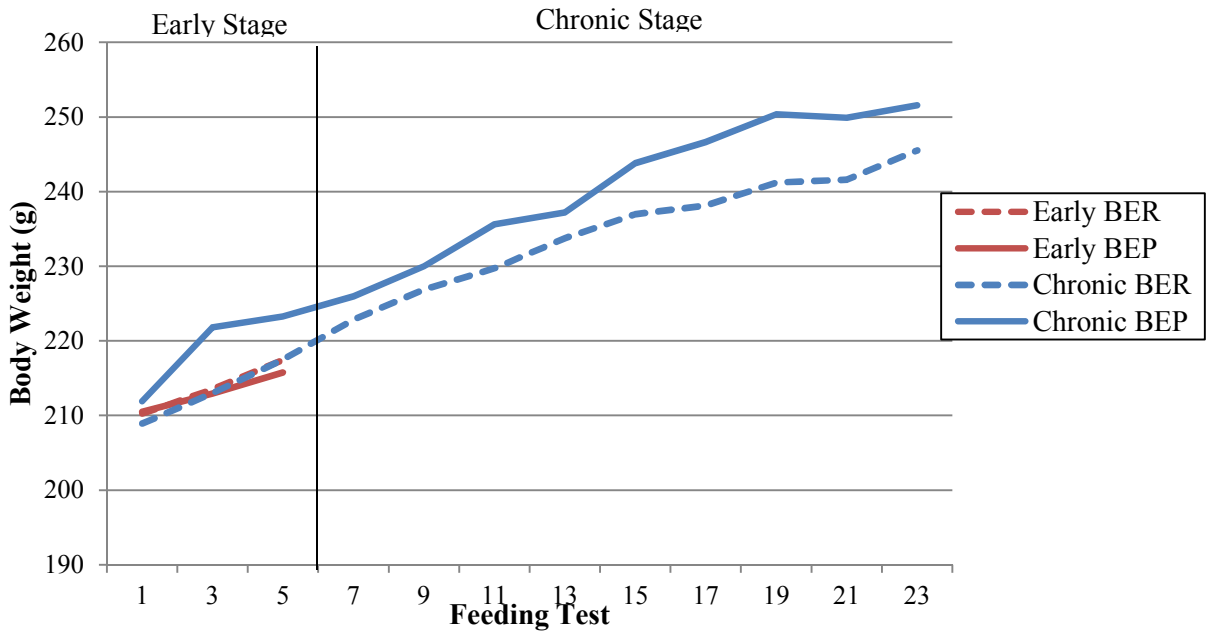


Note: NAC = nucleus accumbens, BEP = binge eating prone. Early Stage = feeding tests 1-6, Chronic Stage = feeding tests 7-24. Total N = 41.

Figure 4

Examining Palatable Food Intake, Chow Intake, and Body Weight across Stage and BER/BEP Phenotypes.

a) Average Body Weight on Feeding Test Days



b) Average Palatable Food (PF) Intake on Feeding Test Days

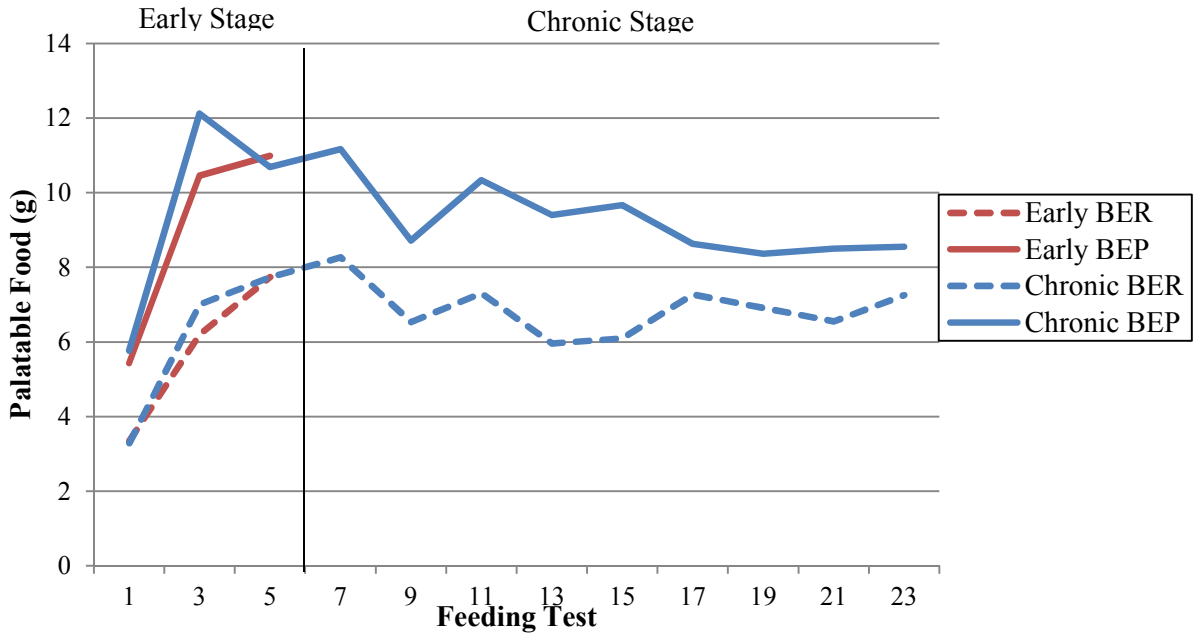
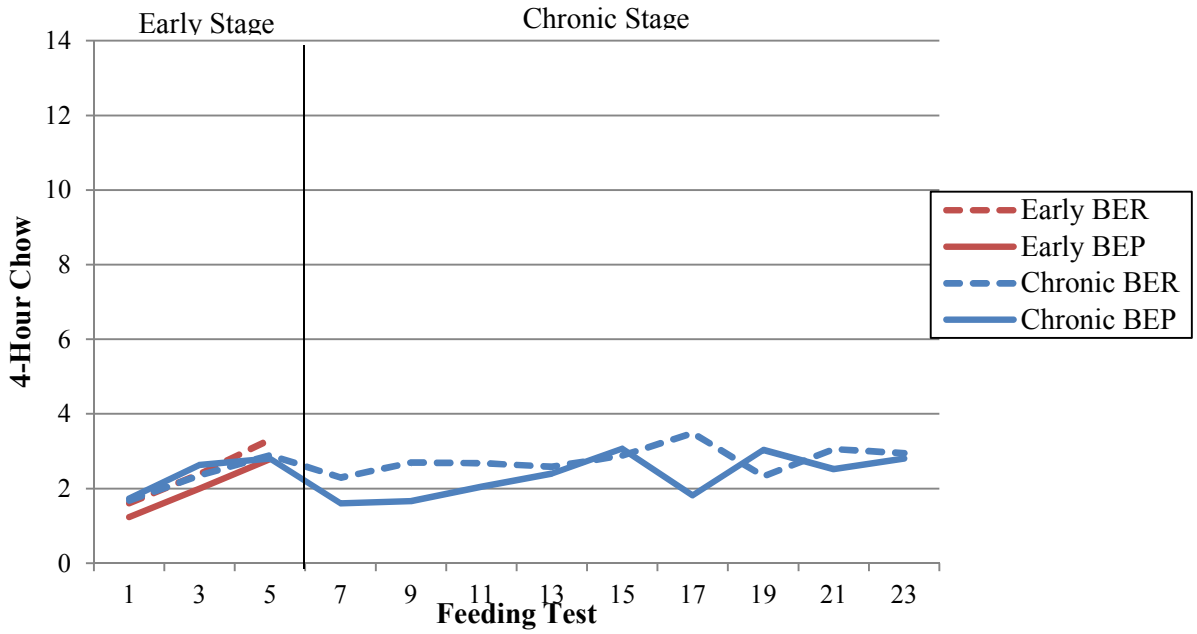
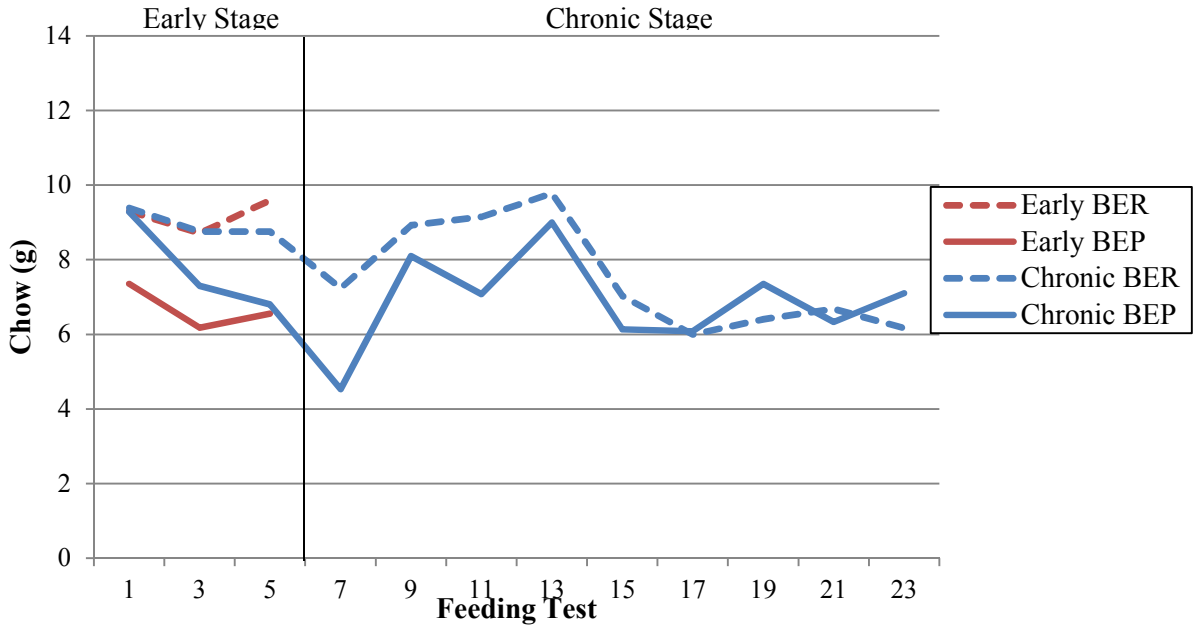


Figure 4 (cont'd)

c) Average 4-Hour Chow Intake on Feeding Test Days



d) Average 24-Hour Chow Intake Post-Feeding Test Days



Note. Group differences by BER or BEP phenotype in a) Body Weight, b) 4-hour feeding test day chow consumption, and c) 4-hour feeding test day palatable food consumption. BER = binge eating resistant, BEP = binge eating prone, FT = feeding test. All values presented are in grams. Because of the large number of feeding tests (i.e., 23 tests), every other feeding test (rather than every feeding test) is pictured in the graph. Animals included in analyses are those that were

Figure 4 (cont'd)
phenotyped as BER or BEP based on FT1 to FT6 data. For chronic stage animals to be included in analyses from FT7 to FT24, they were required to continue to meet their original phenotype of BER or BEP at least one out of the 3 remaining feeding test groupings. Sample sizes: early stage BER n = 18, early stage BEP n = 11, chronic stage BER n = 10, chronic stage BEP n = 6.

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