Repeated binge access to a palatable food alters feeding behavior, hormone profile, and hindbrain c-Fos responses to a test meal in adult male rats.

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Running Head: Bingeing schedule and feed-forward mechanisms of intake

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Abstract

Repetitive cycles of palatable food access and chronic calorie restriction alter feeding behaviors and forebrain neural systems. The purpose of this study was to determine the behavioral, endocrine, and meal-related hindbrain neural activation in adult male Sprague Dawley rats exposed to a binge access feeding schedule. The binge access schedule consisted of repeated twice per week episodes of acute calorie restriction (to 1/3 of the previous day’s intake) followed by 2 h concurrent access to high-calorie palatable food (sweetened fat; 90% vegetable shortening/10% sucrose) and chow. The Binge Access rats consumed more calories during the “binge” period than rats with continuous sweetened fat access (Continuous Access) or repeated acute calorie restriction only (Chow-Restricted). The Binge Access group also exhibited a ~25% increase in sweetened fat intake from weeks 1 to 6. Persistence of the binge phenotype in the Binge Access animals was demonstrated following 2 weeks, but not 4 weeks, after ad libitum chow. The Binge Access and Chow-Restricted groups maintained a similar normal body composition and hormonal profiles, whereas the Continuous Access animals developed an obese phenotype. The Binge Access group did have significantly higher terminal ghrelin levels compared with the Continuous Access group. Consumption of a standardized meal resulted in more c-Fos positive cells, in the Binge Access group compared with Naive controls, along the anterior-posterior nucleus of the solitary tract regions. These results suggest repeated cycles of acute calorie restriction followed by palatable food
Bingeing schedule and feed-forward mechanisms of intake produces physiological alterations that may facilitate overconsumption of a highly palatable food during limited access periods.

**Key Words**: bulimia nervosa, binge eating, nucleus of the solitary tract, area postrema
Introduction

The increased availability of highly palatable foods is one factor strongly implicated in the obesity epidemic of Western cultures (48). The palatability of food is typically a function of its fat and/or sugar composition (31, 79). When given optional access (i.e., free choice) to either a fat, sucrose, or mixed macronutrient solution, chow-fed rodents typically demonstrate a pronounced preference for and increased acceptance of the palatable food (28, 53). Feeding behavior of this type, overconsuming palatable foods without calorie restriction, has been termed “hedonic hunger” or “hedonically driven eating”. Binge-like intake (i.e., excessive intake in a relatively short amount of time) has been demonstrated in energy-replete rats on intermittent scheduled access to palatable foods (e.g., vegetable shortening, high fat diet, sucrose solutions) (7, 9, 19, 26, 28, 59, 60, 87). Scheduled access to palatable food options has been demonstrated to result in a pattern of excess caloric intake on “binge” days and reduced or “compensated” calorie consumption of standard diet on non-binge days. Such hedonically driven feeding is strongly dependent on temporal cues or habitual patterns of food access (17, 63). Alternatively, the reduced consumption following the bout of “bingeing” could represent diminished reinforcing potency or relative decreased taste hedonic of the less palatable standard diets (21, 46).

Exposing animals to entrained feeding schedules (palatable or standard diets) has been demonstrated to result in anticipatory behaviors, expectant increases in insulin and ghrelin levels, elevated gene expression of hypothalamic neuropeptide Y (NPY) and synchronized circadian regulated genes (30, 37, 64-
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66, 88). Thus, optional scheduled access to palatable food results in behavioral and physiological alterations to promote periods of overeating.

Persistent calorie restriction prior to food access also alters the behavioral and neural response to foods (13, 14, 61). Specifically, chronic calorie restriction (< 85% of ad libitum intake) in rodents has been shown to modulate the opioidergic and dopaminergic dependent signaling involved with food reinforcement (5, 6, 10-12, 74). Rats exposed to daily calorie restriction with scheduled optional access (i.e., 12 h access) to standard chow and sugar solutions displayed an escalating binge-like pattern of sugar consumption (16). Prolonged history of calorie restriction in rats also results in binge-like eating of palatable foods (e.g., Oreo cookies) in response to foot shock stress (45). In clinical populations, intermittent calorie restriction or persistent dieting is associated with episodic binge eating and caloric restraint is likely to be involved in the maintenance of bulimia nervosa (BN) (62, 69, 73).

The present studies employ a novel rodent model of binge eating of relevance to human binge eating behavior. This feeding paradigm incorporates intermittent acute food restriction followed by brief periods of access to a highly palatable sugar and fat mixture. These repeated episodes of acute calorie restriction are in contrast to other binge feeding paradigms, which use longer periods of calorie restriction for extended periods of times (e.g., 66% calorie restriction for 5 days (8) or 12 h daily restriction for 8-30 days (15, 16). The sweet-fat ration used in this study has a macronutrient profile resembling “forbidden foods”, characteristic of bulimic binges (51). In addition, the binge
access period occurs during the part of the active eating cycle of the rat during which the greatest amounts of food are normally consumed, resembling binge eating disorder (BED) and BN patients who generally binge more during afternoon and evening meal times (27, 51, 75). Furthermore, the Binge Access rats in this model are exposed to intermittent restriction days alternating with 2 “binges” per week thereby modeling the intermittent behavior demonstrated in bulimics and paralleling the two binge per week minimum frequency diagnostic criterion in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) for binge eating in both BED and BN (3, 18, 25).

The aim of these experiments was to characterize behavioral, hormonal, and neural consequences of exposure to differing dietary and scheduled access feeding constraints. Three groups of animals were either exposed to repeated episodes of acute calorie restriction followed with scheduled access to a palatable food, repeated episodes of acute calorie restriction, or continuous access to a palatable food.

One of the defining features of binge eating is consuming a meal that is of a larger size (1). Multiple lines of research have suggested that the nucleus of the solitary tract (NTS) is the initial central neural site that integrates meal-related viscerosensory afferent information and peripheral hormonal signals (34, 39, 78, 80). To assess whether a history of repeated episodes of acute calorie restriction followed by scheduled access to a palatable food alters neural responses in the NTS, hindbrain c-Fos immunoreactivity following a standardized meal was also characterized in the groups.
Materials and Methods.

Animals. A total of sixty-five adult male Sprague Dawley rats (Charles River), with an initial weight range of 325-350 g were individually housed in stainless steel wire mesh hanging cages and placed on a 12/12 h light dark schedule (lights off @ 1230 h). All rats received ad libitum standard laboratory chow (Global Diet-2018, Harlan Teklad; 3.3 Kcal/g, fixed formula diet of 18% protein, 5% fat) unless otherwise noted. Water was available at all times during the experiment. All the procedures were approved by the Institutional Animal Care and Use Committee of the Johns Hopkins University.

Feeding schedules and experimental groups. The palatable food used in these experiments was “sweetened fat” and consisted of 90% vegetable shortening, (Crisco®, J.M. Smucker, Co.; 1.5 g trans fat) and 10% sucrose; 8.6 Kcal/g. All groups received a 24 h pre-exposure to the sweetened fat 3 days before beginning their respective feeding schedules. The pre-exposure to the sweetened fat was used to determine if the rats had initial difference in their preference for the palatable food. The rats were divided into three groups with initial statistically similar body weight and sweetened fat preference and were designated Continuous Access, Binge Access, or Chow Restricted groups. The Continuous Access group had unlimited optional access to both standard chow and jars containing sweetened fat throughout the experiment. Jars containing sweetened fat were refreshed as needed and completely changed out every third day. The Chow Restricted and Binge Access group were restricted at the beginning of the dark cycle to 33% of the previous day’s chow calorie intake on
Days 2 and 5 of each week (32). On restriction days animals were noted to consume the entire restricted amount of chow within the first 4 h. Hence, on the restriction days, this amounted to the equivalent of a ~20 h food deprivation prior to the re-feeding days. On subsequent days (Day 3 and 6) 2 h into the dark cycle (1430 h) (total deprivation time ~ 22 hrs), the Binge Access groups were given access to both standard chow and sweetened fat, whereas the Chow Restricted groups were re-fed with chow alone. The Binge Access group had access to the jars of sweetened fat only for the first 2 h of each re-feeding period. In this fashion the Binge Access group was exposed to a repeated cycle that consisted of three no restriction days (Day 1, 4, and 7), two weekly episodes of calorie restriction (Day 2 and 5), and two weekly episodes of scheduled re-feeding starting with 2 h access to an optional palatable food (Day 3 and 6). This schedule was chosen to provide the animals with combination of intermittent days of calorie restriction, palatable food access and ad libitum standard chow access within a 7 day period.

Palatable food and chow intake during the feeding schedules. Animals were maintained on each of these three weekly feeding schedules (n= 14 per group) for at least 6 weeks. Food intake and spillage was recorded to nearest 0.1 g and measured separately for the 2 h re-feeding period, the 20 h following the re-feeding period, and the 24 h before caloric restriction (for Binge Access and Chow Restricted groups) throughout the experiment. Intakes for Day 7 were not recorded.
**Persistence of the binge-like behavior following the binge access schedule.** A group of binge access rats (n=6) were removed from the feeding schedule after 6-weeks and placed on ad libitum standard chow. Following 2 weeks of ad libitum chow feeding (i.e., without caloric restriction or palatable food access) these rats underwent one day with a 33% calorie restriction and on the subsequent day were re-fed with chow and sweetened fat for 2 h and spillage and intakes were recorded. Rats were then again placed on ad libitum chow and this procedure was repeated 2 weeks later (4 weeks after the initial 6 weeks cycle).

**Body weight, fat pad weights, and plasma hormone assays.** The remaining eight rats chosen randomly from each group were continued on their respective schedules for two additional weeks. After a total of 8 weeks on the feeding schedule, all three groups (n=8 for each group) were food-restricted beginning at the onset of the dark cycle to 33% of the previous day’s caloric intake. Since the groups were on different feeding schedules, the uniform 33% calorie restriction before sacrifice was employed to eliminate the potential confounding effects of recent food intake. The 8-week time point was chosen because it was after significant differences in feeding pattern emerged and would be representative of the maintenance phase of either obesity (Continuous Access) or an eating disorder (Binge Access). Rats were decapitated on the following day, 2 h into the dark cycle at the time of the expected re-feeding for Binge Access and Chow-Restricted groups. The animals were sacrificed in a counterbalanced staggered fashion for each group. Decapitations were performed in a separate room to
minimize the stress on the remaining animals. Approximately 4 ml of trunk blood from each rat was collected into an EDTA vacutainer tube and 20 µl was removed for blood glucose assay (Freestyle, Abbott Laboratories). The remainder of the blood sample was maintained on ice until centrifugation at 3,000 rpm for 10 min. Standard radioimmunoassay kits (Millipore, St. Charles, MO) were used to determine plasma insulin (sensitivity; 0.1 ng/ml), ghrelin (total) (sensitivity; 100 pg/ml), leptin (sensitivity; 0.5 ng/ml) and corticosterone (sensitivity; 25 ng/ml; MP biomedical, Redding, CA) levels. Epidydmal, retroperitoneal, and subcutaneous fat pads were dissected from carcasses and weighed to the nearest 0.1 g. Because these were representative of visceral and subcutaneous fat depots, the sum of these values were expressed as a consistent proportion of estimated body fat. The value of percent body fat was estimated by multiplying the grams of fat tissue by body weight.

**c-Fos immunohistochemistry of the caudal hindbrain.** A separate group of animals followed the above feeding schedules for 5 weeks. In addition to Binge Access (n=6), Continuous Access (n=5) and Chow-Restricted (n=6), an additional Naive group (n=6) was added to serve as controls for the c-Fos immunohistochemistry. Although the naive group underwent a pre-exposure to the sweetened fat, they were fed ad libitum standard chow (i.e., not food restricted or given any access to the sweetened fat) for 5 weeks. On week 6, all groups were given a 33% caloric restriction (similar to Days 3 and 6 of the Binge Access) and re-fed the following day with a standardized meal 2 h into the dark cycle. The body weight between each group prior to receiving the standardized
meal approached significance \[F(3, 19)=2.6, p=0.081\] and were 464 ± 12 g (Binge Access), 464 ± 19 g (Chow-Restricted), 537± 31g (Continuous Access) and 512 ± 18 g (Naive). The meal was the average intake of chow and sweetened fat consumed by the Continuous Access group during 2 h re-feeding. This amount was chosen to expose the animals to a standardized meal that would be readily consumed by all groups in the time allotted (<20 min; 24 kcal; 2g chow and 2g of sweetened fat). The whole meal was consumed by all the rats. Ninety minutes after the presentation of the standardized meal, rats were deeply anesthetized with a 1 ml/kg intraperitoneal injection of Euthasol (Virbac AH, Inc., pentobarbital sodium and phenytoin sodium; 1 ml/kg) and were transcardially perfused via a 16-gauge needle placed in the left ventricle, with ~200 ml of 0.15 M NaCl followed by ~150 ml of 4% (wt/vol) paraformaldehyde in PBS. Brains were removed, stored overnight in 4% paraformaldehyde with 25% (wt/vol) sucrose, frozen, and sectioned at 40 µm on a cryostat through the rostrocaudal extent of the nucleus of the solitary tract (NTS) and area postrema (AP).

The immunohistochemistry procedure was similar to that previously published by our laboratory (34). Briefly, sections were incubated for 20 h with cFos primary antibody (1:20,000 Oncogene, rabbit polyclonal, catalog No. PC38) and processed with standard immunoperoxidase methods (Vectastain ABC reagent; Vector Laboratories) with Ni-3'-3'diaminobenzidine (DAB; Vector Laboratories) chromagen incubation used to stain Fos-like products black. To control for staining variability, each immunohistochemistry run contained
matched sections from all experimental groups and controls. Quantitative analysis of c-Fos immunoreactivity was done using the IP Laboratory Imaging System (Scanalytics, Vienna, Virginia) image analysis software. The c-Fos positive cells were counted bilaterally for each structure by the imaging program by setting minimum and maximum optical density levels. In order to further normalize background conditions, software counts were compared with visual counts and standard optimal settings were applied to all experimental groups and individual runs.

Coronal bilateral sections from four rostrocaudal levels of the NTS and AP were analyzed per animal. The examined anterior posterior levels were determined by coordinates from the interaural line following Paxinos and Watson (70). The NTS areas consisted of; 3 anatomically –matched sections from caudal (cNTS; -5.6 mm), at the level of the obex, corresponding to the posterior edge of the AP; 4 anatomically –matched sections from medial, (mNTS; -5.06 mm) at the maximal extent of the AP; 4 anatomically –matched sections from intermediate (iNTS; -4.3 mm), anterior to the AP, corresponding to the maximal extent of the gelatinous subnucleus of the NTS; 3 anatomically –matched sections from rostral (rNTS; -3.8 mm) consisting of the area rostral to the gelatinous nucleus and the caudal aspect of the medial vestibular nucleus on the dorsal boundary. This analysis provided a view for the rostral-caudal extent of c-Fos activation. Because the NTS is organized in a viscerotopic fashion, the average number of c-Fos positive cells was ascertained across these rostral-caudal levels (i.e., caudal, medial, intermediate and rostral) rather than within
individual NTS subnuclei (i.e., dorsolateral, medial, commissural, gelatinous, central, etc.).

**Statistical Analysis.** Total Kcal intakes for 2 h, 20 h, and 24 h for the 6-week feeding schedules were analyzed using a two-way repeated measures analysis of variance (ANOVA) with feeding groups as the between-subject factor and weeks as the within subject factor. Separate repeated measures ANOVA were performed to determine the contribution of sweetened fat or chow on 6-week intakes or persistence of the binge-like feeding response. Hormone assays were analyzed with a one-way ANOVA and quantification of the c-Fos activation was analyzed with a one-way ANOVA at each level of the NTS. Post-hoc comparisons were made when appropriate with Neuman-Keuls test, unless otherwise noted. Correlation coefficients and t-test of slope = 0 were used across groups to determine the relationship between body weights and ghrelin levels and total fat mass and leptin levels. All statistical analyses were performed with Statistica 6.0 software (StatSoft Inc.) and significance was set at $\alpha = 0.05$. 
**Results**

*Palatable food and chow intake during the feeding schedules.* There were no significant group differences in the initial body weights or the intake of sweetened fat during the 24 h pre-exposure period. For the pre-exposure all rats demonstrated a preference (Kcal) for the sweetened fat over standard chow (65.5± 1.1%, range: 55.1-84.1%).

For the 2 h “re-feeding” caloric intakes (Days 3 and 6) there was a significantly group effect \([F(2,81)= 218.7, P<0.001]\) with all three groups different from each other \((p<0.001)\). The Binge Access group consumed the most calories during this time. There was a significant Group X Weeks interaction \([F(10, 405) =3.5, P < 0.001]\). At each Weekly time point the Binge Access and the Continuous Access groups consumed significantly more calories than the Chow-Restricted group \((p< 0.05\) or \(p<0.01;\) see Figure 1 A for week differences). For the Binge Access group, post-hoc testing revealed significantly greater intake in Week 6 compared to Week 1 \((p<0.05)\). In the Continuous Access group, intake during Week 1 was significantly greater than all other weeks \((p<0.05;\) see Figure 1 A). A separate within group repeated measures ANOVA demonstrated a significant increase in the calories derived from sweetened fat \([F(5, 135)=3.3, p<0.01]\) from Week 1 at Week 3 (not shown) and Week 6 in the Binge Access groups, see Figure 1B. Over the same time period, the Continuous Access group demonstrated a significant decrease in the calories derived from sweetened fat \([F(5, 135)=2.5, p<0.05]\) from Week 1 at Weeks 2 through 6 \((p<0.05)\), see Figure 1B for Week 6 comparisons.
The 20 h caloric intakes following the 2 h re-feeding on Days 3 and 6 also differed significantly among groups \( F(2, 81)=61.5, P<0.001 \) and again all three groups were significantly different from each other \( (p<0.001) \). The Continuous Access group consumed the most calories during this period, see Figure 2A. There was a significant Weeks effect \( F(5, 405)=9.3, P<0.001 \) and a significant Group X Weeks interaction \( F(10, 405)=6.2, P<0.001 \). At Weeks 1 through 4, intakes in the Continuous Access group were significantly greater than intakes in the Chow-Restricted group \( (p<0.01 \) or \( p<0.05 \); see Figure 1A for week differences). The Binge Access group decreased intake from Week 1 at Week 6 \( (p<0.05) \). The Continuous Access group decreased intake from Week 1 to Weeks 2 through 6 \( (p<0.05, \text{for all}) \), see Figure 2A. For the Continuous Access group the decrease in intake was attributed to decreased intake of sweetened fat \( F(5, 135)=7.0, p<0.001 \). The 24 h caloric intakes on “no restriction” Days 1 and 4 were also significantly different among the groups \( F(2, 81)=21.1, P<0.001 \). Similar to the 20 h intakes, all three groups were significantly different from each other \( (p<0.001) \) and the Continuous Access group consumed the most calories. There was also a significant Weeks effect \( F(5, 405)=5.4, P<0.001 \) and a significant Group X Weeks interaction \( F(10, 405)=6.2, P<0.001 \). Post-hoc testing revealed that Continuous Access group decreased intake from Week 1 at Weeks 2 through 6 \( (p<0.05) \), see Figure 2B. This effect could be attributed to the decrease in the calories derived from the sweetened fat \( F(5, 135)=9.4, p<0.001 \).

*Persistence of the binge-like behavior following the binge access schedule.* After the 6-week feeding schedule, a sub-group of Binge Access animals \( (n=6) \) were
placed on ad lib chow and re-tested for the binge-like feeding phenotype after 2 weeks and after 4 weeks. A repeated measures ANOVA demonstrated a significance group difference in 2 h Kcal intake over this time \[F(3, 15)=5.1, p<0.05\]. Differences were observed between the first re-feeding (“binge” 1; 75 ± 4 Kcals) and last re-feeding periods (“binge” 12; 87 ± 2 Kcals) of the 6-week cycle (p<0.05) and between “binge” 1 and the re-test after 2 weeks of ad libitum chow (89 ± 4 Kcals; p<0.05). After 4 weeks on ad libitum chow, 2h total Kcal intake differed from “binge” 12 and from after the 2 week ad lib chow re-test (p<0.05), but not from “binge” 1. Notably however, the differences at 4 weeks re-test resulted from a decrease in chow rather than sweetened fat consumption from the 2-week re-test time point (p<0.05) from 41 ± 3 to 31±3 Kcals, see Figure 3.

**Body weight, fat pad weights, and plasma hormone assays.** Animals (n=8 for each group) from the 6-week feeding schedule were maintained on their respective feeding protocols for an additional 2 weeks (i.e., 8 weeks total). Table 1 illustrates the body weight and fat pad weights at the time of sacrifice. After the 8-weeks, there were significant differences in final body weights \[F(2, 21)=13.5, p<0.01\], estimated body fat \[F(2, 21)=16.6, p<0.001\], and in the subcutaneous \[F(2, 21)=13.5, p<0.01\], retroperitoneal \[F(2, 21)=16.3, p<0.001\] and epididymal \[F(2, 21)=14.6, p<0.005\] fat pads. In all instances, post hoc tests revealed that there were significant differences between all groups compared with the Continuous Access group (p<0.01), but therewere no differences between the Binge Access and Chow Restricted groups.
Table 1 also illustrates the blood glucose and hormonal measurements after 8-weeks on the feeding regimen. Blood glucose \([F(2, 21)=9.7, p<0.01]\), insulin \([F(2, 21)=14.3, p<0.001]\), leptin \([F(2, 19)=5.2, p<0.05]\), ghrelin (total) \([F(2, 21) = 4.1, p< 0.05]\), corticosterone \([F(2, 21) = 9.2, p<0.01]\) were significantly different among groups. Post hoc testing revealed glucose, insulin, and leptin were significantly elevated in the Continuous Access group compared with the Binge Access and Chow Restricted groups \((p<0.05)\), but there were no differences between the Binge Access and Chow Restricted groups. The correlation between leptin levels and total fat mass across groups approached significance \((R=0.386, p=0.06)\). The Continuous Access group had significantly lower corticosterone levels compared with the two other groups \((p< 0.05)\), whereas the ghrelin (total) levels were significantly reduced in the Continuous Access group compared with the Binge Access group \((p<0.05)\), see Table 1 for values. The ghrelin effect was not entirely accounted for by body weight differences across groups, since ghrelin levels were not significantly correlated with body weight \((R= -0.305, \text{n.s.})\).

**c-Fos immunohistochemistry of the caudal hindbrain.** Representative photomicrographs for the mNTS and the iNTS from the Binge Access (A, C) and Naive (B,D) animals are shown in Figure 4. The number of c-Fos immunoreactive (positive) cells following a standardized meal differed between groups in 3 of the 5 hindbrain regions examined, the cNTS \([F(3,19) = 4.2, p<0.05]\), mNTS \([F(3,19) = 3.7, p<0.05]\), and the iNTS \([F(3,19 = 3.6, p<0.05]\). In the cNTS and the mNTS,
the Binge Access group had more c-Fos positive cells than the Naive group (p<0.05). In cNTS the Binge Access group had more c-Fos positive cells than Chow-Restricted group (p<0.05), whereas in the mNTS the Binge Access group had more c-Fos positive cells than the Continuous Access group (p<0.05). For the iNTS, planned comparison between groups revealed significantly more c-Fos positive cells in animals with a history of access to the sweetened fat, both Binge Access and Continuous Access groups, compared with those without, Chow-Restricted and Naive groups (p<0.01), see Figure 5.
Discussion

The aims of this study were to determine the consequences of a binge access schedule to a palatable food on feeding behavior, hormone profile, and meal-induced hindbrain neural activation in adult male Sprague Dawley rats. The palatable food used in these experiments combined both sweet and fat components with a macronutrient composition similar to that used by others in animal models of binge eating (7, 54). The most prominent differences between this model and others are that the animals underwent repeated schedules of an acute caloric restriction (33% of daily intake) prior to scheduled access to the palatable food. This is in contrast to other binge eating feeding paradigms in rodents that have employed longer periods of intermittent calorie restriction (8, 15, 16). In addition, the palatable food access period used in this paradigm occurred during the time phase associated with greatest food intake (i.e., 2 h into the dark period) (50). These conditions were chosen to maximize the amount of calories consumed during the 2 h access period by engaging both calorie regulating and hedonically driven neural pathways. In this fashion, the binge access schedule in this study utilized the rebound hyperphagia that typically results from an acute food restriction (~20 h), the temporal pattern of rodent feeding behaviors, and the innate preference for a palatable food to promote binge-like feeding in adult male rats. The interpretations of this study are limited in determining the exact contribution of each independent dietary variable on the feeding response and we cannot claim the binge access animals are displaying more than an exaggerated hyperphagic response. An additional feeding condition
that offered the sweetened fat at the same time and frequency as the Binge Access group without intermittent calorie restriction would have allowed for determining the particular contribution of the behavioral and physiological consequences of the scheduled access to sweetened fat. In addition, including a group that had continuous access to the sweetened fat and chow, but had intermittent acute calorie restriction would offer insight into how intermittent acute calorie restriction influenced the re-feeding response. The results of this study are nonetheless beneficial in understanding the consequences of binge-like eating because the Binge Access group demonstrated a caloric intake during a 2 h re-feeding with sweetened fat + chow that was ~ 170% greater than the subsequent 20 h chow only intake on “binge” days and was ~75% of the calories consumed during the 24 h (chow only) non-calorie restriction days. Because the eating patterns occur in repeated episodes and the calorie amount eaten in a short amount of time (2 h) approximates the rat’s daily (24 h) calorie intake, the amount consumed in the measured time period is binge-like. In a laboratory setting, single course binges (e.g., ice cream) of bulimics are ~1300 kcal (84). That is, approximately 65% of the recommended daily allowance of calorie intake for humans. In addition, the Binge Access group increased their relative preference for sweetened fat during the 2h binge access period as measured by the ratio of calories consumed of sweetened fat to chow calories consumed by ~25% across the 6 week experimental period.

A similar binge-like intake of calories has been reported by the Corwin laboratory in adult male and female Sprague Dawley rats exposed to a three time
per week 2-h access to vegetable shortening under non-calorie deprived conditions (20, 28). Even though the calories from the fat option (~40 Kcal) in the Corwin model is similar to the caloric amount of sweetened fat consumed in the present study, the additive calories from chow (~30 Kcal) consumed during the 2 h “binge” in the present study resulted in a total 2h intake that was almost twice (~40 versus ~80 Kcal) the amount consumed with the Corwin paradigm. The caloric intake of the Binge Access rats in the present study during the 2 h access period approached their 24 h ad libitum caloric intake on non-restricted days. In the Corwin model and similar protocols (7, 54) there is an over-consumption of calories on “binge” days, but under-consumption on non-binge days. In the present study, we observe a similar pattern of hyperphagia on “binge” days (~120 Kcal) compared with relative normal calorie intakes on “non-binge” days (~90 Kcal). In a similar fashion, we also observed a hyperphagia and hypophagia pattern of intake within “binge” days. That is, there was an increase in calories during the 2 h binge, but decrease in the following 20 h that emerged after the 6-weeks (See Figures 1 and 2). In a recent study by Cottone and colleagues, a binge-like feeding response to a sucrose-rich diet was produced in female rats when the preferred diet was preceded by access to a standard diet. In that study rats underwent a daily regimen of 1 h standard chow access, 2 h food deprivation, 10 min access to standard chow followed sequentially by 10 min access to either the preferred diet (Chow/Preferred) or standard chow (Chow/Chow) for a period >2 weeks. The Chow/Preferred group displayed an anticipatory negative contrast effect in that they developed a preferred diet
hyperphagia that was dissociable from the standard diet hypophagia (22). Similar to the findings from the present experiment, the bingeing rats also developed a hypophagia of the home-cage standard chow (20 h access) that was temporally related to the onset of the preferred diet hyperphagia. Even though the 20 h hypophagia in this study is likely a compensatory suppression of feeding from overeating, further experiments are needed to determine the relative contributions of hedonic or calorie regulating mechanisms are involved in this post-binge hypophagic response (40, 44). Whatever the cause, these data demonstrate a shift in the eating pattern during the “binge” and post-“binge” feeding periods of rats on the binge access schedule.

The binge-like feeding response demonstrated over the 6-week schedule in the Binge Access group persisted after a 2-week period of ad libitum chow intake. This suggests an entrainment of the physiological substrates involved in the feeding response, as similar examinations of the persistence of binge-like eating used by others have suggested. The Corwin group has reported that an imposed abstinence of vegetable shortening for 5 weeks produced a more pronounced “binge” intake when the vegetable shortening was reinstated (86). A comparable persistence of a binge response was reported in rats exposed to longer periods of calorie restriction. Hagan and Moss exposed female Sprague Dawley rats to 12 restriction-feeding cycles (6-8 days per cycle) consisting of 4-6 days of 75%-50% calorie restriction followed by 2-4 days of chow re-feeding with a palatable food (e.g., vanilla creme cookies). The persistence of a binge-like phenotype after 30 days of ad libitum chow was demonstrated following a 24 h
caloric restriction and under spontaneous feeding conditions (43). In that study under both conditions, the palatable food was given following a 3.5 h period of chow access. In the present study we did not observe a sustained binge-like phenotype after 4-weeks of ad libitum chow. Notably, the reduction in total caloric intake at 4 weeks was due not to a decrease in the palatable food consumed, but rather to a decrease in chow intake during the 2 h re-test. This suggests that although total calories consumed did not increase in response to reinstatement of the schedule at 4 weeks, the relative preference for the palatable food remained intact. In addition, these results suggest that the binge-like phenotype in this model is transient or experience-dependent, opening future possibilities for studying the physiological changes that occur at various time points before, during or after animals are on the binge access cycle and display or lose the phenotype.

Despite differences in feeding patterns between groups, only the Continuous Access group differed in body weight gain or body fat after 8 weeks. There were no differences in body weight and composition between the Binge Access and Chow-Restricted groups. Consistent with their increased body weights, the Continuous Access group also had higher glucose, leptin and insulin levels. This finding is in agreement with the findings from other feeding protocols that have used continuous access to a palatable food to produce diet induced obesity in Sprague Dawley male rats (57, 58). Corticosterone levels were not different between the Chow Restricted and Binge Access, but these levels were~4 fold higher than in the Continuous Access group. Such elevations are
consistent with prior findings of elevated plasma corticosterone levels following acute and chronic food restriction (24, 49). Using adult male Long-Evans rats with 5-week continuous access to a sweetened fat mixture, Kinzig and colleagues demonstrated that rats with intermittent (2 h access, 3-days a week) or scheduled (2 h, 7-days a week) sweetened fat had a blunted stress response and higher palatable food consumption (54). Long-term intermittent exposure to a physical stressor (i.e., foot shock) is also necessary to elicit a robust binge-like feeding response in a rodent model of binge eating using repeated cycles of calorie restriction, palatable food (i.e., oreo cookies) and stress (45). Furthermore, rats exposed to caloric restriction and foot shock had higher baseline corticosterone levels than rats experiencing restriction or stress alone (2). In our model, rats in the Binge Access and Chow-Restricted groups were exposed to repeated acute caloric restriction twice per week and had corticosterone levels that approximated those following a physical stressor (52, 54, 71).

Elevated plasma ghrelin levels in the Binge Access compared with the Continuous Access group may have additionally contributed to the increased consumption during the binge access period. Although the Binge Access group had the highest ghrelin levels, there were not significantly different from Chow Restricted group. In this study there was no significant correlation between body weights and ghrelin levels, suggesting the difference between the Binge Access and Continuous Access groups was not a result attributed entirely to the weight differences between animals. Higher endogenous ghrelin levels are associated
with increased subjective hunger ratings in humans and exogenously administered ghrelin elicits increases in food intake in humans and rodents (23, 56). The increased salience of the “binge” meal, in the binge access schedule may have resulted in an entrainment of the “pre-binge” ghrelin levels in this group as has been reported with habitual eating patterns (29, 37). Elevation of plasma cortisol and ghrelin following an overnight fast has also been demonstrated in clinical eating disorder populations (41, 42, 82, 83), although there are some inconsistencies among these studies (68).

To address how a history of the feeding conditions may drive or facilitate the binge response, we examined the hindbrain neural response of all groups to a standardized meal. The meal was considerably smaller than the Binge Access group typically consumed and all groups underwent 33% calorie restriction the day prior to meal presentation to ensure that all of the meal would be consumed by all groups. Elevated c-Fos labeling in response to the test meal was demonstrated in the Binge Access group, compared with Naive and Chow-Restricted groups, in regions of the NTS that receive vagal afferent input from the gastrointestinal tract (4, 47). We had expected that a history of binge-like eating might lead to decreased rather increased meal induced hindbrain activation. The rationale for this expectation was two-fold. First, the NTS has been demonstrated to play an important role in mediating controls on meal size. Signals that decrease feeding (e.g., mechano, nutrient, CCK, leptin, etc.) have been shown to result in NTS neural activation (33, 55, 78). Second, bulimic subjects have increased gastric capacity, a finding which would be expected to
result in diminished feedback inhibition on the NTS for a given gastric load, potentially facilitating binge intakes (38). However, this was not the result. The number of c-Fos positive NTS neurons was increased in the Binge Access group.

There are a number of potential explanations for the increased NTS activation in the Binge Access group. One possible explanation for the increased c-Fos activation in the Binge Access animals is that there could have been increased sensory signaling and increased activation due to the enhanced salience of the standardized meal (i.e. 2 g of chow and 2 g of sweetened fat) as a consequence of their binge history. Evidence, in part, for this notion can be supported by robust c-Fos activation in medial and intermediate regions of the NTS following intraoral delivery of a standard volume (7.5 ml) of sweet tasting sucrose (0.5 M) solution compared when with bitter tasting quinine (1 mM) solution or distilled water (89). Similar to the c-Fos pattern of activation in our study, there were no differences in the rNTS, a region that receives afferent gustatory information, between the intraoral delivery of sucrose and quinine (89).

An additional contribution could derive from the increased plasma ghrelin levels in the Binge Access rats. Recent work examining the orexigenic role ghrelin has revealed that central and peripheral administration of ghrelin results in increased NTS activation, suggesting a role for the NTS in mediating the feed-forward mechanisms of food intake(36, 56, 81). Since we did not include two additional control groups, a group that had repeated access to sweetened fat at the same time and frequency as the Binge Access group without intermittent calorie restriction and a group with continuous access to the sweetened fat with
intermittent acute calorie restriction, the separate effects of diet and restriction
cannot be discounted in the observed meal-induced c-Fos response. The
increases in NTS activation in the Binge Access group, however, could represent
a feed-forward mechanism, mediated in part by elevated ghrelin, driving the
robust intake during the “binge”. This increased salience is similar to what is seen
in response to 4th ventricle administration of a dose of ghrelin, which induces a
hyperphagic response and increased c-Fos activation in the NTS (35, 36).
Because one of the principal roles of the caudal brainstem in feeding involves the
control of meal size, this pattern of neuronal activation in the Binge Access group
is suggestive of involvement of the NTS in binge-like eating (77). Moreover, the
NTS activation may represent the mechanism involved in facilitating the
accommodation of the larger meal size of the “binge”.

Even though the phenotype of the c-Fos expressing cells was not
characterized in the present study, there are reasons to believe that the activated
cells are different from catecholamine neurons activated by satiety signals. NTS
catecholaminergic (i.e., immunostained for tyrosine hydroxylase; TH-positive) c-
Fos positive cells are not significantly activated in rats when given a smaller than
usual (i.e., 1/3 the amount) volume of a dextrose sweetened liquid diet compared
with unfed rats (76). In fact, Rinaman and colleagues observed the highest
activation of TH-positive c-Fos positive cells in animals that had standard
(unrestricted) access to an unexpected diluted volume of the liquid diet. It is also
the case that the c-Fos activation following 4th ventricle ghrelin does not occur in
TH positive cells (36). Additional experiments are required to determine the
phenotype of NTS cells that are activated in response to the standardized meal in animals with a history with the binge access feeding schedule.

**Perspectives and Significance**

The heterogeneity of onset and multi-factorial aspects of bulimia nervosa (or any psychiatric disease) makes developing an appropriate animal model difficult. Clinical and pre-clinical research has focused on whether dieting, dietary restraint, and/or psychological stress are precursor or risk factors to binge eating in this population (67, 72, 85). Rather than examining factors that initiate or lead to an onset of binge eating, we used a restriction/binge model of bulimia nervosa to determine the physiological consequence of this pattern of eating behavior that may facilitate disease maintenance. Data from these experiments demonstrate that repeated acute calorie restriction prior to access to a highly palatable food resulted in an exaggerated binge-like behavioral response in adult male Sprague Dawley rats. Accompanying the alteration in palatable food preference are hormonal and hindbrain changes that promote a feed-forward feeding response. Other animal models of binge-like intake in rodents have been reported, however the model used in this study is unique in that it combines acute calorie restriction with scheduled access to a highly palatable nutrient. These features make the model and its results more relevant to the consequences of human dieting behaviors including dietary restraint and binge eating, and the clinical syndrome of bulimia nervosa.
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References

36. Faulconbridge LF, Grill HJ, Kaplan JM, and Daniels D. Caudal brainstem delivery of ghrelin induces fos expression in the nucleus of the solitary tract, but not in the arcuate or paraventricular nuclei of the hypothalamus. *Brain Res* 1218: 151-157, 2008.
Bingeing schedule and feed-forward mechanisms of intake


78. **Schwartz GJ and Moran TH.** Leptin and neuropeptide y have opposing modulatory effects on nucleus of the solitary tract neurophysiological responses to gastric loads: implications for the control of food intake. *Endocrinology* 143: 3779-3784, 2002.
84. **Walsh BT, Kissileff HR, Cassidy SM, and Dantzic S.** Eating behavior of women with bulimia. *Arch Gen Psychiatry* 46: 54-58, 1989.
Figure Legends

Figure 1. Total caloric intakes for the 2 h re-feeding period on Days 3 and 6 of the 6-week feeding schedule. A: The 6-week total caloric intake (Kcal ± SEM) for the 2 h period (n=14 per group). A significant differences from the Chow-Restricted at the respective week is indicated by # (p<0.01) and + (p<0.05). A significant difference within groups Week 1 total Kcal intake is indicated by * (p<0.05). B: Over the 6-week period the Binge Access group consumed significantly more calories derived from sweetened fat on Week 6 than Week 1, while the Continuous consumed less over the same time period (p<0.05), which is indicated by *. The striped area represents the portion of calories derived from sweetened fat, while the solid area represents those calories derived from chow.

Figure 2. Caloric intakes for the 20 h following the 2 h re-feeding period on Days 3 and 6 and the 24 h feeding period on Days 1 and 4 of the 6-week feeding schedule. A: The 6-week total caloric intake (Kcal ± SEM) for the 20 h period after the 2 h re-feeding. B: The 24 h intakes on “no-restriction” (ad libitum) days is represented. A significant differences from the Chow-Restricted at the respective week is indicated by # (p<0.01) or + (p<0.05). Significant difference within groups Week 1 total Kcal intake is indicated by * (p<0.05)

Figure 3. Persistence of binge-like feeding following a 2-week and 4-week period of ad libitum feeding with standard chow. The respective contribution of either the sweetened fat (striped area) or chow (solid area) to the total caloric intake during the 2 h refeeding period (“binge”) for the Binge Access group. The intakes of first (1) and last (12) individual binges of the 6-week cycles and after a period of ad libitum chow (i.e., 2 weeks and 4 weeks) are shown. There were significant differences between the total calories consumed at “binge” 1 and those consumed at “binge” 12 or at the re-test after 2 weeks of ad libitum chow (*; p<0.05). A significant difference was observed in the intake of chow from 2 weeks ad lib to 4 weeks ad lib ($; p<0.05).
Figure 4. Representative coronal micrographs of immunohistochemistry c-Fos staining (black) of Binge Access (A and C) and Naive (B and D) rats 90 minutes following the presentation of a standardized meal. Bilateral hindbrain sections from the medial (mNTS, A and B) and unilateral sections from the intermediate regions (iNTS, C and D) of the nucleus of the solitary tract. The bar in each image represents 200 µm. Abbreviations; AP, area postrema; ST, solitary tract; IV, fourth ventricle.

Figure 5. Average immunoreactive c-Fos counts in hindbrain regions of rat with different histories of feeding schedules in response to a standardized meal. All groups were given a 33% caloric restriction (similar to Day 2 of the Binge Access) and re-fed the following day with a standardized meal (e.g., 2 g of chow and 2 g of sweetened fat) 2 h into the dark cycle. The meal was the average intake of chow and sweetened fat consumed by the Continuous Access group during 2 h re-feeding (see figure 1A). In the cNTS, the Binge Access group had significantly more c-Fos positive cells than the Naive (*, p<0.05) and Chow-Restricted ($, p<0.05). In the mNTS the Binge Access group had significantly more c-Fos positive cells than the Naive (*, p<0.05) and Continuous Access (#, p<0.05) groups. Planned comparisons revealed a significant effect for palatable food access was observed in the iNTS region, in that, the groups that with a history of access to the sweetened fat compared with those that did not, had more c-Fos positive cells(**, p<0.01).
Table 1. Body weight and blood parameters after 8 weeks on the feeding schedules

<table>
<thead>
<tr>
<th></th>
<th>Binge Access</th>
<th>Continuous Access</th>
<th>Chow Restricted</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial Body Weight (g)</strong></td>
<td>342 ± 10</td>
<td>343 ± 12</td>
<td>342 ± 10</td>
</tr>
<tr>
<td><strong>Final Body Weight (g)</strong></td>
<td>507 ± 24</td>
<td>639 ± 35</td>
<td>506 ± 21</td>
</tr>
<tr>
<td><strong>Estimated Body Fat (% of Body Weight)</strong></td>
<td>~11%</td>
<td>~22% *</td>
<td>~11%</td>
</tr>
<tr>
<td>Subcutaneous (g)</td>
<td>29 ± 4.7</td>
<td>92 ± 15</td>
<td>32 ± 4.7</td>
</tr>
<tr>
<td>Retroperitoneal (g)</td>
<td>15 ± 2.0</td>
<td>29 ± 3.4</td>
<td>14 ± 1.8</td>
</tr>
<tr>
<td>Epididymal (g)</td>
<td>13 ± 1.9</td>
<td>23 ± 1.9</td>
<td>11 ± 1.3</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.5 ± 0.23</td>
<td>6.1 ± 0.4</td>
<td>4.5 ± 0.18</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>1.1 ± 0.19</td>
<td>5.8 ± 1.2</td>
<td>1.2 ± 0.21</td>
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<tr>
<td>Leptin (ng/ml)</td>
<td>7.8 ± 1.5</td>
<td>13.9 ± 1.4</td>
<td>8.1 ± 1.6</td>
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<td>Ghrelin (total;ng/ml)</td>
<td>2.9 ± 0.3</td>
<td>1.9 ± 0.2</td>
<td>2.5 ± 0.2</td>
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<tr>
<td>Corticosterone (ng/ml)</td>
<td>348 ± 68</td>
<td>83 ± 11</td>
<td>368 ± 60</td>
</tr>
</tbody>
</table>

*  p<0.05 significantly different from Binge Access and Chow-Restricted Groups
#  p<0.05 significantly different from Binge Access Group only