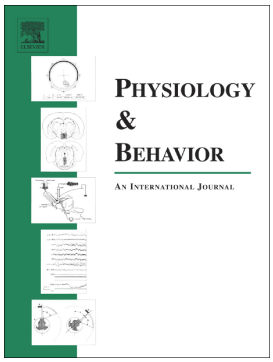

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Relatively Enriched Housing Conditions Delay Binge Onset but Do Not Attenuate Binge Size

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ABSTRACT

Housing and enrichment conditions are essential factors to consider when using animal models of behavior, as they can alter the behavior that is under investigation. The goal of this study was to determine the impact of the relatively enriched environment recommended by current animal care guidelines on development and maintenance of binge-type behavior in rats, using the limited access (LA) binge model. Non-food-deprived rats were divided into two groups, enriched and nonenriched, with all rats housed in shoebox cages. Bedding, nesting material, toys, and a solid floor were provided only to the enriched group to create a state of relative enrichment, or RE, compared to the nonenriched conditions historically used in the LA model. Enriched and nonenriched groups were further divided into control and experimental groups. Control rats received access to an optional source of fat (vegetable shortening) for 30 minutes each day (daily access) while experimental rats received 30-min optional fat access on Monday, Wednesday, and Friday only (intermittent access). The four groups were designated C-E (Control-Enriched), C-NE (Control-Nonenriched), I-E (Intermittent-Enriched), and I-NE (Intermittent-Nonenriched). Bingeing in the LA model is established when a group with intermittent access (i.e., I-E or I-NE group) consumes significantly more vegetable shortening during the limited access period than a group with daily access (i.e., C-E or C-NE group). Access sessions continued for 8 weeks under these conditions, at which time the housing conditions of the I-E and I-NE groups were reversed for an additional 8 weeks of access sessions. Intakes of the C-E and C-NE groups were similar and data from these two groups were combined. Relative to this Combined Control Group (CCG), the I-NE group began bingeing in week 3 while the I-E group binged during weeks 6 and 8. Following the reversal at the beginning of week 9, the newly enriched I-NE group ceased bingeing in week 9 but resumed bingeing in weeks 10-16. The newly nonenriched I-E group continued bingeing through the remainder of the study. Intakes of the I-E and I-NE groups were not significantly different at any time during the study. These results indicate that RE delays binge onset; that is, RE increases the time between the first fat access session and the first occurrence of bingeing. However, RE does not significantly alter the amount of fat consumed during binge sessions. Furthermore, addition of RE to a nonenriched group of animals (I-NE) does not reverse established binge behavior. Thus it appears that regardless of enrichment condition, intermittent access to vegetable shortening induces greater consumption of fat than does daily access. However, it is clear that a certain level of austerity in housing conditions is required for rapid development of lasting binge-type eating to occur. In addition, results suggest that it is unlikely that enrichment, to the degree provided in this study, can prevent or reverse binge-type eating in rats.

Key Words: Binge eating; dietary fat; enrichment; food intake; ingestive behavior

1. Introduction

About 5% of Americans binge eat, which is associated with a variety of psychological and physical comorbidities [1, 2]. To study binge-type behavior, Corwin and colleagues developed the limited access (LA) rat model of binge-type eating [3, 4]. The LA model utilizes an intermittent schedule of brief access to a palatable food, typically vegetable shortening, to induce binge-type eating. Intermittent-access rats are designated as bingeing when they consume significantly more of the palatable food during the limited access period than control groups receiving daily brief access. Rats are not food-deprived at any time and are therefore eating in the absence of hunger [5], a common feature of binge eating disorder (BED) as described in the DSM-V [6]. Furthermore, body weights of rats maintained on this protocol are similar to those of rats maintained on chow only [5]; thus, this model allows for the examination of factors critical to binge behavior in the absence of confounds associated with obesity. This also has relevance to human binge eating, as only about 35% of people who regularly binge are overweight or obese [1].

Traditionally, rats used in the LA model are singly housed in hanging stainless steel wire cages with no enrichment or other additions to the cages. Since the origination of the LA model of binge-type eating, the Guide for the Care and Use of Laboratory Animals and the Office of Laboratory Animal Welfare (OLAW) have published stronger language in support of enrichment, solid flooring, bedding, and social housing for laboratory rodents [7, 8]. Thus, it is desirable to be able to study binge-type eating using the LA model with rats housed in the relatively enriched environments recommended under current animal care guidelines, though whether or not binge eating will develop under these conditions has not been previously studied.

The Guide's recommendations for the use of enrichment are the result of studies showing that environmental enrichment (EE) positively affects the physical and psychological well-being of laboratory animals [7]. Research in the field of EE also has shown that EE provides broad-spectrum protection against the development of behavior in animals relevant to addiction and depression, and improves recovery from various neurological disorders [9] and injuries [10-16]. Some specific addiction-related parameters that are reduced by EE in animal models include sensitization to repeated exposure to psychostimulants, nicotine, and morphine, self-administration of psychostimulants and ethanol, motivation to obtain drug (amphetamine), extinction resistance, and tendency to relapse [9, 17]. Research on the effects of EE on depression-like behavior in rats reveals decreases in anhedonia-type behavior, social withdrawal, and behavioral despair, as measured by sucrose consumption in a sucrose preference test, grooming time in a social contact test, and mobility time in a forced swim test, respectively [9].

A number of other studies have focused on the effects of EE on ingestive behavior in rats [18-26]. Enrichment can affect sucrose consumption [24, 26-28] and reduces resistance to extinction of responding for sucrose or food [19, 20], attribution of incentive salience to a stimulus associated with food [18], anticipatory responding for sucrose-paired stimuli [22, 23], and sucrose cue-reactivity [21, 24]. To our knowledge, no studies exist on the effects of enrichment on binge-type eating specifically. Given the overlap in neurobiological, genetic, and psychobehavioral characteristics of addiction and disordered eating [29-35], as well as evidence supporting the influence of EE on multiple behavioral and neural parameters (cited above, and [36]), we hypothesized that an enriched environment would also attenuate binge-type eating in the LA model.

Historically, enrichment studies have utilized novelty, exercise, social contact, and frequently a large and/or complex environment as the components of EE. Facets of one, two, or more of these components are used in various combinations and are usually compared to standard or isolated housing conditions. While these components of EE have seen widespread usage, Crofton et al. [9] notes that at a fundamental level, EE must simply have a positive effect on the animal such that the enriched condition is set apart from conditions that have a negative effect. In the present study, 'enriched' rats were provided with bedding, nesting material, non-caloric chew toys, retreats, and solid flooring, while 'nonenriched' rats were housed on wire flooring with no bedding, nesting material, toys, or retreats. Thus, in order to distinguish between traditional styles of enrichment and the set of enrichment conditions used in the present study, we refer to the former as 'environmental enrichment', or EE, and the latter as 'relative enrichment', or RE. Because RE can be viewed as a mild form of EE, it may be reasonably expected that behavioral and/or physiological effects of these forms of enrichment would be similar, though possibly varying in degree and dependent on the specifics of the enrichment conditions used.

The investigation described here examined the effect of relatively enriched housing conditions, using current animal welfare guidelines for the housing of laboratory rats to represent a state of relative enrichment, on fat intake in the LA model of binge-type eating. Our objective was to determine whether a relatively enriched environment would a) reduce or prevent the establishment of bingeing, and b) attenuate or abolish bingeing once it is established. In short, we sought to determine if RE could prevent as well as treat binge-type eating in rats.

2. Methods

2.1 Animals

Sixty-four female Wistar rats were housed individually in standard shoebox cages (48.3 cm x 26.7 cm) fitted with wire rack inserts upon receipt. This represented the nonenriched condition, as described below (section 2.2). Rats were kept in these nonenriched conditions until completion of group assignment procedures (section 2.3.1). Upon receipt, rats weighed 194-248 g and were approximately 60 days old. A reverse 12:12 light cycle with lights out at 11 AM was implemented on the day of the rats' arrival. Rats had *ad libitum* access to water and nutritionally complete rat chow (Purina 5001 Rodent Lab Chow, Gray Summit, MO, USA). Chow was removed during the 30-minute shortening access sessions but access to water was maintained. Cage changes were performed once weekly. During the transfer to clean cages, rats were handled for about 15 seconds and body weights were recorded. The temperature of the animal room was maintained at 68-72°F. The humidity varied between 30-70%, though efforts were made to keep humidity levels as close as possible to the preferred range of 40-45% [37]. All procedures were approved by the William Beaumont Army Medical Center Institutional Animal Care and Use Committee.

2.2 Housing Conditions

Nonenriched conditions consisted of standard shoebox cages fitted with wire rack flooring inserts to imitate the cages traditionally used in the LA model [3, 4, 37-39]. No bedding materials, toys, or other

enrichment items were added to these cages. Again, this was done to mimic typical LA housing conditions. Relatively enriched conditions consisted of the same shoebox cages as used for the nonenriched environment but with Paperchip® rodent bedding (Shepherd Specialty Papers, Watertown, TN, USA), Enviropaks™ for nesting (W.F. Fisher and Son, Somerville, NJ, USA), Nylabone® non-caloric chew toys (TFH, Neptune, NJ, USA), rectangular retreats, and no wire rack flooring insert (i.e., a solid floor). This environment meets but does not exceed the standard recommendations of OLAW and the Guide for the Care and Use of Laboratory Animals for rodent housing. These standard recommendations represent a relatively enriched environment in comparison to the nonenriched housing conditions.

It is important to note that the rats were singly housed regardless of environment. This enabled us to keep the rats in their home cages during shortening access sessions, to calculate the consumption of food by individual rats, and to mimic the established LA procedures. The shoebox cages are transparent and were arranged with 4 rats per row x 4 columns per rack, with all 4 racks located in the same room. Rats therefore had visual, auditory, and olfactory contact with other rats throughout the study.

2.3 Design

Limited access procedures were based upon previously published procedures [37, 39], and are described below.

2.3.1 Pre-experimental Procedures

Following a one-week acclimation period, rats were given overnight access to 25g of Crisco® vegetable shortening (a semi-solid fat; The J.M. Smucker Company, Orrville, OH, USA) on 3 occasions that were separated by 24 hours. This was done in order to (a) prevent neophobia, (b) determine individual rats' propensity to eat the shortening, and (c) possibly induce faster establishment of bingeing. Water and chow were freely available during overnight access sessions. Any rats that did not consume Crisco during overnight access sessions were excluded (n=4). The remaining 60 rats were matched by body weight and average Crisco consumption and placed into four groups of n=15 each: Control-Enriched (C-E), Intermittent-Enriched (I-E), Control-Nonenriched (C-NE), and Intermittent-Nonenriched (I-NE). Rats in C-E and I-E groups were placed into the shoebox cages containing bedding, nesting material, chew toys, and retreats, per the relatively enriched conditions described above (section 2.2). Rats in C-NE and I-NE groups remained in the shoebox cages containing only wire rack flooring inserts, per the nonenriched conditions described above (section 2.2). Groups were distributed evenly throughout the rows, columns, and racks used for cage storage. Twenty-four hours after animals were placed into their experimental environments, they were given one additional overnight Crisco access session. Experimental procedures began on the third day following this fourth and final overnight access session.

2.3.2 Experimental Procedures

The control groups, C-E and C-NE, were given access to Crisco for 30 minutes every day of the week. The intermittent groups, I-E and I-NE, were given access to Crisco for 30 minutes on Monday, Wednesday, and Friday (MWF) only. The access time was shortened relative to the 1 or 2 hours typically used in previous publications. The choice of 30 minutes was based on observations during a refinement

phase that the majority of Crisco consumption occurred during the first 10-15 minutes. Occasional additional intake by a few animals after the first 15 minutes created only nominal differences in overall 1-hour consumption. Previously, the LA procedure has been successfully implemented with 20-min and 30-min access periods [40, 41]. Access sessions occurred 2 hours before lights out each day and all groups received access simultaneously. Fresh shortening (60 g) was given at least once weekly, and bowls were refilled any time they fell below approximately 30 g. Shortening bowls were protected from excessive bedding contamination by suspending the bowls against the wall of the cages using metal hangers. The height of the bowls was sufficient to keep bedding out but still allow for easy access by the rat. Chow was removed from the cages during access sessions and replaced immediately after. Rats had continuous access to water during access sessions.

Groups with intermittent access (I-E and I-NE) could hear, smell and see when access sessions were given to groups with daily access (C-E and C-NE) on Tuesday, Thursday, Saturday, and Sunday. Any potentially disruptive procedure was performed on Tuesday or Thursday to minimize impact on MWF intakes. For example, regular cage changes took place on Thursdays.

Average weekly consumption was converted from grams to kilocalories and normalized to body weight$^{2/3}$ (providing a mass-independent expression of energy metabolism [42]) using the following formula:

$$\text{Normalized MWF Crisco Intake} = \text{MWF kcal Crisco}/(\text{g Body Weight}^{2/3})$$

Normalized shortening intakes were used for all groups to determine weekly binge status. After 8 weeks, housing conditions were reversed for the intermittent groups only. Control groups remained in their initial environments. Crisco access procedures were maintained for an additional 8 weeks. Following the reversal, the I-E group was renamed x-I-E, and the I-NE group was renamed x-I-NE.

Bingeing was operationally defined in the following two ways: (a) I-E and I-NE groups were designated as bingeing when their average MWF shortening consumption for the week significantly exceeded that of the control group, and (b) bingeing was considered to be firmly established once two consecutive weeks of significant differences between the I-NE or I-E groups and the control group were observed.

2.4 Statistics

Body-weight normalized Crisco intakes were used for all analyses of relative consumption. Rats that did not eat Crisco during the first few 30-min access sessions were excluded from the statistical analyses (n=6; of these, 3 were from C-NE, 2 from I-NE, and 1 from C-E), in addition to the rats that had already been excluded for non-consumption during pre-experimental procedures (n=4). One additional rat (from the C-NE group) was excluded from the analyses beginning in week 13 due to health concerns. Interestingly, the health issues resolved once RE was instituted. A total of 53 rats were included in the final analyses (n per group: C-E, 14; C-NE, 11; I-E, 15; I-NE, 13).

Statistical Analysis Software (SAS v. 9.4, Cary, NC, USA) was used for all statistical analyses. Initially, the I-E group was compared to the C-E group and the I-NE group was compared to the C-NE group. However, housing conditions were not switched for the control groups in week 9 in order to keep the independent variable (housing condition) constant throughout the study for the controls. Therefore, comparing each experimental group to its respective control during the last 8 weeks of the study

necessitated either comparing each experimental group to a control group in a different housing condition (for instance x-I-NE to C-NE), or to a different set of rats than pre-reversal that were in the same housing condition (for instance x-I-NE to C-E). For these reasons, we sought to determine if the control groups, C-E and C-NE, could be combined by determining whether intakes and body weights differed between them. No statistically significant differences existed between the intakes of the two control groups for the entire study ($p>0.05$ for all weeks; see Figure 1). Likewise, body weights of the C-E and C-NE groups did not differ significantly throughout the study (data not shown; $p>0.05$ weekly). Therefore, the C-E and C-NE groups were treated as a single combined control group (CCG) for analysis, in order to provide continuity throughout the 16 weeks in the comparisons performed. Both shortening intake and body weight were assessed by the general linear mixed model for repeated measures across 16 weeks to test for group effect, week effect, and group by week interaction. Comparisons were made both before and after the reversal of housing conditions took place. For the reversal, I-E and I-NE groups were switched starting at week 9. In the analyses, the rats stayed in their respective groups, with the reversal noted.

With significant group x week interaction, post-hoc tests were conducted to check for the significance of group effects for each week and significance of week effects for each group. Significance for any of these effects was followed by simple effect comparisons, to determine differences among groups, using the multiple comparison Holm-Tukey method for post-hoc analyses. All tests were conducted with a significance level of $\alpha = 0.05$.

3. Results

For body weight-normalized Crisco intake, a 3 x 16 general linear mixed model for repeated measures over time revealed a significant group x week interaction ($F(30,761)=4.31$, $p<0.0001$). Further post-hoc testing showed significance of group effect starting at week 3 ($F(2,761)=4.96$-14.58, $p\leq0.0072$). Simple effect comparisons between the intermittent and control groups for each week are described in 3.1 and 3.2 below.

3.1 Nonenriched Housing Conditions

Shortening consumption normalized to body weight is shown over time in Figure 2 for the I-E, I-NE and CCG groups (see Table 1 for intake in grams). Intake of the I-NE group became significantly different from the CCG beginning in week 3 and remained so for the rest of the 8-week pre-reversal period ($p\leq0.0136$ weekly). Thus, according to the operational definition of bingeing used in this study, bingeing first occurred in the I-NE group in week 3 of the experimental period, was firmly established in week 4, and continued through week 8.

Following the addition of RE to the cages after week 8, the I-NE group was referred to as x-I-NE. The previously established binge behavior was attenuated for the x-I-NE group in week 9 ($p>0.05$) but resumed in week 10 ($p=0.0087$) and continued for the duration of the study ($p\leq0.0026$ weekly).

Results were similar when the individual control groups were used for the comparisons, i.e., when the I-NE group was compared to the C-NE group (instead of the CCG) for the first 8 weeks. That is, bingeing

occurred during week 3 and became firmly established by week 4, continuing through week 8. Following the addition of RE after week 8, bingeing was temporarily attenuated when compared to both the C-NE and C-E groups, but then resumed for the duration of the study (see Table 2 for p- values).

3.2 Relatively Enriched Housing Conditions

Normalized intakes for the I-E group were not statistically different from the CCG for weeks 1-5 ($p>0.05$ weekly). The normalized intake for the I-E group was significantly greater than that of the CCG ($p=0.0382$) in week 6 but this did not continue in week 7 ($p>0.05$). Thus, although bingeing occurred in week 6 in the I-E group, it was not firmly established during the pre-reversal period per the operational parameters used in this study (2 consecutive weeks of bingeing). Bingeing again occurred in the I-E group in week 8 ($p=0.0292$), and became firmly established in week 9 ($p=0.0005$); however, week 9 is when the housing conditions were reversed. Bingeing then continued for the remainder of the study ($p\leq0.0011$ weekly). Note that the I-E group is referred to as x-I-E following the reversal at week 9.

While intakes of the I-E group were not statistically different from control for most of the pre-reversal period, the intakes of the I-E group also did not differ from the I-NE group. Specifically, there were no significant differences between the I-E and I-NE groups throughout the entire study ($p>0.05$ weekly). Thus, although RE delayed binge onset, it did not significantly reduce binge size.

Results for the I-E group during the first 8 weeks differed somewhat when the individual control groups were used for the comparisons; i.e., when the I-E group was compared to the C-E group (instead of the CCG). Specifically, operationally defined bingeing did not occur during the entire 8-week pre-reversal period. However, bingeing did occur in week 9 when compared to both the C-NE and C-E groups, and continued for the remainder of the study (see Table 2 for p- values).

3.3 Body Weights

Prior to the start of experiments, animals were matched by body weight (as well as shortening consumption during overnight sampling sessions). Consequently, there were no significant differences in body weights among groups at the start of the study ($p>0.05$). However, the group x week interaction was significant ($F(30,761)=7.38$, $p<0.0001$). Post-hoc testing revealed significance of the group effect starting at week 9 ($F(2,761)=4.72 – 11.24$, $p\leq0.0091$). Beginning in week 9, body weight of the x-I-E group became significantly lower than the CCG ($p=0.0147$; see Figure 3). In week 11, body weight of the x-I-NE group also became significantly lower than the CCG ($p=0.0301$). Body weights then remained significantly lower than the CCG to the end of the study ($p\leq0.0010$ weekly for the x-I-E group; $p\leq0.0132$ weekly for the x-I-NE group).

Similar results were obtained when the analysis was performed using the separated control groups, C-NE and C-E. That is, body weights of the intermittent groups, I-NE and I-E, did not differ from their respective controls during the first 8 weeks, but did diverge from controls after the reversal. However, body weights became consistently lower than controls at a later time point than when the CCG was used. Specifically, body weights of both intermittent groups were consistently lower than those of both control groups in weeks 13-16 ($p< 0.05$ weekly; data not shown). In week 12, body weight of the x-I-E group

was also significantly lower than the body weights of both control groups, but the body weight of the x-I-NE group was significantly less than only the C-E group ($p < 0.05$ weekly; data not shown).

4. Discussion

4.1 Binge eating is readily established in shoebox cages with wire flooring inserts

Results demonstrate that the wire flooring inserts used in shoebox cages for the nonenriched housing conditions of the present study create an acceptable substitute for the hanging wire cages traditionally used in the LA method. The visual, olfactory, and auditory contact with neighboring animals could be viewed as limited social contact and therefore as an element of EE. However this did not prevent binge-type eating from developing, which is similar to results reported in previous studies using animals individually housed in hanging wire cages [3, 4, 38].

4.2 RE delays the development of binge-type eating

Given a relatively enriched environment including non-caloric chew toys, nesting and bedding material, retreat cubicles, and solid flooring, female rats in the I-E group did not commence enduring binge-type eating of an optional source of dietary fat inside of the 8-week pre-reversal timeframe provided. This is in contrast to the I-NE group, which commenced enduring binge-type behavior in week 3. According to the operational definition used for this study, bingeing was considered to be firmly established once the behavior occurred for two consecutive weeks. When the I-E group was compared to the CCG, binge-type eating was seen in the I-E group at week 6 but disappeared in week 7 and then reappeared in week 8; however, when the I-E group was compared to the C-E group, bingeing did not operationally occur at all during the first 8 weeks. This was likely due to the size of the control group used in the analyses. Regardless of the control group used for the analyses, however, bingeing occurred during week 9 and then continued through the end of the study. Because housing conditions were reversed at week 9, it is not known whether the I-E group would have manifested firmly established binge-type eating given additional time under relatively enriched conditions. Given the weekly increase in mean body weight-normalized fat intakes of the I-E group in all weeks except week 7, and the flat intakes of the CCG, it seems likely that over time the I-E group would reach enduring binge status. Future studies with RE provided for several additional weeks would resolve this.

One possible explanation for the observed differences between the I-E and I-NE groups is that rats provided with EE have been reported to be more adept at learning [43] and may have a greater ability to discriminate the availability of reward [21]. The I-E group may have been less likely to binge as a result of their advantage in learning that reward, e.g. Crisco, will be available again even after a delay of 2-3 days.

Because all groups in the current study were housed in the same room, the I-E and I-NE groups were exposed to visual, olfactory, and auditory stimuli associated with food reward even on days when only the daily groups, C-E and C-NE, were given fat access sessions (i.e., Tuesdays, Thursdays, Saturdays, and Sundays). Beckman and Bardo [18] demonstrated a reduction in incentive salience ascribed by

environmentally enriched rats, relative to isolated nonenriched rats, to a stimulus associated with a food reward. Exposure to the procedures of the daily access sessions may have had a greater effect on the I-NE group than on the I-E group, in that the I-NE group may have ascribed greater incentive salience to the fat-related cues. This in turn might have contributed to the earlier occurrence of bingeing in the I-NE group than the I-E group.

Furthermore, in the Beckman and Bardo study [18], results from a Pavlovian conditioned approach task indicated that environmentally enriched rats tended to goal-track whereas nonenriched rats tended to sign-track. Sign-trackers previously have been reported to have elevated corticosterone levels [44]. This observation, along with evidence that environmentally enriched animals have lower basal corticosterone levels [36], lead Beckman and Bardo to suggest that decreased activity of the HPA stress axis in environmentally enriched animals may be a mechanism by which EE reduces incentive salience ascribed to reward-associated stimuli. Interestingly, rats in that study were equally adept at learning responses regardless of their environmental conditions. It previously has been suggested that uncertainty induced by an intermittent access schedule may encourage bingeing due in part to the activation of the stress axis [45]. Thus, it is feasible that intermittent access together with a lack of any type of enrichment could exacerbate the HPA stress axis more than intermittent access in an enriched environment (RE or EE). Our results indicate an earlier occurrence of operationally defined bingeing in the I-NE than the I-E group, which is consistent with this possibility. Interestingly, nonenrichment alone (i.e., no intermittency) in the C-NE group did not induce increasing or elevated intakes as compared to the C-E group (Figure 1). This combined with the delay of binge behavior in the I-E group suggests that intermittency is an independently critical factor in binge development, while RE imparts limited attenuation of the behavior.

Neurobiological changes are likely responsible for the behavioral differences seen in this study. For instance, brain structures of the mesocorticolimbic system are affected by EE [36]. In addition, uncertainty with respect to binge opportunities may activate the stress axis as previously mentioned and alter signaling within mesocorticolimbic pathways [45]. In particular, differential neuronal processing within the ventral tegmental area and prefrontal cortex (PFC) between intermittent and daily rats was recently reported in a study using the LA model [46]. Given the interconnectivity between the PFC and other regions of mesocorticolimbic circuitry, the combined effects of uncertainty and nonenrichment could create neurobiological states that may ultimately promote the development and maintenance of binge-type eating behavior.

4.3 RE does not attenuate binge size

Although binge onset (i.e., the first occurrence of bingeing) was delayed, binge size was not attenuated by RE. Furthermore, intake during the limited access period never differed significantly between the I-E and I-NE groups even before bingeing was established in the I-E group. This points to the powerful effect that intermittency can have on intake, even under relatively enriched environmental conditions.

Several studies that examined the effects of EE on voluntary intake of other substances such as alcohol, nicotine, methylphenidate, amphetamine, cocaine, and morphine suggest that EE can reduce intake of these substances [27, 47-60]. The results of the present study appear to be inconsistent with these findings, since RE did not significantly reduce binge size in spite of the delay in binge onset, nor were

intakes in the C-E and C-NE groups differentially affected. Several factors may account for these apparent discrepancies. For instance, there is some evidence that EE may protect against self-administration of methylphenidate, amphetamine, and cocaine at lower doses but not at higher doses [27, 50, 51, 54, 55] . The fat in the current investigation was undiluted, and thus intake may not have been sensitive to the potentially protective effects of RE due to the high fat concentration. Future studies using fat emulsions would resolve this issue.

Mixed results have been reported in studies using natural rewards. Several detected no differences in sucrose [20, 28]or food consumption [19] when environmentally enriched, standard, or isolated rats were compared, while another found that environmentally enriched rats consumed less sucrose than isolated rats in two of three test sessions (the third session occurred soon after a forced swim test) [26]. In contrast, Bardo et al. [27] reported an increase in sucrose earned by environmentally enriched rats relative to isolated rats, though this effect was temporary. Two experimental parameters that often vary in such studies are sucrose concentration and deprivation state of the rats, though neither of these factors consistently account for the disagreement among results. For instance, in two studies that found no effect of EE, one used sucrose pellets [20] while the other used 1% sucrose [28]. Another study using sucrose pellets found that EE temporarily increased the number of pellets earned [27]. EE decreased consumption of 32% sucrose [26] and responding for 26% sucrose [49], though the results with 26% sucrose occurred only in the second of two test sessions, while no effect was found in the first session. Studies using both sucrose pellets [56] and 20% sucrose [25] reported an influence of deprivation state, in that EE reduced intake in free-fed rats but increased intake in food-deprived rats in both studies. While some studies that did not vary deprivation state report results that support this relationship [26, 27], still others found no effect of EE on food or sucrose self-administration whether rats were food deprived [19, 28] or not [20]. In the current study, rats were never food-deprived, and no significant effect of RE on the amount of fat consumed during access sessions was seen between the intermittent groups or between the daily groups.

In addition to substance, dose, and deprivation state, a number of other variables are meaningful for interpreting results on the effects of RE on voluntary intake in the context of previous research (e.g., sex and strain of the rats, the timing and duration of enrichment provided, the nature of the reward, method of group assignment [random vs matched for baseline consumption of reinforcer and/or body weight], whether or not intake results are normalized to body weight, and the method of reward delivery). Specific enrichment conditions used for experimental and control groups are highly diverse, and definitions often overlap or disagree (thus the utility of the phrase ‘relative enrichment’). In the present study, relatively enriched conditions correspond most closely to conditions frequently used in EE studies as an intermediate condition. The nonenriched groups of the present study correspond most closely to isolated conditions utilized in other studies, and the present study did not utilize any group that closely parallels the environmentally enriched conditions of most studies in the field. As previously described, EE conditions in most studies typically consist of exercise, novelty, social housing, and (often) larger and more complex cage environments; none of these were employed in the present study. Future studies will need to determine whether results regarding binge size as well as binge development or reversal in the LA model would be different in a group that included the full set of environmental enrichment conditions.

4.4 Binge-type behavior in the LA model is not reversible with added RE

In the second half of the study, RE was removed from the I-E group (x-I-E) and added to the I-NE group (x-I-NE). In the x-I-NE group, the binge behavior that was observed from weeks 3-8 was temporarily attenuated upon addition of RE to the cages. Intakes for the x-I-NE group were not statistically different from the CCG for week 9 but bingeing resumed in week 10 and persisted for the remainder of the study. Perhaps a neurobiological transition in behavioral control from the ventral to the dorsal striatum occurs with establishment of binge eating, similar to that demonstrated in addiction studies [61, 62]. This transition accompanies a shift from voluntary to compulsive intakes and likely signifies a change in neuronal processing such that the original controlled behaviors become difficult if not impossible to restore. As reviewed by Moore et al. [35], binge-type eating is one of several possible manifestations of compulsive eating behavior, which has neurobiological roots that underlie persistent behaviors and lack of inhibitory control. In addition, recent findings using the LA model indicate that mesocortical signaling differs between rats with intermittent and daily fat access, suggesting a shift in neuronal processing in circuits mediating behavioral control under binge-type conditions [46].

Our results suggest that RE does not reverse or 'treat' binge eating in an appreciable way, while the conditions that promoted bingeing in the first place (intermittent access) are still in place. These results contrast with a number of other studies on seeking and intake of rewarding substances. For instance, EE has been shown to reverse a variety of addiction-related behaviors after they have been established [17]. It is possible that different mechanisms may be involved in 'treatment' of binge-type eating versus 'treatment' of compulsive drug intake and related behaviors with enrichment (EE or RE). In addition, the intermittent access schedule was ongoing after the addition of RE to the I-NE group, again indicating the powerful effect of intermittency on ingestive behavior.

4.5 Body weights in the bingeing groups become lower than that of the control group

Body weights were not statistically different among groups for the first 8 weeks of the study, but body weights of the x-I-E and x-I-NE groups became significantly lower than those of the control groups post-reversal. While the time point at which significant differences occurred depended upon the control group that was used in the analysis, consistently lower body weights in both intermittent groups emerged after the reversal and then persisted through the end of the study. To our knowledge, this is the first study using the LA model in which body weights of groups with intermittent access were significantly lower than those of groups with daily access.

Possible explanations for this deviation include differences in energy expenditure and/or energy intake between groups with intermittent versus daily access. Since neither was empirically determined in the present study, we can only speculate regarding what may have occurred. One possibility is that the overall energy intake in the long term may have been greater for the controls than for the I-E and I-NE groups in this study, though this has not been reported in previous studies using the LA protocol in female rats [4, 63]. Studies utilizing the LA model usually last up to 8 weeks; our group differences in body weight emerged beginning in week 9 when using the CCG, and in weeks 12 and 13 when using the separated control groups. It is possible therefore that subtle increases in total energy intake of the daily groups (C-E and C-NE, or the CCG) did not influence body weight initially but manifested as increased body weight as time went on. One previous study using the LA model lasted 30 weeks and still no group differences

in body weight were reported [38]. That study however subjected animals to 3 different sets of shortening access procedures across 3 consecutive time periods, making a comparison difficult. Importantly, the rats used in that previous study were males whereas the present study utilized females. Perhaps more importantly, the present study used Wistar rats, whereas previous studies using the LA model in females used Sprague-Dawley rats [4]. Recent evidence indicates that Wistar rats are more susceptible to high-fat diet-induced obesity relative to Sprague-Dawley rats [64]. Therefore, the daily fat access in the CCG may have promoted greater weight gain in the present study due to the strain of rat that was used.

In addition, the reversal of housing conditions in the I-E and I-NE groups should be considered, given the timing of the divergence in body weights. Housing conditions were altered on Monday of week 9 and the first statistical differences were noted in body weights taken on Thursday of that week when the CCG was used in the analysis. Of particular relevance to this study is a report by Teske et al. [65] that switching rats between solid- and wire-bottom caging every 2 weeks affected chow intake, although the order of the switch had opposite effects. That is, switching from solid to wire stimulated intake, whereas switching from wire to solid reduced intake. Thus, based upon those results, one might also have expected opposite effects in the I-E and I-NE groups in the present study, which was not the case. That said, the strain and sex of the rats as well as the dietary conditions in the Teske et al. study differed from those of the present study. Thus, while it is unlikely that the reversal of housing conditions in the present study can explain the long-lasting and similar effects on body weight in the I-E and I-NE groups, we cannot rule this out as a possibility.

5. Conclusions

It is evident that there is a need to maintain a certain level of austerity in housing conditions to support rapid development of binge eating using the LA model. In addition, larger groups may be necessary when relatively enriched environments are used, compared to what is required under nonenriched conditions. For future experiments, depending on the study aims, it may be preferable to forego RE and establish bingeing more quickly. On the other hand, it may be possible to extend the study by a number of weeks in order to attempt to firmly establish bingeing in animals housed in the recommended, relatively enriched conditions. A third option may be to begin a study without RE but to add it at a later time following establishment of bingeing, provided this does not interfere with protocol aims. Because binge size is not affected statistically by the provision or lack of RE, this may be an acceptable option.

The results of this study suggest that mild (i.e., relative) enrichment may be useful as part of a battery of tools to help prevent or slow the progression of disordered eating in patients showing an inclination toward bingeing. However, mild enrichment may not be helpful once bingeing has been ongoing for an extended period of time. Studies in human subjects are needed to determine the utility of various degrees of enrichment for both the prevention and the treatment of binge eating. While the present study did not include neurological endpoints, these results in combination with other published reports support the importance of preventing binge-related eating disorders, due to a possible permanent shift in neuronal processing that may contribute to treatment challenges.

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ALL FIGURES TO BE REDUCED TO 1.5 COLUMN WIDTH

Figure 1. Intake of the optional shortening over time for the separated control groups. Data are expressed as kcal normalized to body weight$^{0.67}$. Vertical bars indicate SEM. Housing conditions remained the same throughout the study for these rats. There were no significant differences between the Control-Enriched and Control-Nonenriched normalized intakes ($p>0.05$ for all weeks).

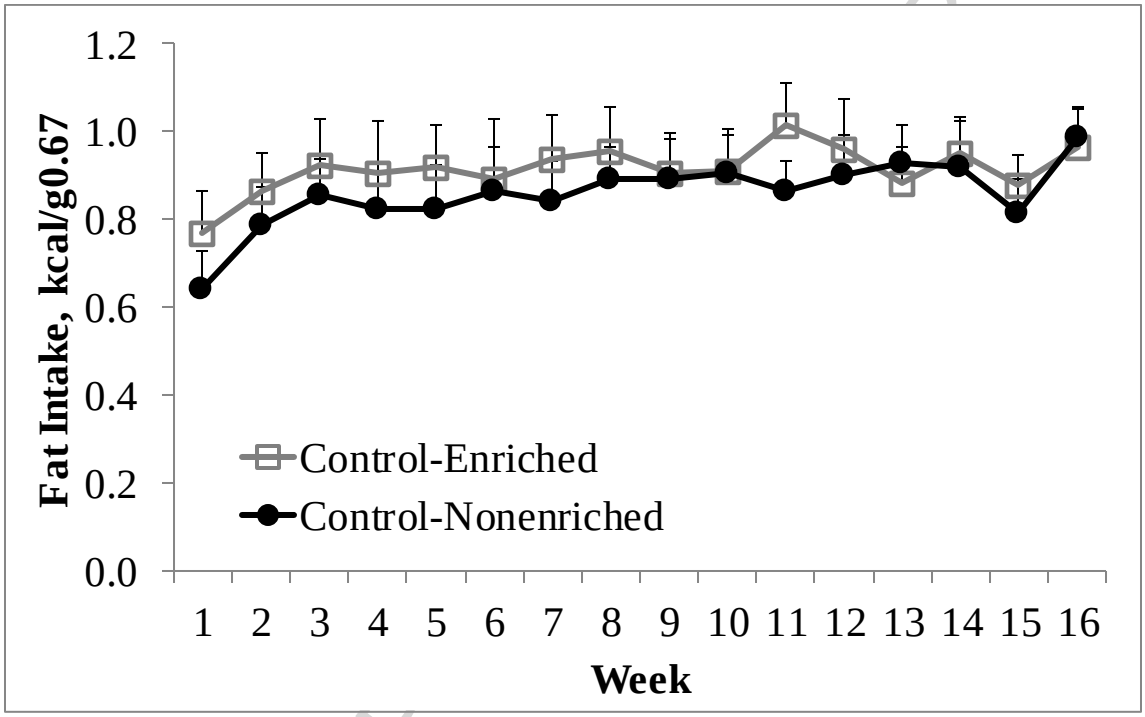

Figure 2. Intake of the optional shortening over time. Data are expressed as kcal normalized to body weight$^{0.67}$. Vertical bars indicate SEM. Beginning in week 9, RE was removed from the Intermittent-Enriched group and added to the Intermittent-Nonenriched group. Different letters indicate significant differences among groups within each week ($p \leq 0.0382$).

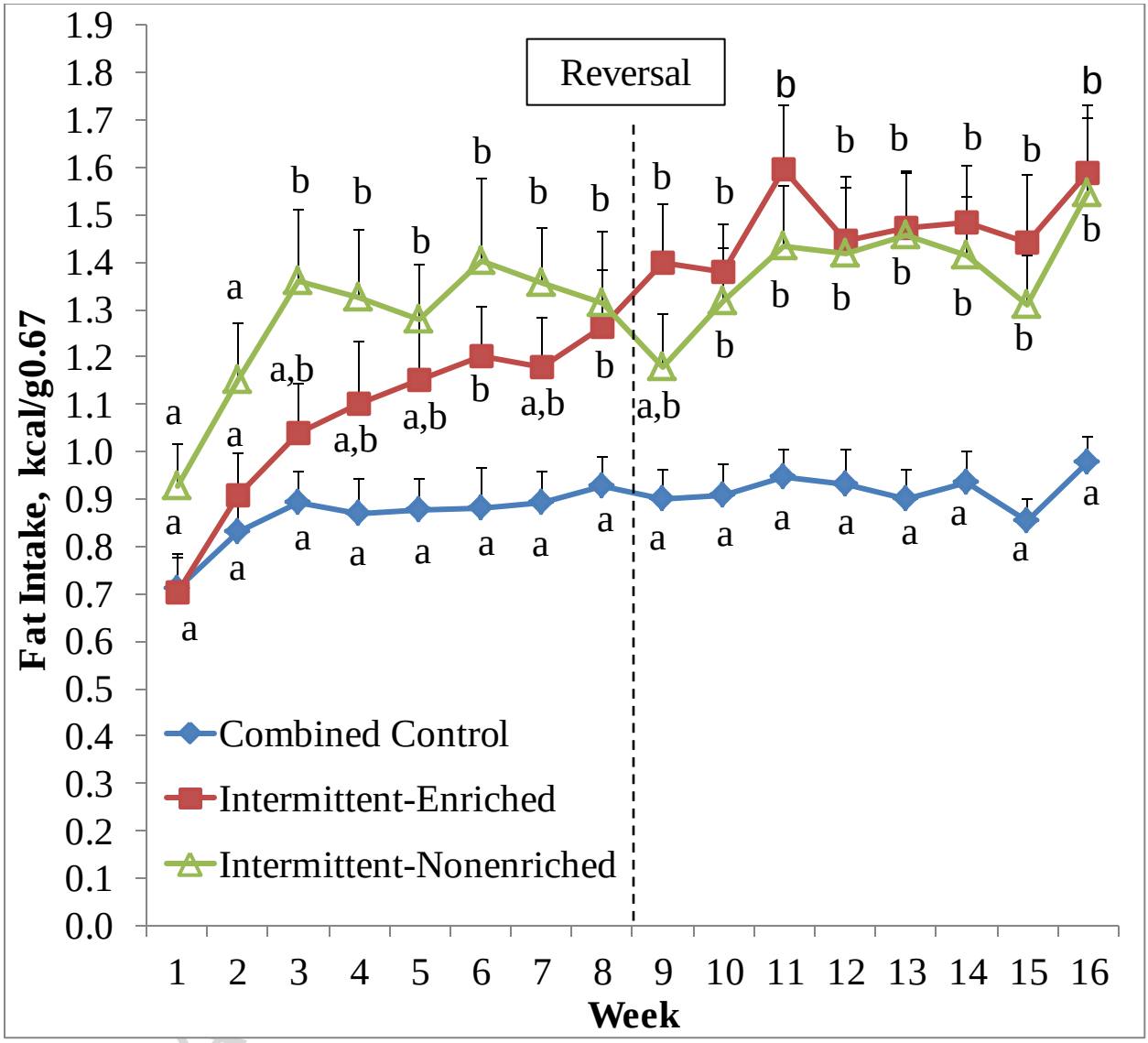

Figure 3. Body weights of the 3 groups: Combined Control (CCG), Intermittent-Enriched (I-E/x-I-E), and Intermittent-Nonenriched (I-NE/x-I-NE). Vertical bars indicate SEM. The reversal of housing conditions occurred at Week 9. There were no significant differences among groups in Weeks 1-8 ($p>0.05$). For Weeks 9-16, significant differences among groups within each week are noted with different letters ($p\leq 0.0301$).

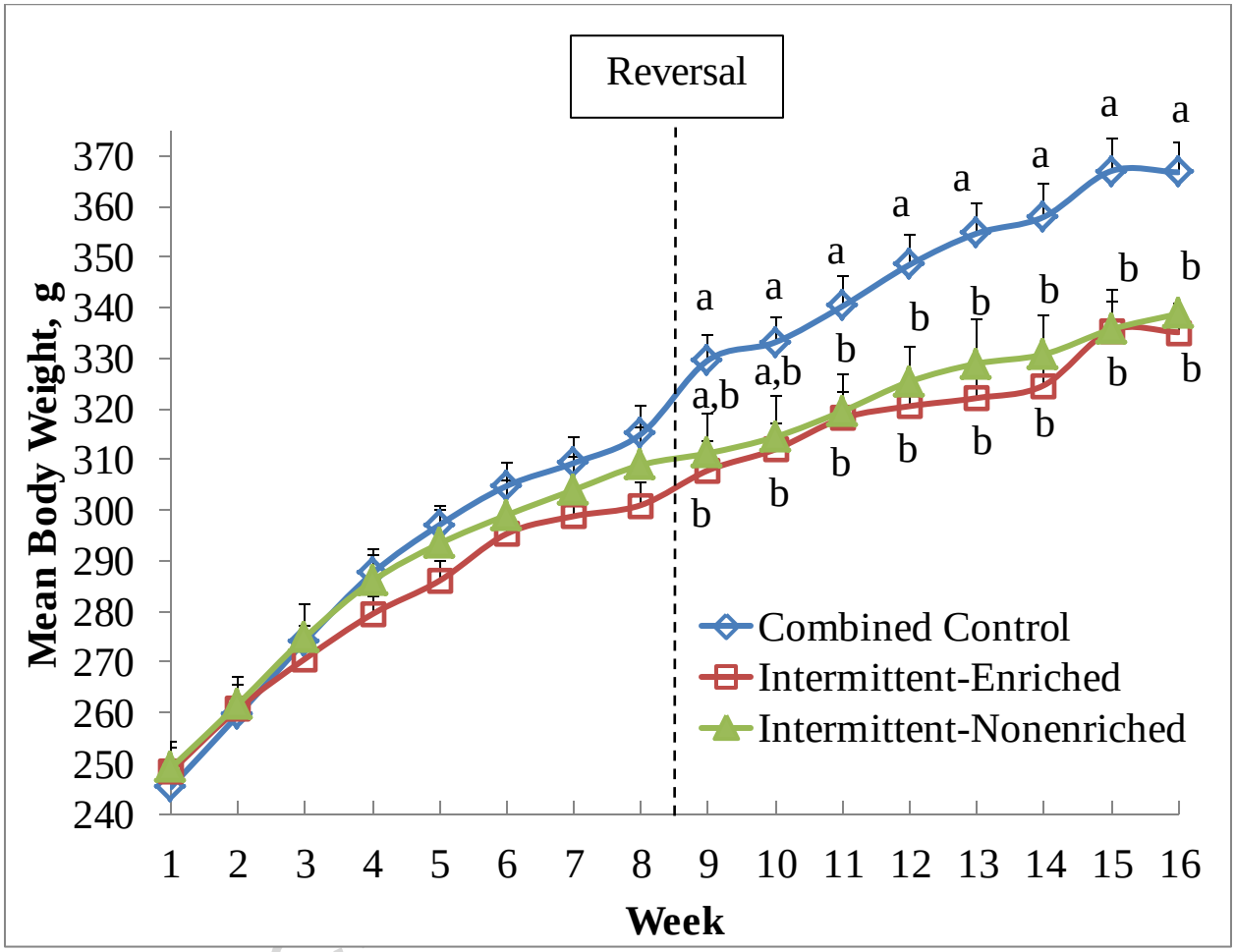

Table 1. Average weekly shortening consumption by group (+/- SEM).

Average MWF Crisco Intakes, g (+/-SEM)					
Week	**I-E**	**I-NE**	**CCG**	**C-E**	**C-NE**
1	3.10 (0.37)	4.07 (0.36)	3.10 (0.30)	3.36 (0.45)	2.79 (0.38)
2	4.09 (0.38)	5.20 (0.53)	3.77 (0.28)	3.95 (0.40)	3.57 (0.39)
3	4.82 (0.47)	6.35 (0.66)	4.22 (0.33)	4.37 (0.53)	4.05 (0.40)
4	5.20 (0.58)	6.41 (0.72)	4.25 (0.38)	4.46 (0.63)	3.99 (0.41)
5	5.56 (0.58)	6.29 (0.58)	4.38 (0.37)	4.63 (0.54)	4.09 (0.51)
6	5.93 (0.51)	6.95 (0.85)	4.50 (0.49)	4.61 (0.81)	4.38 (0.54)
7	5.85 (0.52)	6.82 (0.59)	4.59 (0.39)	4.83 (0.60)	4.32 (0.50)
8	6.30 (0.60)	6.68 (0.78)	4.80 (0.36)	4.93 (0.57)	4.66 (0.41)
9	7.09 (0.64)	6.03 (0.59)	4.81 (0.35)	4.84 (0.50)	4.78 (0.51)
10	7.05 (0.51)	6.76 (0.58)	4.90 (0.37)	4.87 (0.45)	4.93 (0.61)
11	8.29 (0.70)	7.42 (0.67)	5.15 (0.36)	5.54 (0.55)	4.70 (0.41)
12	7.52 (0.69)	7.45 (0.75)	5.19 (0.45)	5.36 (0.68)	4.98 (0.57)
13	7.70 (0.63)	7.78 (0.81)	5.07 (0.37)	4.94 (0.51)	5.24 (0.54)
14	7.78 (0.64)	7.51 (0.65)	5.29 (0.39)	5.32 (0.48)	5.25 (0.67)
15	7.73 (0.77)	7.02 (0.60)	4.88 (0.30)	5.02 (0.38)	4.70 (0.48)
16	8.53 (0.77)	8.33 (0.85)	5.59 (0.34)	5.50 (0.51)	5.72 (0.43)

TABLE 2. P-values for comparisons of intermittent groups to the separated control groups for body weight-normalized shortening intakes.[1]

Week	I-NE vs. C-NE[2]	I-E vs. C-E[2]		
1	0.2915	0.9743		
2	0.1225	0.9932		
3	0.0116*[3]	0.8662		
4	0.0119*	0.5796		
5	0.0289*	0.4123		
6	0.0057*	0.1751		
7	0.0090*	0.3806		
8	0.0478*	0.1844		
	x-I-NE vs. C-NE[2,4]	**x-I-E vs. C-E[2,4]**	**x-I-NE vs. C-E[2,5]**	**x-I-E vs. C-NE[2,5]**
9	0.2882	0.0073*	0.3108	0.0077*
10	0.0571	0.0118*	0.0499*	0.0151*
11	0.0030*	0.0008*	0.0417*	<.0001*
12	0.0087*	0.0078*	0.0200*	0.0032*
13	0.0068*	0.0006*	0.0015*	0.0034*
14	0.0129*	0.0027*	0.0178*	0.0020*
15	0.0139*	0.0014*	0.0343*	0.0005*
16	0.0037*	0.0003*	0.0014*	0.0009*

[1]Housing conditions for the intermittent groups were reversed at week 9, while the control groups stayed in the same housing conditions through all 16 weeks.

[2]Abbreviations: I-NE, Intermittent-Nonenriched; I-E, Intermittent-Enriched; x-I-NE, Intermittent-Nonenriched with enrichment added for weeks 9-16; x-I-E, Intermittent-Enriched with enrichment removed for weeks 9-16; C-NE, Control-Nonenriched; C-E, Control-Enriched.

[3]*Indicates significant differences at α=0.05 after adjustment for multiple comparisons using the Holm-Tukey method.

[4]These comparisons have the advantage that each intermittent group (x-I-NE and x-I-E) is compared to the same set of rats in weeks 9-16 as in weeks 1-8 (C-NE and C-E, respectively). The disadvantage of these comparisons is that the housing condition of each intermittent group (x-I-NE and x-I-E) is different from that of the control group to which it is compared for weeks 9-16 (C-NE and C-E, respectively).

[5]These comparisons have the advantage that each intermittent group (x-I-NE and x-I-E) and the control group to which it is compared (C-E and C-NE, respectively) are experiencing identical housing conditions in weeks 9-16. The disadvantage of these comparisons is that each intermittent group is now compared to a different set of rats in weeks 9-16 than it was compared to in weeks 1-8.

HIGHLIGHTS

- A relatively enriched environment delayed the first occurrence of bingeing.
- A relatively enriched environment did not attenuate binge size.
- Enrichment after binge establishment briefly attenuated bingeing.
- Enrichment may increase the time and cost of studies using this model.