

Chapter 2

Binge-Prone Versus Binge-Resistant Rats and Their Concomitant Behavioral Profiles

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Abstract

Binge eating is a recalcitrant symptom of bulimia nervosa, binge-eating disorder (BED), and the binge/purge subtype of anorexia nervosa. Binge eating is rooted in gene–environment interactions, but the biology of these interactions is largely unknown. This chapter describes a simple and reliable animal model of binge eating that is based on such an interaction: a significant inherent difference in eating patterns when palatable food (PF) is encountered in the environment. Roughly one-third of rats exhibit a binge-like pattern of intake of PF despite normal intake when only chow is available. The PF intake of these binge-eating prone (BEP) rats is significantly and consistently greater than that of binge-eating-resistant (BER) rats. Also described are subsequent experimental manipulations that reveal additional parallels between BEP rats and human binge-eating behavior, including preference for and abnormal intake of PF when stressed, binge eating in the absence of hunger and despite evidence of satiety, motivation to obtain and eat PF despite punishing consequences, and age of onset shortly after puberty. The model also dissociates binge eating from obesity proneness such that four subgroups can be obtained that resemble bulimia nervosa (binge eating with compensatory restriction to prevent obesity), BED (binge eating with propensity for obesity), frank obesity (obesity proneness without binge eating), and healthy controls (non-binge-eating, obese-resistant rats). These behavioral profiles render the BEP/BER model a useful tool to uncover some of the genetic and epigenetic substrates distinguishing BEDs. It can also be used to develop and test more targeted treatments against these life-threatening conditions.

Key words: Binge eating, Diet-induced obesity, Eating disorder, Obesity, Rat

1. Introduction

This chapter describes an animal model of binge-eating prone (BEP) versus binge-eating resistant (BER) rats. Binge eating in humans is a highly distinct pattern of overeating that is characterized by the intake of an abnormally large amount of food in a discrete period of time and is accompanied by a sense of lack of control over the ability to limit the amount of food eaten or to stop eating (1).

Binge eating is a stubborn symptom in the binge/purge subtype of anorexia nervosa, of bulimia nervosa, and of binge-eating disorder (BED) (1), which collectively afflicts approximately 8% of the U.S. adult population (2). For brevity, these three disorders will be referred to here as “binge-eating disorders.”

In its most basic form, the BEP rats most closely model BED out of all of the eating disorders. This is because caloric restriction and/or weight loss are not required to produce BEP rats. However, this chapter describes subsequent manipulations of the model that can be easily conducted to yield behavioral responses with parallels to features of bulimia nervosa and binge/purge anorexia. The BEP/BER model originated from the observation that, while rats of the same age and sex eat relatively equal amounts of standard lab chow, their intake will vary when offered highly palatable food (PF). The PF is high in sugar and/or fat and is given intermittently (e.g., 2–3 times per week vs. daily) to simulate the “forbidden” regard of these foods and diagnostic frequency in clinical binge eating (1, 3, 4). The key observation is that approximately one-third of the rats *consistently* eat the highest, and one-third eat the lowest, amount of the PF (5). This stable pattern is consistent with the established chronic and stable nature of binge eating in BED (6). Importantly, the expression of binge eating in the BEP rats is not observed until they come in contact with PF. That is, BEPs and BERs are indistinguishable until exposed to an environment containing PF. BEPs also do not have to learn to overeat PF; they inherently overeat during the first exposure. This is important because it represents an example of a gene–environment interaction. Gene–environment interactions are pathogenic of eating disorders, yet are not researched as aggressively as they need to be (7, 8).

Our work suggests that the only environmental factor needed to elicit the BEP/BER model is PF. The salience of PF in human binge-eating behavior cannot be understated. Since these foods are typically high in refined sugars and fat, they are rewarding and calorie dense, and are therefore regarded as “forbidden foods” outside of binges. They are obsessed over, craved, and overconsumed during binges (4, 9, 10). Intake of just a morsel of PF or simply the smell of PF is known to trigger relapses back to binge eating (11–13). It is not possible to estimate the extent to which exposure to PF is necessary for the development of BEDs because of its ubiquitous nature in the modern world. We are exposed to these foods from childhood and even as neonates (14, 15). Nonetheless, the reliance of the BEP/BER model on PF exposure does not devalue the model as a research tool in BEDs, especially considering the salient role of PF in these disorders.

The numerous parallels between BEP rat behavior and clinical binge eating validate its use as a preclinical tool. These parallels go beyond binge eating in a discrete period of time and the stable nature of the binge-eating pattern. Here we describe additional parallels that emerge when the rats are subjected to factors relevant

to life experiences of those with BEDs. These factors include stress, hunger, tolerance of painful consequences in order to binge on PF even when sated, and exposure during puberty. In all cases, BEP rats respond with striking similarity to individuals with BEDs (5, 16, 17). The BEP/BER model also recapitulates the independence between binge eating and propensity to develop obesity. Given the large weight range of patients with BEDs, it is not surprising that familial studies confirm a strong genetic contribution for binge eating that is independent of the obesity phenotype (18, 19). Here too is described how it is possible to obtain four subgroups from the BEP/BER model that are behaviorally similar to bulimia nervosa, BED, non-BED obesity, and healthy controls (5). These subgroups could prove useful in the discovery of genes that distinguish propensity for each of these conditions. In so doing, more targeted treatments can be designed. Lastly, the model promises to help identify the exact physiological changes that take place when modern “super-hedonic” food ingredients and predisposing genes intersect. This type of research is needed to learn how to best prevent or decrease the recidivistic nature of BEDs.

2. Materials and Procedures

2.1. Animals

Young adult female Sprague–Dawley rats (Harlan, IN and WI) are used in keeping with the higher female incidence and age of onset of BEDs (2, 7). The rats may be older in age but not pre-pubertal (17). The typical results presented here are those obtained with 60-day-old rats. Regarding the number of rats required at the onset, the BEP/BER model relies on *extreme amounts* of PF consumed. These extremes are best observed from an initial group that is three times the number of rats desired in the BEP and BER group. For example, for $N=10$ BEPs and $N=10$ BERs (a typical N per group in the author’s and others’ rodent studies), one would start with $N=(10 \times 3)$ or 30 rats. Of course, the number of BEP and BER rats required will depend on the complexity of the study design; e.g., drug versus control conditions may require 20 rats per group if working with a between-groups design in which case a good start number would be (20×3) or 60 rats. Multiple studies confirm the reliability of the “ $N \times 3$ ” formula because roughly one-third of female rats will meet BEP and one-third will meet BER criteria (5, 16, 17, 20, 21). The rats can be single or pair-housed when not being tested and should be acclimated to standard colony conditions with a 12:12-h dark/light phase. Lights should be timed to turn off at the onset of the feeding tests; e.g., if feeding tests wish to be conducted at 1000 hours then lights should go off at 10 a.m. (1000 hours). This captures intake during the initial dark period. Rats should be well acclimated to any new light/dark schedule as for any other study.

2.2. Diet

The rats are maintained on ad libitum water and standard rat chow (e.g., Harlan Teklad Global Diets, IN; 3.3 kcal/g) throughout the studies. To identify BEP and BER groups, a PF must be introduced. Most studies have used Oreo Double-Stuf[®] cookies (4.8 kcal/g; Nabisco, NJ) (5, 16, 20). Oreos[®] have worked well in other models of binge eating (5, 20, 22–24) and include the high-fat and sugar contents that are typically craved and overeaten by humans who are binge eating (3, 4). Other PFs have been used successfully (5, 17, 21), but the results given here are those resulting with the use of Oreos[®]. The use of other PF types is discussed in Sect. 3. The PF is always given alongside standard rat chow.

2.3. Identifying BEP and BER Rats

BEP and BER rats are identified by a series of “feeding tests.” All rats are first allowed to overcome neophobia by introducing a few grams of the PF (e.g., half a cookie) in home cages prior to the feeding tests. The rats are then subjected to four measured feeding tests. Each test consists of placing a generous premeasured amount of the PF (e.g., two Oreo[®] cookies, ~29 g) and chow pellets (e.g., 10 g) inside of or on the lid of the home cages just prior to lights out. Intake is measured after 4 h under red or dim lighting. The cookies remain in the cage for 24 h. Care should be taken to include any spillage, although it tends to be minimal with these types of foods. The 4-h interval is a discrete period of time that provides measurable differences in intake between groups in this and other models of binge eating (20, 22–25). Food intake can be measured at any other intervals up to 24 h, but is not necessary for the identification of BEP/BER status. Body weights can be recorded periodically to confirm no change in weight between the groups over time. However, body weights are also not necessary in determining BEP/BER status. Importantly, the feeding tests are separated by at least 1 day of only ad libitum chow. Typically the PF and chow feeding tests occur 2–3 times per week. This renders PF intake as an intermittent event, simulating the two times per week criteria for binge eating (1) and the “forbidden food” regard for PF in BEDs (4, 9, 10). Periodic 24-h measures of chow intake on the chow-only days serve to confirm that there is no significant difference in amount of this food eaten between BEP and BER rats. Differences are only observed with PF. Once BEP/BER status is established using the criteria described in Sect. 2.4, the feeding tests can occur less frequently (e.g., one time per week) for subsequent experimental manipulations.

2.4. Criteria Used to Classify BEP from BER Rats

For each of the four feeding tests, the kcal intake of PF of each of the rats is grouped into tertiles. That is, the values and their corresponding rat identification are evenly distributed into three groups: a lowest, a middle, and a highest PF-intake group. Rats in the lowest PF-intake tertile across all four of the feeding tests, or in three out of the four tests, are assigned BER status. Those in the highest PF-intake tertile across all four, or three out of the four

tests, are assigned BEP status. How consistently a rat appears in a particular tertile is more important in determining status than the absolute kcal value of PF consumed—extreme as it might be if the rat appears in more than one tertile. Rats falling into the middle tertile do not need to be retained for further BEP versus BER tests unless one wishes to maintain the rats on chow throughout as a chow-control group. This is an appropriate control because all rats, regardless of BEP or BER or “middle” status eat equivalent amounts of chow when only chow is available.

Alternatively, the middle group can be treated as a third “middle PF-eating” group, but the caveat must be considered that some of the rats making up this group were placed in the group because of their inconsistent amount of PF intake. Another option, and one used by the author, is to retain the middle rats to pilot test any experimental variables before they are tested on the BEP and BER rats. This is useful given that the rats are of the same age, sex, and body weight as the BEP and BER rats. But again, if time, labor, and cost are an issue, and if the study aims permit, the middle tertile rats need not be used at all. At the end of the feeding tests, there should be an equal number of BEP and BER rats. Subsequent PF+chow feeding tests can be conducted to confirm the stability of the BEP/BER patterns, but these should be preceded by at least 1 day of only chow with no experimental manipulations. The typical BEP/BER intakes one can expect are described in the Sect. 3.

2.5. Time Required

If the feeding tests are administered 2–3 times per week, BEP and BER rats can be identified within two weeks’ time.

2.6. Data Analysis

Frequency descriptive statistics set at 33.3% percentiles will yield tertile groups of PF intake for each feeding test. Cronbach’s alpha can be used to verify consistency of high versus low PF intake within rats across the feeding tests. Student’s *t*-test or ANOVA is used to compare differences between BEPs and BERs on PF intake, chow intake, and body weights. Bonferroni or Tukey post hoc tests are used if more than two groups are compared, e.g., if the middle group is analyzed. The alpha level for all comparisons is 0.05. More complex designs have been used, such as mixed within-subject and repeated measures ANOVAs (20), and mixed linear models when measuring changes over time (17). All food intake data should be converted to and analyzed as kilocalories, especially when combining measurements of intake of various energy-dense foods.

3. Notes and Anticipated Results

The following variables are *not necessary* to obtain the BEP/BER model, but they yield additional behavioral profiles in BEP rats that parallel features of binge eating. These can serve as additional

“models” according to the characteristics one wishes to explore further. Given the simplicity of obtaining BEP and BER groups, these subsequent manipulations do not require much additional time to perform. All of the variables described below were tested in a between-groups design starting with $N=60$ rats for $N=20$ BEP and $N=20$ BER young adult female Sprague–Dawley rats, except where noted. If rat labor and upkeep, but not time, are issues, half the rats per group (e.g., $N=8-10$ /group) is acceptable for most within-subjects designs. The results of these manipulations and their relevance to understanding binge-eating behavior in humans are described below, as are alternate methods of conducting these tests.

3.1. Effect of Acute Food Deprivation on BEP Versus BER Rats

Once rats are classified as BEP or BER and following at least 2 days of chow-only feeding, half of the rats from each group are given 50% of their normal 24-h chow intake at lights out. The reduced amount is 50% of the mean of all the rats’ previous day’s 24-h chow intake. The other half of the rats of each group remain on ad libitum chow. On the following day just prior to lights out, all rats are given a premeasured amount of PF and chow, as in the feeding tests, and intakes are recorded after 1, 4, and 24 h. More frequent recordings can be taken if needed.

3.2. Effect of Stress on BEP Versus BER Rats

Stress typically has anorectic effects on laboratory rats (26, 27). The exception is when stress is combined with a “history of dieting,” which the author used to develop a different model of binge eating (22, 24) (also see Chap. 3 for innovative variations on this model). The BEP/BER model does not require caloric restriction or dieting simulations, so the rats are not expected to overeat when stressed. Nonetheless, stress evokes interesting and clinically relevant differences between the BEP and BER rats. Once the rats are classified as BEP or BER and following at least 2 days of chow-only feeding, half of the BERs and half of the BEPs are individually subjected to four 3 s bouts of 0.6 mA of scrambled foot shock in a shock alley apparatus, prior to lights out. The other half of the rats in each group is placed in the shock alley for the same amount of time without shock.¹ The rats are then returned to their home cages while lights are still on, with a premeasured amount of PF and chow as in the feeding tests. Intakes are recorded after 2 and 4 h of feeding.

3.3. Effect of Suffering Consequences for PF in BEP Versus BER Rats

This test of motivation for PF uses the same foot shock apparatus as in the stress procedures above. Here $N=10$ BEP and $N=10$ BER rats naïve to foot shock are allowed to eat ad libitum amounts of chow in their home cages during the first 2–4 h in the dark. This precludes hunger from confounding this test, which is intended to

¹ Additional details on this manipulation are in (5); shock apparatus details can be found in (22).

measure motivation for the rewarding versus metabolic properties of PF intake. The rats are then allowed to individually roam in the shock alley under red light to acclimate to the space and to learn that one end of the alley is baited with PF. Plain M&M's® candy (Mars, McLean, VA) has been used, but it is likely that another PF such as Froot Loops (Kellogg, MI) or small flavored pellets (Research Diets, NJ) would work as effectively (28). Acclimation to the alley is confirmed when all rats take at least one bite of an M&M® during the first minute after being placed into the shock-free end of the alley. This typically occurs after three 10-min sessions in the alley (over 3 days). On the first day of actual testing, the rats are placed in the alley for 10 min, but with no shock, in order to obtain a baseline measure of PF intake under these conditions. On the second day, the lowest level of shock (0.10 mA) is administered for 3s immediately following retrieval of an M&M®. The candy must be completely removed from the food hopper by paw or mouth before shock is delivered. This level of shock is readministered for as many times as the rat returns and retrieves an M&M® during a single 10-min session. In each 10-min session thereafter (on the following days), the shock level is increased by 0.05-mA increments until the rat no longer retrieves PF. On the test day following a session where the rat chooses not to retrieve an M&M®, the rat is given a last chance to retrieve and if there is no attempt within the 10-min session, the rat is no longer put into the alley for the duration of the study. When placed into the alley, the rats are always placed in the end of the alley that is not baited with food or wired to shock.² Measures recorded include number of M&M's® retrieved, amount of M&M's® kilocalories consumed per session at each shock level, and the highest shock level tolerated per rat.

3.4. Effect of a High-Fat Diet on the Propensity of BEP Versus BER Rats to Develop Obesity

Clinical binge eating occurs in individuals that maintain a wide range of body weights, e.g., binge eating occurs in underweight anorexia nervosa, normal weight bulimia nervosa, and overweight or obese BED patients (1). Hence, the susceptibility to develop obesity should be independent of binge-eating status. To test for this, $N=20$ BEP and $N=20$ BER, which never significantly differ in body weight, are subjected to a traditional diet-induced obesity (DIO) protocol (29, 30). Under the DIO protocol, all the rats are provided with a daily sole ad libitum diet of 35% fat pellets (Research Diets, Diet # D12266B, New Brunswick, NJ) in their home cages for a minimum of 14 days. Body weights and 24-h food intakes are recorded daily or at minimum on days 1 and 14. During statistical analyses, body weights on day 14 of half of the rats that gain the most weight are compared to weights of the other half of the rats that gain the least weight, regardless of BEP/BER status.

²Refer to (16) for additional details.

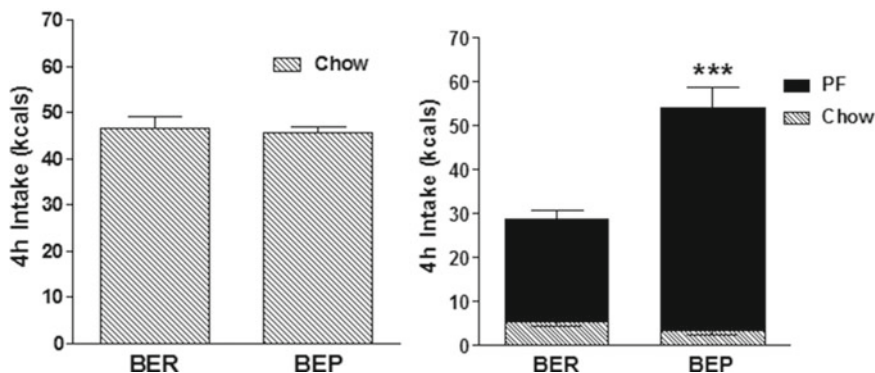


Fig. 1. Typical 4-h intake patterns of binge-eating resistant (BER) versus binge-eating prone (BEP) rats; $N=20$ /group. (a) When only chow is available the groups are indistinguishable by their intake (ns). (b) When PF is available with chow, BEPs consistently consume >40% more PF kilocalories than BERs ($***p<0.001$). The effect is also observed with a smaller $N=8-10$ rats/group (16, 17, 20, 21). Reproduced, with permission, from (5).

A statistically significant difference in the means will confirm the success of the DIO protocol to identify obese-prone from obese-resistant rats. Then a chi-squared test can be used to determine if there are a different number of BEP versus BER rats in the obese-prone versus obese-resistant groups. Equal numbers of BEPs and BERs are expected in each weight group given the independence of binge eating from obesity proneness.

3.5. Effect of Developmental Factors on the Expression of BEP Versus BER Patterns

One may wish to use this model to examine environmental or physiological correlates of early-life experience, puberty, or aging on binge eating. Klump and colleagues investigated the age of onset and effect of estradiol removal on BEP/BER patterns in female Sprague-Dawley rats. The PF for the feeding tests was 15–20 g of Betty Crocker Vanilla Frosting (General Mills, MN) in a dish suspended inside the cage. More was added as needed with the rats' growth over time. The feeding tests were conducted as described above and occurred three times per week from postnatal day P23 through P69. Puberty onset occurred at P34–P39 (defined as vaginal opening), during which time feeding tests were conducted only one time per week. In sum there were six feeding tests in pre-early puberty, four in mid-late puberty, and five in adulthood. Mixed linear models analyses were used to compare PF intake, chow intake, and body weight during age development in BEP versus BER rats (17). In a separate study, adult BEP and BER rats were ovariectomized (OVX) at age P70 or P71 and subjected to four additional feeding tests on day P79 through P86. A second study controlled for any effects due to the surgery by including sham-operated rats (21).

3.6. Typical/Anticipated Results

The feeding tests yield two groups of rats that never differ in the amount of plain chow intake if they only have access to chow (Fig. 1), but that differ consistently (Cronbach's $\alpha=0.86$) and significantly in the amount of PF they consume. As shown in Fig. 1,

the BEP group typically consumes >40% more PF kilocalories by 4 h (55% shown here) than do the BERs. The statistical difference in PF intake can actually be observed as early as the first hour of eating (5) (not shown) but the 4-h period assures that rats have eaten to satiety and it is also a time interval when BEP/BER differences are the largest. By 24 h, the BERs approach but do not quite match the BEPs' PF intake (5). Replications of the model have obtained similar BEP/BER differences with as few as $N=8-10$ rats per group (16, 17, 20, 21). Tests using the middle PF eaters show that they eat an amount of PF intermediate with that of the BER and BEP groups (5). There is never a significant difference in body weights between the two groups due to the intermittent access to PF. In line with the stable nature of clinical binge eating (6), the BEP/BER patterns are stable. Consistent patterns have been observed even after multiple manipulations, some noxious, including acute and cyclic food deprivation, foot shock, contextual-cue conditioning, exposure to other PFs (5, 16, 20), and surgeries (21). Eating a larger amount of food than normally expected, within a discrete period of time, and with a sense of lack of control to limit intake are diagnostic features of binge eating (1). Likewise, BEPs consume an amount of food clearly larger than normal, in a discrete period of time. This is not only due to the fact that their intake is being compared to the extreme lowest PF-eating rats because they can also eat a significantly greater amount of PF than the middle PF eaters (21). They also seem unable to regulate the amount of PF they consume despite the fact that, when only chow is available, they consume as much as BERs, which hints of normal satiety function. Hence, exceeding this level of food intake suggests that they ignore satiety signals when bingeing on PF. The results from the hunger test below support this assumption.

3.6.1. Effect of Acute Food Deprivation on BEP Versus BER Rats

As shown in Fig. 2, a period of caloric restriction causes BERs to eat significantly more food then when sated. This increase consists of greater chow intake, which is typical of rats hungry from metabolic deficit (25, 31). BEPs, too, eat proportionally more chow than when sated and so appear to respond normally to hunger. Also, because hungry BEPs do not eat more total kilocalories than hungry BERs, it can be implied that they also have normal satiety. However, as also shown in Fig. 2, the amount of total kilocalories that BEPs eat under restricted conditions matches the amount of calories they take in under sated conditions. This behavior is clearly abnormal especially given the behavioral indices of normal hunger and satiety cues in these rats. The responses just described are observed in the 4th hour of feeding but can all be observed as early as after the 1st hour of feeding (5). Clinical binge eating also appears to be unaffected by hunger and satiety cues. Indeed, hunger is one of the weakest triggers of binge eating and binges are diagnostically defined as occurring in the absence of hunger (1, 32–34). In humans, a more potent trigger is stress (34–38).

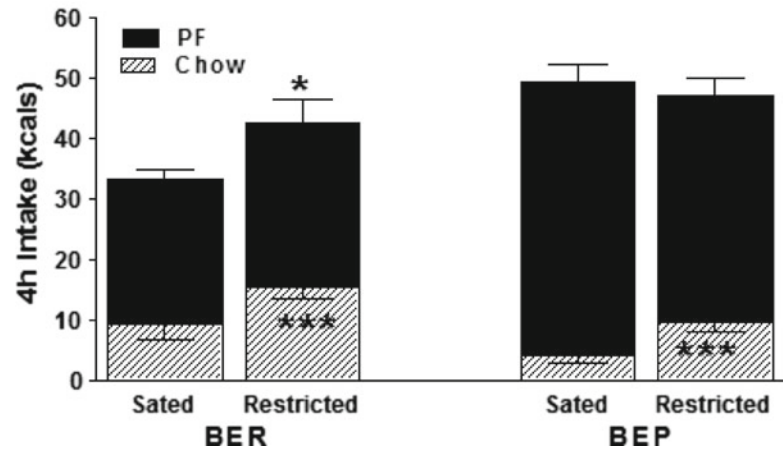


Fig. 2. Amount of chow and PF consumed by BEPs and BERs ($N=10$ /per condition) under calorically restricted or hungry versus ad libitum or sated conditions. BERs eat more total kilocalories after a period of deprivation, $*p<0.05$, and both groups eat proportionately more chow kilocalories under food deprived than sated conditions, $***p<0.001$. However, BEPs eat as many kilocalories when sated as when they are hungry after a period of food deprivation (ns). Reproduced, with permission, from (5).

*3.6.2. Effect of Stress on
BEP Versus BER Rats*

As seen in Fig. 3, at 2 h following foot shock, stressed BERs consume less food than when not stressed. This is the expected and normal response of rats to laboratory stressors (26, 27). However, stressed BEPs fail to display this normal hypophagia and in fact appear to be completely unaffected by shock. By 4 h, stress causes BERs to remain hypophagic. Notably, they are eating less PF, not less chow. By this time BEPs appear somewhat affected by the stress, but their decrease in intake is due to forsaking the healthy chow over PF. Their PF intake remains abnormally elevated. By 24 h, the intake of each group normalizes to match their counterparts' intake under nonstressed conditions. The behavior of BEPs under stress resembles that of human binge eating in that stress triggers overeating versus undereating and is associated with increased consumption of PFs (34–37). In human binge eating, stress may actually make PFs more rewarding (38). Just how rewarding BEP rats find PF can be seen by how much punishment they are willing to tolerate for it.

*3.6.3. Effect of Suffering
Consequences for PF in
BEP Versus BER Rats*

As shown in Fig. 4, BEPs make significantly more M&M[®] retrievals than BERs. This difference reaches significance at shock levels of 0.25 mA and higher. At 0.40 mA and higher, only one BER versus 8 BEP rats braved shock for M&M's[®]. Only BEP rats continue to cross at 0.60 mAs (Fig. 4). As also seen in Fig. 4, the retrieved M&M's[®] are consumed as evidenced by the 2-fold greater kcal intake of M&M's[®] by BEPs versus BERs across all shock levels (16). In sum, the BEP rats' willingness to tolerate increasing pain and anxiety associated with foot shock models the addictive-like

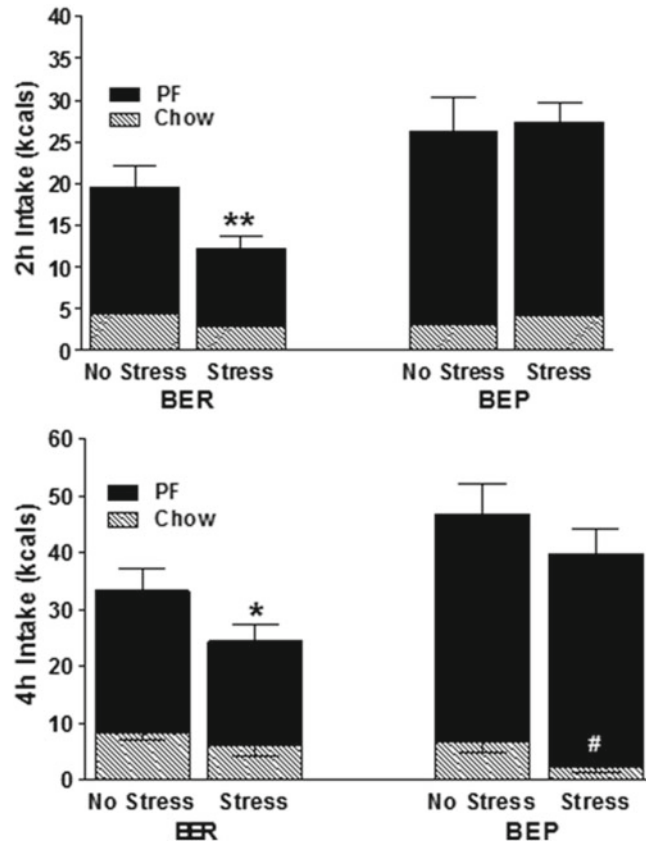


Fig. 3. Amount of chow and PF consumed by BEPs and BERs following foot shock stress or no stress ($N=10/\text{condition}$). (a) In the first 2 h, only BERs show a normal anorectic effect to stress, $**p < 0.01$ versus unstressed BERs. At this time, BEPs are not affected by stress. (b) By 4 h, there is evidence of a stress-induced anorectic effect in BEPs but the decreased intake is on chow, not PF intake ($\#p < 0.05$). Conversely, at 4 h, BERs forsake PF, not chow, when stressed ($*p < 0.05$). Reproduced, with permission, from (5).

nature of human binge-eating where motivation to binge-eat persists despite the mounting psychological and physical consequences directly associated with this behavior (1, 39–41).

3.6.4. Effect of a High-fat Diet on the Propensity of BEP Versus BER Rats to Develop Obesity

When BEP and BER rats are fed a high-fat diet for 2 weeks, exactly half of the BERs and half of the BEPs develop obesity while the other half of each BEP and BER group resist weight gain. The obese-prone rats gain approximately 8.3% of initial body weight vs. a 1.9% gain by the obese-resistant rats ($p < 0.01$) (5). This is due to the obese-prone rats' failure to decrease their normal volume of food when forced to eat the more calorie-dense high-fat diet. Importantly, BEPs are as likely to do this as BERs. Each group is also as likely to voluntarily restrict the amount of the high-fat diet which results in maintaining normal weight.

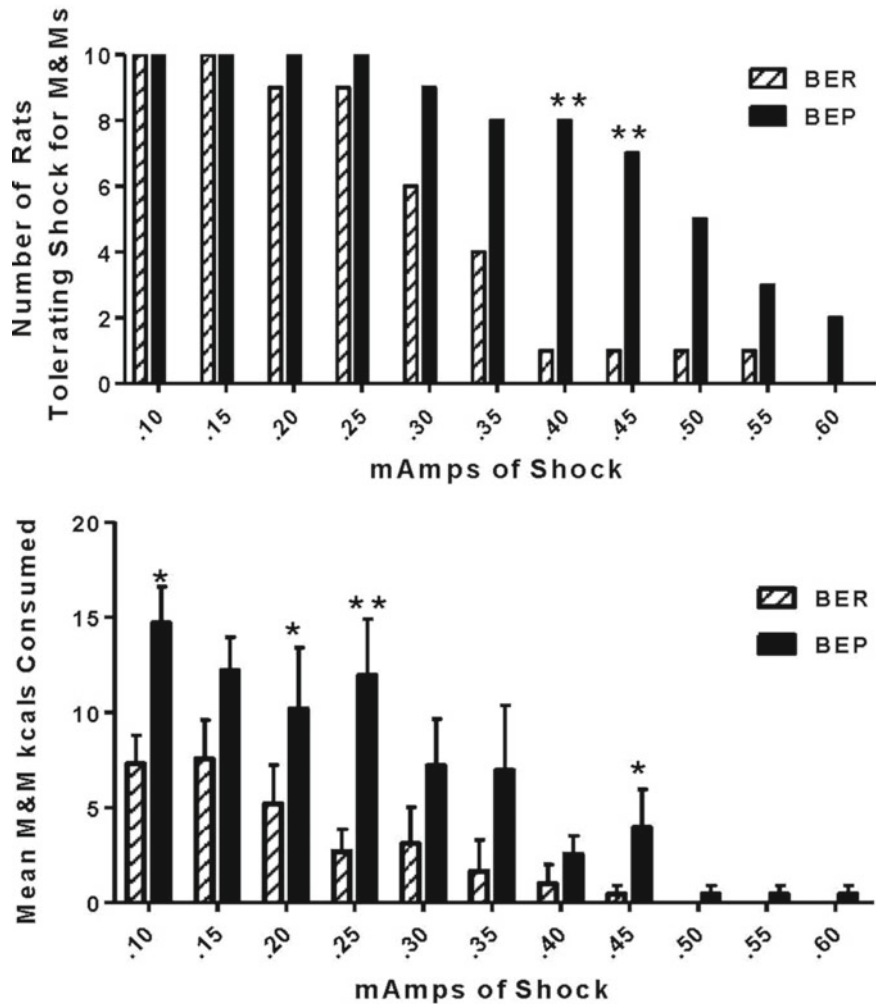


Fig. 4. (a) More BEP versus BER rats are willing to cross incrementing levels of foot shock to obtain M&M® candies; $**p < 0.01$; $N = 10/\text{group}$. (b) The retrievals are also consumed by the BEPs for a final greater intake of M&M's® vs. BERs across all levels and statistically significant at some of the levels denoted by $*p < 0.05$; and $**p < 0.01$. Reproduced, with permission, from (16).

At the end, the DIO protocol yields four subgroups that can be used to investigate possible biological differences between bulimia nervosa, BED, non-binge-eating obesity, and healthy controls (see Table 1). For example, some individuals with bulimia or BED resist obesity through compensatory behaviors, including limiting caloric intake (1, 42). Similarly, the obese-resistant BEP rats reduce their volume of high-fat intake, thereby reducing total kilocalories consumed to maintain normal body weight. Not all human motivations to restrict caloric intake can be modeled in rats, but there may be a common physiology between bulimia nervosa patients and BEP-obese-resistant rats that enables them to reduce caloric intake amidst PF, a physiology possibly compromised in BEP-obese-prone rats and obese individuals with BED.

Table 1

Clinical conditions represented by the four subgroups that result from placing BEP and BER rats on a traditional diet-induced obesity (DIO) protocol

	BER	BEP
Obese-Resistant	<p>Healthy</p> <p>These rats do not have a binge pattern on intermittent PF and when placed on a forced high-fat diet stay lean by voluntarily eating less</p>	<p>Bulimia Nervosa</p> <p>These rats have a binge pattern on intermittent PF but remain lean when placed on a forced high-fat diet by voluntarily eating less or by “compensating” for the increased calories</p>
Obese-Prone	<p>Frank obesity</p> <p>These rats do not have a binge pattern on intermittent PF but gain weight on a forced high-fat diet because they fail to adjust their intake for the additional calories of that diet</p>	<p>Binge-eating disorder</p> <p>These rats have a binge pattern on intermittent PF and gain weight on a forced high-fat diet because they fail to compensate for the additional calories of that diet</p>

BER binge eating resistant, *BEP* binge eating prone rats based on difference in intake of palatable food (PF) during feeding tests used to identify the groups (see text for procedures). Obese-Resistant and Obese-Prone groups ($p < 0.01$ difference in weight gain) emerge from switching BEP and BER rats to a no-choice high-fat diet. Exactly $\frac{1}{2}$ of BEP and $\frac{1}{2}$ of BER rats develop obesity while the other $\frac{1}{2}$ of BEP and BER rats resist weight gain. $N = 10$ per subgroup (5)

3.6.5. Effect of Developmental Factors on the Expression of BEP Versus BER Patterns

As is typical of the BEP/BER model in the author's hands, Klump and colleagues found that roughly one-third of an initial group of thirty rats could be clearly classified as BEPs and one-third as BERs based on their significant difference in amount of PF intake (17). Of major importance is that when they observed PF intake patterns across time, they found that the onset of the BEP phenotype does not appear until mid-late puberty (P39-P58). This seminal change in PF intake occurred as chow intake during chow-only days, and body weights, remained the same for both groups. Only PF intake differed. The emergence of PF binge eating shortly after puberty was replicated in a separate squad of $N = 36$ rats that were exposed to more frequent feeding tests during puberty (three times per week vs. one time per week) (17). The results are a compelling parallel to the age of onset for eating disorders, which is after puberty (1, 2, 43). Results from the OVX study revealed that OVX caused an expected increase in general food intake and body weight across all hormone-depleted rats, regardless of BEP or BER status. However, the OVXed BEP rats still continued to eat significantly more PF than the OVXed BER rats (21). The fact that BEP/BER patterns remained stable despite removal of estradiol indicates that other—yet unknown—signals activated at puberty are needed to express binge eating. While these results were at first surprising given that the BEP pattern appears after and not before puberty, they are consistent with the fact that a significant number of men, and not only women, develop BEDs (2).

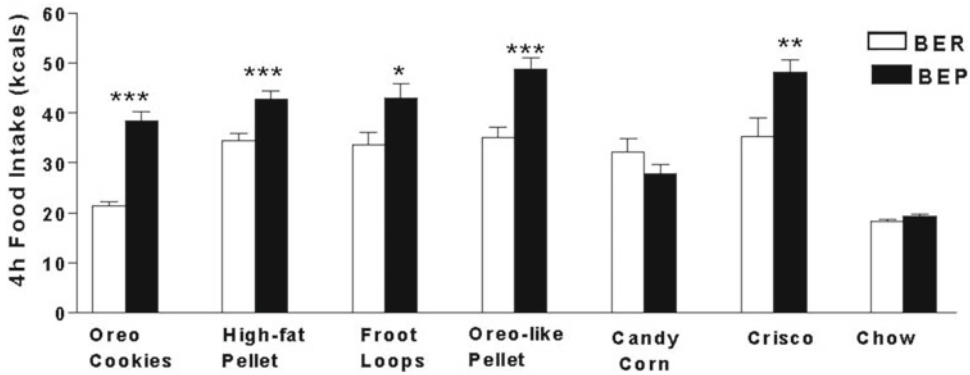


Fig. 5. The BEP/BER patterns generalize to other PFs including those containing mainly sugar (Froot Loops)[®], mainly fat (Crisco)[®], or combinations of both (cookies, pellets). All are preferred by both groups ($N=20$ /group) over chow but, with the exception of candy corn, BEPs eat significantly more of them than do BERs; * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Reproduced, with permission, from (5).

3.7. Troubleshooting and Guidelines if Altering Variables

3.7.1. Identifying BEP/BER Status

In the rare event that not enough rats meet the four-out-of-four or three-out-of-four feeding test criteria for consistency in PF intake, additional tests should be conducted. When choosing BEP rats, if, after selecting the most consistent eating animals, the choice must be made between selecting a rat that ate in the highest quartile three times and once in the lowest quartile versus a rat that ate three times in the highest quartile and once in the *middle* quartile, the latter should be chosen into the BEP group because the middle values are closer to the high end of intake. The same applies to selection of BER rats (middle intake is closer to the lowest tertile than the highest tertile). There is no specific amount of kilocalories that determine BEP or BER status. As pointed out by Klump et al., this also parallels the method by which clinical binge-eating was first defined; it was based on comparing women in the high vs. low ends of the binge eating distribution (1, 17).

3.7.2. Using Other Palatable Foods to Identify BEP/BER Status

Most studies have used either Oreo cookies (5, 16, 20) or Betty Crocker[®] Vanilla Frosting (General Mills, MN) (17, 21). Figure 5 illustrates that rats first identified as BEP/BER with Oreos[®], exhibit the same patterns of intake on other PFs, including high-fat pellets (Research Diets, Diet # D12266B, NJ), Oreo-flavored pellets (Research Diets, NJ), Froot Loops[®] (Kellogg, MI), Crisco[®] (Proctor & Gamble, OH) (5), and M&M's[®] (16). Hence, it may be possible to identify BEP and BER rats with nonfat sugary (e.g., Froot Loops) or nonsugar fatty PFs (e.g., Crisco[®]) and not just mixed macronutrient PFs like Oreos[®] and frosting. Still, one is warned to first test any new PF. For example, Fig. 5 illustrates that one of the PFs tested, Candy Corn (Brach's Confections, TN), failed to yield a significant difference between groups although it

was still preferred over chow by both groups (5). Hence not all PFs may dissociate BEPs from BERs. It may be that some PFs produce negative alliesthesia more quickly than others and BEPs may be as sensitive to this as BERs. Salty snack foods have not been tested, but are predicted to discern BEP/BER groups given that rats find them rewarding (44).

3.7.3. Using Alternate Modes of Stress-Induction

If foot shock is not practical, there is no reason that other standard laboratory stressors, including immobilization, cold temperature or water exposure, social defeat, noise, or emotional stress, should not yield the same differences in responses to stress observed in the BEP and BER rats. A particular “human-like” stressor, to the extent that craving can be regarded as stressful, is to dangle the PF in front of the rats without allowing them access to it. This has been used successfully in replications of the author’s *stress + dieting model* of binge eating, which originally used foot shock (45).

3.7.4. Intermittent Versus Chronic Access to PF

The intermittency of PF used in this model is integral. If instead, rats are allowed *daily* access to PF and chow, the model is compromised because over time (within 2 weeks) the PF intake of BEPs and BERs become comparable. This is due to an eventual decrease in PF intake among the BEPs (5). The model relies on the intermittent, not daily, access to PF, which closely models how individuals with BEDs eat (1). PF is regarded as “forbidden” (4, 9, 10) and there is evidence that sporadic access to PF may exacerbate binge eating by increasing its rewarding quality (46).

3.7.5. Using Male Versus Female Rats

Currently there are no published studies using male rats with this model. However, Klump et al. report that age-matched males do not ingest as much PF (vanilla frosting) as females, and hence do not exhibit the wide range of PF intake needed to be classified as BEPs or BERs (personal communication, October 9, 2011). While male rats still prefer the PF to chow, they eat proportionately more chow during meals than do young females, likely because of increased protein needs. The lower incidence of male rats to achieve BEP status may offer future explanations for the lower male-to-female ratio in BEDs. However, the effects in the male rats may be confounded by the type of PF used. Gender is known to influence PF preferences. Male rats and humans prefer palatable fat/protein or “savory” combinations, and female rats and humans prefer carbohydrate or “sweet” combinations (47–49). Therefore, attempts to replicate this model with male rats should first test the PF to be used; it should yield a range of consistent intakes wherein the highest and lowest amounts consumed are statistically different.

3.7.6. Conducting Pharmacological Tests

Drug studies have not yet been conducted with this model. Since the BEP/BER patterns remain stable and robust even after surgical procedures and aversive manipulations like foot shock, it is not

expected that drug administration procedures will compromise the model so long as the rats are first acclimated to the procedures. Acclimation to injection procedures should be assured and followed with another “feeding test” to confirm the BEP/BER patterns prior to any drug testing.

3.7.7. Using Other Rat Strains or Species

Only Sprague–Dawley rats have been used so far with this model, but it is expected that results replicate in other inbred or selectively bred strains of rats. Mice have not been used, but since they show clear preferences for PF, including food used in the BEP/BER model here with rats (50), it may be possible to use mice if their patterns of PF intake are determined to be stable.

4. Conclusion

The BEP/BER model offers a simple, quick, and reliable method by which to study human binge eating. Pavlov posited that a simple reflex could give clues as to the mechanisms behind some of the most complex reactions between humans and their environment (51). Eating disorders are certainly complex reactions to the environment. The BEP/BER model was developed by targeting one simple “reflex-like” symptom: that of overeating once PF enters the mouth. Once rats with an inherent penchant to do this (BEPs) are identified and discerned from those without (BERs), it is discovered that there are many more behavioral parallels to human binge eating than eating abnormally larger amounts of food in a discrete period of time. Like clinical binge eating, BEPs binge in the absence of hunger, the binges override satiety, PF intake remains high under stress, BEPs tolerate aversive consequences for PF, and only some are prone to obesity. Also as is typical of human BEDs, the age of onset for the BEP binge pattern is shortly after puberty.

The BEP/BER model also offers a tool with which to investigate a variable that warrants much more attention in eating disorders research, namely the biological changes that take place to explain how factors in the environment interact with predisposing genes to express eating disorder symptoms (7, 8). PF is ubiquitous in the environment, as are stress and dieting, yet not all develop eating disorders when subjected to these. The BEPs never learn to overeat PF, but instead do this upon first encountering PF. Therefore, binge eating is a preexisting disposition that is expressed when exposed to PF. The identification of genetic and epigenetic markers that confer the BEP versus BER phenotypes once PF is eaten (and once puberty sets in) should help clarify the physiology of binge eating. Similarly, identification of gene markers in the four subgroups obtained from subjecting BEPs and BER to a high-fat diet should shed light on the physiology that predisposes some

who binge eat to develop BED vs. bulimia nervosa, and to develop obesity with and without binge eating.

Lastly, the BEP/BER model attests to the incredible value of animal models in eating disorder research. BEP rats display behaviors such as willingness to cross painful shock for M&M's®, inability to limit PF intake despite normal hunger-satiety cues, and in some, an ability to restrict calories and prevent weight gain despite the stable trait to binge eat. They also do not start bingeing until they reach puberty. These are responses that in humans with BEDs are commonly attributed to processes only capable in humans (e.g., cognitive dysregulation, irrational thinking, concern with body weight and shape, judgment by peers and the opposite sex). Clearly, researchers cannot mimic all motivations that drive human binge eating in rats, but it is clear that there is a more basic "reflexive" biology underlying binge eating when many of these complex behaviors are observed in rodents. This biology can be exploited with the help of the BEP/BER model, as well as with other animal models in this book, to prevent the expression of eating disorders altogether, or, at minimum, to develop superior treatments for the millions that suffer from them.

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