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ASSESSING BINGE EATING: AN ANALYSIS OF DATA PREVIOUSLY COLLECTED IN BINGEING RATS

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Abstract

As interest in the study of binge eating has increased, several measures of bingeing have been developed for use in animal models. Two of the measures that have been used to distinguish binge-type from normal intake in animal studies are: (1) comparing intake at a given point in time between groups, and (2) assessing escalation of intake across time within groups. Here we use both of these measures to reanalyze data from 10 previous bingeing experiments conducted in our lab. Additionally, the data from two of these studies were then restructured in order to evaluate the use of these measures in binge eating prone (BEP) and resistant (BER) rats, as described by others. Analyses comparing intake at a given point in time indicated bingeing in all 10 studies, while comparisons of escalation indicated bingeing in 9 out of 10 studies. The goal of this study was to compare and contrast the two measures, identify the strengths and weaknesses of each, and determine their appropriateness for a given set of potential outcomes. The results indicate that both intake and escalation are useful measures. However, their limitations need to be taken into consideration when attempting to operationalize binge-type eating in animal models.

Keywords

Animal Model; Binge; Bulimia; Eating Disorder; Escalation; Food Intake

INTRODUCTION

Defined clinically, bingeing is the consumption of larger amounts of food in a brief period of time than would normally be consumed under similar circumstances. In addition, bingeing is often accompanied by a sense of “loss of control” (APA, 2000). Defining bingeing operationally in animal studies does not include measures of “loss of control”, but does include some way to distinguish bingeing from normal consumption. Two of the measures that have been used to distinguish binge-type from normal intake in animal studies are: (1) comparing intake at a given point in time between groups, and (2) assessing

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escalation of intake across time within groups. When using the first measure, “normal” eating is represented by a control group, while “binge-type” eating is represented by an experimental group (Boggiano & Chandler, 2006; Cifani et al., 2010; Cifani, Polidori, Melotto, Ciccocioppo, & Massi, 2009; Corwin, 2004; Corwin, Boan, Peters, Walsh, & Ulbrecht, 2010; Corwin & Wojnicki, 2009; Corwin et al., 1998; Czyzyk, Sahr, & Statnick, 2010; Davis et al., 2007; Dimitriou, Rice, & Corwin, 2000; Hagan & Moss, 1997; Hagan et al., 2002; Hancock, Menard, & Olmstead, 2005; Kinzig, Hargrave, & Honors, 2008; Placidi et al., 2004; Thomas, Rice, Weinstock, & Corwin, 2002; Wojnicki, Charny, & Corwin, 2008; Wojnicki, Johnson, & Corwin, 2008; Wojnicki, Stine, & Corwin, 2007; Wong, Wojnicki, & Corwin, 2009; Yu, Geary, & Corwin, 2008, 2011). This establishes “normal” intake for a given “brief period of time” and contrasts it with “higher than normal” intake, i.e. bingeing.

When using the second measure, “normal” is represented by initial intake in one group or individual, usually during the period of binge induction, while “binge-type” eating is represented by intake in the same group or individual at a later point in the study that is greater than it was initially (Avena, Rada, & Hoebel, 2008; Avena, Rada, Moise, & Hoebel, 2006; Babbs, Wojnicki, & Corwin, 2011; Bello & Hajnal, 2006; Berner, Avena, & Hoebel, 2008; Colantuoni et al., 2002; Rada, Avena, & Hoebel, 2005). Evidence for binge escalation is lacking in human studies, primarily because people usually present clinically once bingeing is already fully established. However, escalation has proven useful as a measure of compulsive drug intake and addiction in animal studies (Koob & Kreek, 2007). It has been argued that binge eating and addiction share certain characteristics (Avena, 2010; Blumenthal & Gold, 2010; Corsica & Pelchat, 2010; Corwin & Grigson, 2009; Hetherington & MacDiarmid, 1993). Therefore, escalation is of interest when modeling binge eating in animals. It is important to note that both measures (intake at a given point in time between groups, as well as escalation within groups) are often used in conjunction (Avena, et al., 2008; Avena, et al., 2006; Babbs, et al., 2011; Bello & Hajnal, 2006; Berner, et al., 2008; Colantuoni, et al., 2002). However, the appropriateness of when to use either or both of these measures to characterize bingeing in animals has not been systematically investigated.

In the current study, data from 10 previous bingeing experiments conducted in our laboratory were reanalyzed using both of the measures described above. Additionally, the data from two of these studies were then restructured in order to evaluate the use of these measures in binge eating prone (BEP) and resistant (BER) rats, as previously described by others (Boggiano et al., 2007; Klump et al., 2011; Klump, Suisman, Culbert, Kashy, & Sisk, 2011; Oswald, Murdaugh, King, & Boggiano, 2011). The goal of these analyses was to compare and contrast the two measures, identify the strengths and weaknesses of each, and determine their appropriateness for a given set of potential outcomes.

MATERIALS AND METHODS

Animals

Data were derived from 10 previous experiments conducted in this laboratory over a five-year period. Five of these studies are published (Corwin & Wojnicki, 2009; Puhl, Cason, Wojnicki, Corwin, & Grigson, 2011; Wojnicki, Babbs, & Corwin, 2010; Wojnicki, Charny, et al., 2008; Wojnicki, Johnson, et al., 2008), and five of them are unpublished pilot studies or are in preparation. For each study, all of the conditions were identical as follows: Male Sprague Dawley rats (Harlan, Indianapolis, IN), 60 days of age, were individually housed in hanging stainless steel wire cages in a temperature- and humidity-controlled environment placed on a 12:12 light:dark cycle. All rats had *ad libitum* access to a nutritionally complete commercial laboratory rodent diet at all times during the study (Laboratory Rodent Diet 5001, PMI Feeds, Richmond IN; percent of calories as protein: 28.05%, fat: 12.14%,

carbohydrate: 59.81%; 3.3 kcal/g) placed in hanging metal food hoppers at the front of the cage. Tap water also was freely available. All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

After approximately one week of adaptation to the vivarium, body weights were recorded and solid vegetable shortening (Crisco® All-Vegetable shortening, J.M Smucker Co., Orrville, OH) was provided during a single overnight period, in addition to the continuously available chow. In all of the studies, rats were then matched by body weight and the amount of shortening consumed during the single overnight exposure. Eight of the studies utilized two groups of rats, and two studies used three groups of rats.

Bingeing Procedure

After grouping, rats were given limited access (1-hr) to shortening in a glass jar clipped to the front of the cage 2–3 hours prior to the start of the dark cycle. Chow and water were available during the shortening access period and at all other times. Thus, the shortening was an optional food, and was not necessary for energy homeostasis. One group was given shortening for one hour every day (D) and the other group was given shortening for one hour intermittently, i.e. only on Mondays, Wednesdays, and Fridays (INT). Intake of the optional shortening in the D group historically has represented normal consumption in our studies, whereas intake of the optional shortening in the INT group has represented bingeing. Because some studies have used 24/7 access to represent “normal” intake (Czyzyk, et al., 2010) we also included two studies, one published (Wojnicki, Charny, et al., 2008) and one unpublished, in which a third group was allowed access to shortening, chow, and water at all times (24/7).

Statistical Analysis

Data were taken from the first five weeks of each study and were analyzed using the two different measures described in the introduction, i.e. intake at a given point in time and escalation. First, within each study D and INT mean 1-hour shortening intake from the final two binge days of week 5 (Wed. and Fri.) were compared with between-group t-tests. These analyses were conducted using SAS v.9.1 (SAS Institute, Cary, NC). Second, within each study linear regression analyses were conducted using GraphPad Prism 4 (GraphPad Software, Inc.) to compare escalations in shortening intakes between D and INT groups in each study. Additionally, linear regressions were conducted on the 24-hour shortening intakes of the 24/7 groups in the two studies where this was applicable. Escalation was assessed in two ways: 1) slopes were compared between INT and D groups, and 2) slopes were compared to a zero slope within each group. For the linear regressions, shortening intakes were normalized to body weight using the formula: kcal/body mass^{2/3} (Heusner, 1985) to control for the possible influence of body weight on intake across time.

To determine if proneness to consume shortening affected the ability to operationalize bingeing using intake and escalation, an additional analysis was conducted. For this analysis, it was necessary to combine two studies to increase power. Therefore, two studies were selected in which there was no difference in mean shortening intake and escalation (defined by slope) between the two D groups and between the two INT groups. The data from these studies were pooled into a Combined D (CD; n = 30) and Combined INT (CINT; n = 30) group. Then, within each of the combined groups, rats were ranked from high to low for 1-hour shortening intake on each of the first three binge days (Mon., Wed., and Fri. of week 1). The rankings were combined across the three days and based on these rankings, rats were separated using a median split into a High D group (HD; n = 15), a Low D group (LD; n = 12), a High INT group (HINT; n = 15), and a Low INT group (LINT; n = 15). Three rats were removed from the analysis due to no Day 1 intake. Thus, the high intake groups (HD,

HINT) were more prone to consume shortening relative to the low intake groups (LD, LINT). These groups were then compared for mean shortening intake on the final 2 binge days using a 1-way ANOVA (Tukey's post hoc analysis), and for escalation as described above. This was done in order to determine if bingeing, as defined by intake and escalation, was influenced by proneness to consume shortening.

RESULTS

Mean 1-Hr Shortening Intake

The results of the analyses of mean 1-hour intake for all 10 studies are shown in Table 1. In all studies, t-tests revealed differences in shortening intake between INT and D groups. That is, the INT group in all studies ate significantly more shortening than did the respective D group. Both 24/7 groups (Studies 1 and 2) ate only 0.4g of shortening during the INT and D groups' 1-hour access period, which was not included in the statistical comparisons.

Escalation of Shortening Intake

Escalation of shortening intake (slope) is reported in Table 2 using two kinds of analyses: 1) comparison between INT and D groups, and 2) comparison to a zero slope within each group. In 9 of the 10 studies, intake escalated to a greater extent in the INT rats (i.e. the slopes were higher) than in the respective D rats. D and INT slopes for Study 7 were not significantly different. Furthermore, slopes were greater than zero in all 10 INT groups, but only for 6 of the D groups (Studies 1, 2, 4, 5, 6, 7). That is, shortening intake in all INT groups escalated during the 5-week binge induction period, but this was not true for all of the D groups. In addition, neither 24/7 groups' shortening intake escalated during the induction period. In fact, both groups had significantly non-zero negative slopes. Restated, the 24/7 rats decreased their 24-hour shortening consumption over time.

Effect of Proneness to Consume Shortening on Binge Intake and Escalation

As shown in Table 3, analysis of the mean shortening intake of the final two binge days revealed no difference between LD, HD, or LINT groups (2.6 ± 0.5 g, 3.6 ± 0.3 g, and 3.6 ± 0.3 g, respectively). The HINT group had significantly higher mean shortening intake than did the three other groups [6.7 ± 0.5 g; 1-way ANOVA $F(3,54)=20.14$, $p<0.001$; Tukey's HSD $p < 0.05$]. Slope analyses of escalation of shortening intake found differences among the groups [$F(3,847)=3.2305$, $p=0.0219$]. Post-hoc pair-wise comparisons revealed that LINT (0.3078) > HINT (0.1787) > HD (0.088). The LD group's slope (0.2667) was not significantly different from any other group.

DISCUSSION

In all 10 of the studies that were analyzed, the INT rats ate significantly more shortening in the 1-hour period than did the D rats (Table 1). This indicates that in all 10 studies, our established operational definition of bingeing was met. That is, the INT rats ate "larger amounts of shortening in a brief period of time than is considered normal", with the D intakes representing "normal". Average 1-hour intakes ranged from 2.2 to 4.0 g for the D groups and from 3.9 to 6.7 g for the INT rats, indicating an overlap of intakes between D and INT groups across studies. Importantly, this suggests that bingeing should not be assessed by examining the intake of a single group without comparison to another group, i.e. a control group, within the same study. In addition, it may not be appropriate to compare intakes across studies if there are differences in housing location, environmental conditions, and time of year. Indeed, early research has shown differences in food intake in rats across seasons (Campbell, 1945), which may have contributed to the overlap in intake in the data sets here.

Previous reports have shown that the 24-hr intakes of the 24/7 rats are equivalent to the 1-hr intakes of INT rats (Puhl, Cason, Corwin, Wojnicki, & Grigson, 2009; Wojnicki, Charny, et al., 2008; Wojnicki, Johnson, et al., 2008). This type of comparison is useful for demonstrating the large amount that is consumed by INT rats within the limited access period. However, in order to meet the “brief period of time” criterion for bingeing, it was necessary to consider the 1-hour shortening intake in the 24/7 groups, not the total daily intake. In both of the 24/7 groups, average 1-hour shortening intake was only 0.4 g. When compared to the 1-hour intakes in the D and INT groups, it is obvious that differences exist (a 10-fold difference, in fact), but the direct comparison of intakes of either D or INT groups to the 24/7 groups’ intakes was not included, due to the differences in time of shortening availability. Restated, the 1-hour intake for the 24/7 groups represents only a small portion of their total shortening access time, whereas this same 1-hour period is the only time when D and INT rats have access. Therefore, for the purposes of this study, it was not deemed appropriate to compare the 1-hour intakes of the 24/7 group to those of the D and INT groups.

Analyses of escalation of shortening intakes (normalized to body weight; Table 2) revealed differences in 9 of the 10 studies. Only in study 7 were the slopes of the INT and D groups not significantly different from each other across the 5-week binge induction period. Similar to the intake analyses, there was overlap in the slope values across studies with the D groups’ slopes ranging from 0.01 to 0.239 and those of the INT groups ranging from 0.048 to 0.277. Again, this suggests the importance of using direct comparisons of bingeing and control groups instead of operationalizing bingeing with a specified slope value. Furthermore, although shortening intakes escalated across time in all of the INT groups, this was not the case for the D controls, as they only exhibited positive, non-zero slopes (i.e. escalation) in 6 out of 10 studies. Thus, the daily brief access protocol used in these studies is not a reliable way to induce binge-type consumption. Indeed, even in the studies in which escalation occurred in the D group, intake was still reliably less than that of the respective INT group. In both of the 24/7 groups, calculated slopes were significantly less than zero, indicating that intakes actually decreased over time. This finding is similar to those previously reported (Bello et al., 2009) and indicates that a 24/7 group is not the optimal control against which bingeing should be compared.

Intake and escalation comparisons were affected differently by proneness to consume shortening (Table 3). Comparison of week-five intakes found that only the HINT group had significantly higher intakes than any other group. That is, only the HINT group binged. However, slope analyses indicated that the LINT group had the highest escalation in intake, followed by the LD, HINT, and HD groups, respectively. Slopes among the LINT, HINT, and HD groups were statistically different ($LINT > HINT > HD$), but the LD slope did not differ from any of the other groups. Based upon this analysis, one could conclude that the LINT group binged relative to HINT and HD groups, and the HINT group only binged relative to the HD group. Thus, the escalation analysis results in a conclusion that is quite different from that obtained with the intake analysis. The disparity in interpretation between the two assessment measures (intake vs. escalation) can be explained mathematically. Because the binge eating prone (BEP) groups (HD and HINT) started with significantly higher intakes than the binge eating resistant (BER) groups (LD and LINT), the likelihood of showing statistically higher escalations in the BEP groups’ intake is diminished. To clarify, a rat whose initial intake is high has to eat a great deal more to show significant escalation than a rat whose initial intake is low. For this reason, a slope analysis may not be a useful metric for assessing bingeing when rats have already been sorted by initial intake.

The BEP/BER model operationalizes bingeing based upon a median split of the rats into binge eating prone and resistant groups, with all rats having the same access to the binge

food (Boggiano, et al., 2007; Oswald, et al., 2011). The present study extends this approach by applying it to the two different access conditions commonly used by this laboratory (D, INT). We show that access conditions can influence intake, even in rats with different initial propensities to consume the binge food. This is consistent with another report in which a history of energy restriction stimulated intake in rats classified as binge eating resistant (Oswald et al., 2010). In short, while individual traits undoubtedly contribute to binge eating, this and other studies attest to the powerful contribution of access conditions, as well.

Despite their differences, both assessment measures have strengths and weaknesses. Between-group intake measures that compare intake in a binge group to that of some “normal” control more closely mimic the manner in which bingeing is defined clinically, i.e. consuming more in a discrete period of time than is normally consumed under similar circumstances and within the same period of time. In addition, intake measures provide temporal specificity in that it is possible to determine at which time point the groups separate statistically. Additionally, this type of analysis permits assessment of bingeing at any given point in time, i.e. it does not require multiple time points. A weakness of this measure is that it relies on comparisons among groups of animals, and is ineffective at the determination of bingeing in any given individual animal. Therefore, sample size is a major factor when using this measure.

Unlike assessing intake between groups, slope analysis does allow for comparisons between very small groups or even within subjects. For instance, one can ask if an individual animal binges after a given manipulation, relative to its own pre-manipulation baseline by determining if the intake slope differs from zero. That said, we again want to point out that even our D control group exhibited intake escalation in some of the studies analyzed. Therefore, including appropriate control groups against which bingeing can be operationalized is critical. Slope analysis may also prove more useful than intake analysis when comparing groups with different body weights or sexes, or in studies separated by time (Babbs, et al., 2011). Additionally, this measure is relevant in research showing similarities (i.e. escalation) between addiction and bingeing, e.g. (Rada, et al., 2005). As we have shown with the present BEP/BER analysis, it does have limitations when the groups that are compared have different initial intakes, or when the initial intakes are large, and this should be a consideration when using this technique.

Here we have examined two binge assessment measures (intake and escalation) that have been used to operationalize binge eating in animal models. One limitation of the present analysis is that it was applied to a single established model. Whether similar results would also be seen in other approaches is an open question. Based on the current analyses, as well as published results from other laboratories, both intake and escalation appear to be useful. However, their limitations need to be taken into consideration when attempting to operationalize binge-type eating in animal models.

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RESEARCH HIGHLIGHTS

- 2 measures (intake, escalation) to assess bingeing in rodent models are evaluated.
- Data generated with the Limited Access model of bingeing were used.
- Intake and escalation are both useful measures.
- Limitations of each are discussed and need to be taken into consideration.

Table 1

Average 1-h fat intake (g) on the last two binge days of wk 5.

Study	D Mean	SE	n	INT Mean ^a	SE	n	24/7 Mean	SE	n
1	3.6	0.3	14	5.5*	0.5	14	0.4	0.1	14
2	4.0	0.5	11	6.7*	0.9	14	0.4	0.2	12
3	2.8	0.4	12	4.1*	0.4	12			
4	2.9	0.4	12	4.9*	0.4	12			
5	2.9	0.5	10	5.3*	0.9	14			
6	3.3	0.3	16	5.6*	0.6	17			
7	3.2	0.4	11	4.7*	0.5	13			
8	2.9	0.4	12	5.1*	0.5	14			
9	3.0	0.4	10	7.2*	0.3	10			
10	2.2	0.3	12	3.9*	0.3	30			

^aIndicates significantly different from D group (p<0.05).

Table 2

Escalation of 1-h fat intake over the 5-wk binge induction period.

Study	D Slope	Non-Zero?	INT Slope ^a	Non-Zero?	24/7 Slope	Non-Zero?
1	0.06	Y	0.17*	Y	-0.017	Y
2	0.04	Y	0.15*	Y	-0.025	Y
3	0.01	N	0.05*	Y		
4	0.06	Y	0.16*	Y		
5	0.03	Y	0.12*	Y		
6	0.12	Y	0.22*	Y		
7	0.24	Y	0.28	Y		
8	0.07	N	0.53*	Y		
9	0.02	N	0.15*	Y		
10	0.02	N	0.08*	Y		

^aIndicates significantly different from D group ($p < 0.05$).

Table 3

The effect of proneness to consume shortening on intake and escalation.

Group	n	Mean 2-day Intake, g ^a	SE	Slope ^a	Non-Zero?
LD	12	2.6 ¹	0.5	0.27 ¹²³	Y
HD	15	3.6 ¹	0.3	0.09 ¹	Y
HINT	15	6.7 ²	0.5	0.18 ²	Y
LINT	15	3.6 ¹	0.3	0.31 ³	Y

^aDifferences among groups (p<0.05) are indicated by different numbers.