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The basolateral amygdala mediates the effects of cues associated with meal interruption on feeding behavior

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Abstract

Considerable evidence shows that environmental cues that signal food delivery when rats are food-deprived can substantially potentiate feeding later when rats are food-sated. Similarly, cues associated with meal interruption, food removal or impending food scarcity may also induce increased eating. For example, after learning the association between a discrete “interruption” stimulus and the unexpected termination of food trials, sated rats show enhanced food consumption when exposed to that stimulus. In Experiment 1, unlike sham-lesioned controls, rats with bilateral excitotoxic lesions of the basolateral amygdala (BLA) failed to display such cue-potentiated feeding. In Experiment 2, potentiation of feeding by an interruption signal was found to be food-specific. That is, a stimulus that signaled interruption of trials with one food but not trials with a second food later only facilitated consumption of the first food. These studies extend our knowledge of the psychological and neural processes underlying cue-induced feeding. Understanding these mechanisms may contribute our understanding of the etiology and treatment of binge eating disorders.

Keywords

amygdala; conditioning; cue-potentiated feeding; scarcity; bingeing

1. Introduction

During the last three decades, obesity rates have doubled for adults and tripled for children (e.g., CDC, 2009). Over 72 million people in the United States are now obese. The increasing prevalence of obesity has serious health implications, for example, augmenting the risk of Type II diabetes, cardiovascular diseases, hypertension, joint disorders and some forms of cancer. Additionally, obesity has an exorbitant monetary cost. It has been estimated that the total yearly costs of obesity in the United States are approximately \$117 billion per year (CDC, 2009). Thus, understanding the etiology of obesity is of the utmost importance.

Prolonged binge eating may lead to obesity (Yanovski, 1993; 2003), and binge episodes can compromise compliance to weight control treatments in obese patients. Although there has been considerable research on metabolic and pharmacological contributions to overeating,

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there has been less investigation of the role of environmental triggers in binge-like behaviors. Environmental cues play a key part in the initiation, maintenance and termination of feeding behaviors. For example, sights and smells of palatable food can induce eating, regardless of hunger state. Interestingly, cues that signal the absence or removal of food, such as cues for dieting in adolescents and adults, or for prohibition or removal of palatable food items in children, can also lead to increased eating (e.g. Herman, Olmsted & Polivy, 1983; Herman & Polivy, 1990; Polivy & Herman, 1985; 2002; 2006). Better characterization of the environmental, psychological and neurobiological mechanisms underlying the influence of external triggers on eating is essential to progress in developing therapies for eating disorders. The research described in this article examined the role of the basolateral amygdala (BLA) in the control of feeding initiated by environmental cues that signal meal interruption in rats.

Normal food-sated rats are prompted to eat by presentation of a cue that had been previously associated with food while the rats were food-deprived (Holland, Petrovich & Gallagher, 2002; Holland & Gallagher, 2003; Petrovich et al., 2002; Petrovich, Holland & Gallagher, 2005; Petrovich et al., 2007a; Weingarten, 1983; 1985; Weingarten & Martin, 1989; Zamble, 1973). This cue-induced feeding by food-sated rats can be substantial (e.g., as much as 7 grams of food in a 10 min test; Petrovich et al., 2007b), and can come under strong phasic control by discrete auditory or visual cues, waxing and waning precisely with the repeated initiation and termination of those cues (e.g., Galarce, Crombag & Holland, 2007). Thus, this example of cue-dependent overriding of satiety cues shares characteristics of binge eating (Herman & Polivy, 1990; Sobik, Hutchison & Craighead, 2005).

By contrast, rats with lesions of the basolateral amygdala or disconnections of BLA and the lateral hypothalamus fail to show such potentiated eating (Holland et al., 2002; Holland & Gallagher, 2003; Petrovich et al., 2002). This observation is consistent with the widely-recognized role for the BLA in various forms of emotional learning related to food. For example, in rats with BLA lesions, Pavlovian cues for food fail to acquire the ability to reinforce new Pavlovian or instrumental learning in second-order conditioning (Hatfield et al., 1996) or conditioned reinforcement tasks (Everitt & Robbins, 1992). Similarly, rats with BLA lesions are unable to access updated information about reward value to alter previously-acquired learned responses from those rewards. In devaluation experiments (see Pickens & Holland, 2004, for a review) normal rats and monkeys will spontaneously reduce responding to previously-established cues for food, after the value of a food reinforcer is reduced by either satiety or pairing with illness (Hatfield et al., 1996; Johnson, Gallagher & Holland, 2009; Malkova, Gaffan & Murray, 1997). Furthermore, rats with BLA lesions fail to show Pavlovian-instrumental transfer when multiple food reinforcers are used (Corbit & Balleine, 2005). That is, when previously-trained Pavlovian cues for a specific reinforcer are presented while rats are performing an instrumental response to earn that same reinforcer, the instrumental response rates are enhanced in normal rats, but not in rats with BLA lesions.

Interestingly, potentiation of feeding seems to be specific to the food signaled by the potentiating cue (Fedoroff, Polivy & Herman, 2003), consistent with suggestions that the BLA is critical to the integration of sensory and motivational information (e.g., Corbit & Balleine, 2005). For example, Petrovich et al. (2007a; 2007b) found that when sated rats were placed in a context previously associated with the delivery of flavored food pellets, they consumed more of the pellets that had been previously presented in that context, but not of either novel or familiar pellets of a different flavor. Likewise, Delamater and Holland (2008) and Galarce et al. (2007) presented food-deprived rats with pairings of two different auditory cues with two distinctive food reinforcers (sucrose and maltodextrin). After satiation on chow, rats were given access to each of the reinforcers in separate sessions, in

which one or the other auditory cue was presented. Consumption was enhanced when the presented cue had signaled the available food, but not when it had signaled the other food.

Some studies have shown that uncertainty about food availability can also have orexigenic properties (e.g. Herman et al., 1983; Herman & Polivy, 1990; Polivy & Herman, 1985; 2002; 2006). Recently, Galarce and Holland (2009) found that cues that signaled interruption of a meal also potentiated feeding later when rats were sated. In those studies, food delivery occurred randomly during presentations of one auditory cue (CS+), but when another auditory “interruption signal” (IS) cue was presented at random times during the first cue, both that cue and food delivery was canceled. Subsequently, the IS was found to potentiate feeding of satiated rats.

Experiment 1 was designed to begin a neural analysis of IS-induced feeding. Because previous experiments showed that feeding induced by IS and CS+ share common characteristics, such as phasic stimulus control (Galarce & Holland, 2009), and the BLA is important to CS+ induced feeding, we sought to describe the role of the BLA in IS-induced feeding. If BLA plays a role in IS-induced feeding, then, given BLA's role in integrating sensory and motivational information, it is likely that this type of cue, like a CS+, also controls feeding in food-specific manner. Thus, Experiment 2 was designed to determine whether control of food consumption by an IS is food-specific.

2. Results

2.1. Experiment 1

Experiment 1 was designed to determine the role of the BLA on IS-induced feeding. First, all animals underwent bilateral BLA infusions of N-methyl-D-aspartate (BLA-lesioned group) or PBS vehicle (sham group). Two weeks later, all rats were food deprived to 85% of their ad-lib weights. During the first stage of Pavlovian training, rats learned to associate presentation of an auditory stimulus (CS+) with sucrose (US). Later in training, CS+ trials were interrupted by another cue (IS). After IS presentation, the CS+ was terminated and no more food was delivered until the following trial. After one week of ad-lib access to lab chow, three consumption tests evaluated the influence of CS+, no cue, or IS presentations on US intake.

Of the 11 BLA-lesioned rats, 2 were excluded because their lesions covered less than 75% of BLA. The 9 remaining rats had $91 \pm 3\%$ bilateral BLA damage, which included lateral, basal and accessory basal nuclei. Most lesions spared CeA, and damage to that region was minimal when observed ($2.7 \pm 0.5\%$). Some animals showed ventricular enlargement at the caudal portion of the lesions. No rat manifested recovery complications after surgical procedures. At the end of the recovery period, there was no significant difference between the weights of the BLA-lesioned (433 ± 6 g) and sham-lesioned (441 ± 4 g) rats ($F(1,13)=1.10$, $p=0.313$). Figure 1 shows a photomicrograph of representative BLA (a) lesion and (b) sham brains, and Figure 2 illustrates the extents of BLA lesions for all 9 animals used in this experiment.

Pavlovian conditioning- Phase I—Training was evaluated with a 3-way ANOVA which included group (sham or BLA), session and period (CS+ or ITIs). All rats, except one sham, which was removed from the study, learned to discriminate between CS+ trials and ITIs. Learning became more evident as sessions progressed ($F(6,78)=8.68$, $p<0.001$). During the first session, the proportion of time spent in the food cup was similar during both ITI ($24.3 \pm 3.1\%$) and CS+ periods ($25.6 \pm 4.0\%$). However, on the last phase I session this difference was ample (ITI= $13.2 \pm 2.9\%$; CS+= $46.0 \pm 4.3\%$). No learning differences were found between groups ($F_s<1.078$, $p_s>0.383$).

Pavlovian conditioning- Phase II—Training was evaluated with a 3-way ANOVA which included group (sham or BLA), session and period (CS+, IS or ITIs). During this training phase rats learned to discriminate the three different epochs: ITI, CS+ and IS ($F(2,26)=75.77$, $p<0.0001$). Planned orthogonal contrasts confirmed that responding during CS+ presentations was higher than during ITI or IS, and that IS responding was greater than ITI responding (*see* figure 3) ($F(1,13)=90.91$, $p<0.0001$; and $F(1,13)=65.23$, $p<0.0001$, respectively). Rats in the sham and BLA groups did not differ in their behavioral patterns either within or between training sessions ($F_s<1.223$, $p_s>0.272$).

Cue-potentiated feeding tests—Figure 4 and table 1 show the results of the three consumption tests. Figure 4 summarizes responding during cue and ITI periods for each group during the last half of each consumption test. Table 1 provides the data from both halves of each test. Each test was analyzed separately using a 3-way ANOVA which included group (sham or BLA), period (cue or ITI) and test half (first or second), followed by separate 2-way ANOVAs for each group.

First, during the IS test (figure 4a) rats in the Sham group consumed more during cue periods than during ITIs, whereas BLA-lesioned rats did not (group $\times$ period interaction $F(1,13)=6.54$, $p=0.024$). This increase of consumption during IS presentations was significant in sham rats ($F(1,5)=9.75$, $p=0.026$) and was most evident during the second half of the test session (period $\times$ test half interaction $F(1,5)=12.0$, $p=0.018$). By contrast, BLA-lesioned rats failed to show any change in food consumption ($F(1,8)=0.18$, $p=0.683$).

Second, when rats were tested in the absence of discrete cue presentations (empty test), sham and BLA rats consumed food in a similar pattern (figure 4b). A comparison of both groups' consumption during ITIs and dummy cue trials showed no differences ($F(1,13)=0.56$, $p=0.467$). Individual 2-way ANOVAs confirmed that no dummy cue-ITI difference was found in either group ($F_s<0.323$, $p_s>0.594$).

Third, sham animals increased their consumption during CS+ presentations compared to ITI consumption, but BLA-lesioned rats did not (group $\times$ period interaction $F(1,13)=30.58$, $p<0.0001$; figure 4c). Two-way analyses corroborated that sham animals increased their level of consumption during CS+ trials, but BLA animals did not show such a pattern ($F(1,5)=27.83$, $p=0.003$; $F(1,8)=0.74$, $p=0.414$, respectively). Unlike with IS, sham animals showed this potentiated feeding from the outset of testing. Although it might be argued that the difference between CS+ and IS effects was confounded with test order (IS testing preceded CS+ testing), it is notable that the same difference in within-session pattern was reported in a previous study, in which separate groups of rats were tested with IS and CS+ in the same session (Galarce & Holland, 2009).

Lastly, consumption during the pre-test periods was analyzed as a measure of spontaneous unconditioned food consumption (table 1). For this purpose, a 2-way ANOVA was used (group $\times$ test). Even though consumption differed marginally from test to test ($F(2,26)=3.29$, $p=0.053$), these variations did not interact with group identity ($F(2,26)=1.20$, $p=0.319$). Altogether, no differences in consumption were found between sham and BLA rats ($F(1,13)=0.95$, $p=0.348$).

Extinction test—After the consumption tests, a subset of animals (3 sham and 7 BLA) underwent an extinction test which confirmed and extended the observation in Phases I and II that BLA and sham animals did not differ in conditioned approach and entry to the food cup. In this test, in which no sucrose was presented, we examined responding to CS+ alone, IS alone, a compound of CS+ and IS, and the ITI. A 2-way ANOVA which included group (sham or BLA) and trial type (ITI, CS+, IS or CS+/IS compound) was used to analyze these

data. This analysis was followed by pairwise comparisons with Least Significant Difference (LSD) corrections which were applied to adjust for multiple comparisons.

Overall, rats' approach behavior varied depending on the identity of the trial type ($F(2,24)=13.86$, $p<0.001$), but not on the lesion conditions ($F(1,8)=2.46$, $p=0.155$). As in Galarce and Holland's (2009) study, pairwise comparisons revealed virtually no difference in the time rats spent in the food cup during IS ($21.7\pm5.6\%$) versus during the ITIs ($19.6\pm6.0\%$; $p=0.764$). Furthermore, presentation of the IS in compound with CS+ suppressed responding ($51.3\pm10.0\%$); compared to responding during CS+ alone ($68.0\pm7.4\%$; $p<0.01$). Thus, it is clear that the ability of IS to enhance food consumption was not attributable to its provoking food cup entry or possessing excitatory properties; indeed the opposite was evident. Furthermore, these effects did not differ between sham and BLA-lesioned rats ($F(3,24)=.17$, $p<0.915$), showing that the lesion effects on food consumption observed earlier were not accountable in terms of differences in simple food cup approach tendencies. It is tempting to ascribe conditioned inhibitory tendencies to IS from the results of the extinction test (a passed summation test); however, in the absence of control conditions, we cannot distinguish this outcome from external inhibition or generalization decrement. Nevertheless, it is fair to assert that the results of this test indicate that both sham and BLA rats associated CS+, but not with IS, with the presentation of food.

2.2. Experiment 2

In *Experiment 2* we examined the sensory specificity of IS-potentiated feeding. Hungry rats were trained with two reinforcers, sucrose and maltodextrin, each signaled by a different auditory cue (CS+). Only one of those cues –with its corresponding reinforcer delivery– was then interrupted by an interruption signal (IS). After satiation on standard lab chow in their home cages, rats' consumption of sucrose or maltodextrin was examined in the experimental chambers in the presence of the IS. Thus, IS-elicited consumption of a food that had been previously interrupted by the IS (consistent), or of a food that was experienced in the same context but not interrupted by IS (inconsistent), was examined. Control rats received training with the same cue-reinforcer trials, but the stimulus used as IS in the experimental rats was presented unpaired (CS-) with those trials.

Pavlovian conditioning- Phase I—During this initial stage of training, the rats spent more time in the food cup during reinforced cue (CS+) presentations ($35.7\pm2.1\%$) than during ITIs ($19.7\pm1.5\%$) ($F(1,20)=101.11$, $p<0.0001$). This difference became more apparent as the training sessions progressed ($F(3,60)=9.42$, $p<0.0001$). The effects of group, type of food and their interaction were not significant ($F_s<1.412$, $p_s>0.249$).

Pavlovian conditioning- Phase II—When both types of cue-reinforcer trials (CS +1→reinforcer 1 and CS+2→reinforcer 2, e.g., noise-sucrose and tone-maltodextrin) were first intermixed in the same sessions, the rats continued to respond more during the reinforced cues ($39.6\pm2.2\%$) than during the ITIs ($13.9\pm1.6\%$), ($F(1,22)=189.83$, $p<0.0001$). Discrimination between these two periods continued to increase across sessions; although this change was only marginally significant ($F(3,66)=2.51$, $p=0.066$). Rats' food cup responding to sucrose or maltodextrin cues did not differ, nor were there significant group differences in that responding ($F_s<.709$, $p>0.409$).

Pavlovian conditioning- Phase III—During this third phase of training, the IS was introduced into CS+1→reinforcer 1 trials. During these trials, rats in the experimental and control groups showed a different response pattern when exposed to the CS, IS/CS- or ITIs ($F(2,40)=24.78$, $p<0.0001$). Rats in the experimental group spent more time in the food cup during CS+1 presentations ($45.7\pm3.3\%$) than during the IS ($33.1\pm3.0\%$) or ITI ($11.55\pm2.0\%$)

($F(2,28)=131.47$, $p<0.0001$) periods. Rats in the control group showed a similar pattern of responding ($CS+1>IS/CS->ITI$) but, in this group, $CS-$ responding ($16.3\pm1.8\%$) was closer to ITI ($14.1\pm1.6\%$) than to $CS+1$ behavior ($51.39\pm4.0\%$) ($F(2,12)=163.69$, $p<0.0001$). No between-subject differences regarding the counterbalancing of reinforcer identity or overall group performance were found ($F_s<0.528$, $p_s>0.476$).

Training conditions for the $CS+2\rightarrow$ reinforcer 2 trials were identical for both groups. All rats showed more food cup responding when $CS+2$ was presented ($49.3\pm2.5\%$) than during ITIs ($14.6\pm1.3\%$) ($F(1,20)=339.29$, $p<0.0001$). Type of food or group assignment did not affect training performance ($F_s<2.264$, $p_s>0.148$).

IS-potentiated feeding test—For this test the experimental group was divided into groups *consistent* ($n=8$) and *inconsistent* ($n=8$). For the rats in the consistent group the reinforcer present in the liquid cups was the one that had been interrupted by IS in training (reinforcer 1), whereas in the inconsistent group, reinforcer 2 was present. Data from the consumption test were analyzed with a 4-way ANOVA, with variables of group (consistent, inconsistent and control), period (cue or ITI), test half (first or second), and food reinforcer (maltodextrin or sucrose). Given the difficulty of interpreting 4-way interactions, the data for each group were subsequently analyzed with 3-way ANOVAs including period, test half and food as variables.

The 4-way ANOVA showed that each group displayed a unique pattern of consummatory responding during cue presentations or ITIs (*see* figure 5 and table 2) (group $\times$ period interaction $F(2,18)=10.42$, $p=0.001$). Furthermore, these differential consumption patterns differed across the first and second halves of the test sessions (group $\times$ period $\times$ test half interaction $F(2,18)=8.16$, $p=0.003$). No overall group differences were found ($F(1,18)=1.89$, $p=0.18$).

Confirming the data from *Experiment 1* and Galarce and Holland's (2009) studies, a 3-way ANOVA indicated that the consistent group showed increased food consumption during IS presentations ($F(1,6)=15.98$, $p=0.007$). This effect, however, was mostly evident during the second half of the test session ($F(1,6)=24.04$, $p=0.003$). The other two groups, inconsistent and $CS-$, failed to show this effect. On the contrary, during the second half of the test session, rats in the inconsistent group showed a marginal decrease in food consumption when presented with IS ($F(1,6)=4.97$, $p=0.067$). In addition, when exposed to the $CS-$ cue, rats in the control group suppressed food consumption, throughout the test session ($F(1,6)=11.35$, $p=0.015$). Lastly, rats in consistent group consumed more maltodextrin (13.0 ± 1.7 deliveries/min) than sucrose (7.7 ± 1.7 deliveries/min) ($F(1,6)=10.60$, $p=0.017$).

3. Discussion

In two experiments, intact sated rats showed enhanced food consumption under the strong phasic control of cues that had been previously associated with either food delivery ($CS+$) or the termination of $CS+$ and food availability (IS), relative to consumption during ITIs, empty test sessions, or during a cue that had been unpaired with food delivery. Thus, consumption was enhanced by cues correlated with either the availability or meal termination. Consistent with prior research (Galarce & Holland, 2009), for $CS+$, this enhanced consumption pattern was evident from the beginning of the tests, whereas with IS, it was not apparent until the second half of the test session. At the same time, although IS enhanced food consumption by the end of the consumption test, a subsequent assessment of food cup entry in the absence of food suggested that the IS was primarily suppressive of food cup entry. Thus, the ability of IS to enhance feeding was not attributable to its controlling food cup entry. Indeed, the tendency for IS to suppress food cup approach may

account for its failure to potentiate feeding in the early portions of the consumption tests. Because during training the rats never received food when the IS was presented, they might withdraw from, or at least fail to approach, the food cup when the IS was initially presented. Hence, the rats would not have the opportunity to display enhanced consumption until that inhibitory tendency was overcome, perhaps via some within-test learning to maintain proximity to the food cup during IS delivery. Even though the present data does not provide information about the mechanism by which rats change their behavior within the test session, it is possible that this is the result of a learned excitatory association between IS and US during such test.

In Experiment 1, we found that, unlike intact rats, rats with bilateral excitotoxic lesions of the BLA showed no evidence for enhancement of feeding by either CS+ or IS cues. Nevertheless, these rats showed no differences in the acquisition or expression of food cup entry responses during CS+ or IS. Thus, the effects of BLA lesions were limited to motivational consequences of learning about CS+ and IS. Although previous studies have shown that potentiation of feeding by a cue paired with food depends on BLA function, the observation that potentiation by a cue paired with interruption of food and food cues is also BLA-dependent is novel.

The comparable effects of BLA lesions on CS+ and IS-induced feeding suggest commonalities of mechanism. BLA has often been implicated in aspects of cue-outcome learning, especially conveying information about the motivational value of reinforcers to learned cues, and integrating sensory aspects of reinforcer representations with that value (e.g. Blundell et al., 2001; Corbit & Balleine, 2005; Holland & Gallagher, 2004). Each of these characteristics seem applicable to feeding potentiated feeding by IS as well as by CS+, especially given our observation of food-specificity of IS-potentiated feeding in Experiment 2. Furthermore, data from other domains suggest that IS's ability to potentiate feeding may derive considerably from CS+ (Lysle & Fowler, 1985). Galarce and Holland (2009) found that IS's ability to induce feeding is influenced not only by its relationship to food interruption but also with CS+ termination. Thus, if IS's orexigenic properties are derived from CS+, then it would be expected that BLA lesions would interfere with IS's acquisition of such properties.

Further investigation of circuitry involved in IS-induced feeding would be of interest. Previous investigation showed that potentiation of feeding by CS+ depended on circuitry that included connections among BLA, medial prefrontal cortex (mPFC) and the lateral hypothalamus (Holland & Petrovich, 2005; Petrovich et al., 2005), a key link to the arcuate nucleus and other hypothalamic regions with key roles in feeding (Bouret, Draper & Simerly, 2004; Horvath, 2005; Hurley et al., 1991; Sesack et al., 1989). Given the commonalities between CS+ and IS-induced eating, it would not be surprising if an amygdalo-hypothalamic circuit served as a final common path mediating both. Although it is premature to speculate as to brain circuitry that might be specific to IS-induced feeding, it is notable that a number of brain regions often thought to be involved in emotional responses to reward omission (e.g., central amygdala and the ventral striatal nucleus accumbens, (Abler, Walter & Erk, 2005) are not critical to the display of CS+ potentiated feeding (Holland & Petrovich, 2005; Petrovich et al., 2005). It would be interesting if these regions, known to be important in other aspects of feeding (e.g., Baldo et al., 2005; Petrovich et al., 2009; Zhang, Gosnell & Kelley, 1998) played some role in IS-induced feeding.

Experiment 2 was designed to assess a crucial aspect of scarcity-related cues: their capacity to convey food-specific information. Here, we found that enhanced consumption by an IS was specific to the food whose termination was indicated by that cue. Indeed, rats in the Inconsistent test group slightly reduced their intake of a different, but equally familiar food

when the IS was presented (inconsistent group). There is ample precedent for Pavlovian CS + cues' coding specific attributes of their associated outcomes. For example, experiments using selective devaluation (Johnson et al., 2009), Pavlovian-instrumental transfer (Corbit & Balleine, 2005), and differential outcome expectancy (Blundell, Hall & Killcross, 2001) procedures all show that cue-evoked reinforcer representations can code detailed, specific sensory information. Notably, all of these phenomena depend on BLA function (see Holland & Gallagher, 2004, for a review). Most important, using foods and signals similar to those used in Experiment 2, we found similar food-specificity for potentiation of feeding by CS+ cues (Delamater & Holland, 2008; Galarce et al., 2007).

Evidence for comparable specificity of cues that signal the removal or absence of a particular outcome is less common (Delamater, Lolordo & Sosa, 2003; Holland, 1989). In our experiments, IS might acquire food-specific information either by its close backward temporal relation with the US (a 3 to 8-s gap, which might permit forward associations between IS and food aftertastes or backward associations with memorial aspects of food), or indirectly, via its temporal relationship with the CS+ (i.e., backward second-order conditioning). Regardless of mechanism, IS seems to acquire information about the nature of the US at the same time that it signals its absence or imminent removal.

Why does a cue paired with meal interruption elicit food consumption, and why should that consumption be food-specific? From an allostatic perspective, the ability for cues to inform about present and future availability and unavailability of specific foods may be critical for the modulation of ingestive behaviors. Responding to impending scarcity cues with gorging or bingeing may provide an adaptive advantage over animals that do not use those cues in that manner. Moreover, maximum advantage would be conferred if cues that signal food scarcity also conveyed particular sensory information about foods, especially in cases of particular nutrient deficiencies. Otherwise, an animal's attention and motivation might be scattered among all available foods. Thus, if cues that signaled food scarcity were nonspecific, the chances to compensate for future specific needs would be reduced. The results of Experiment 2 support the notion that an IS allows animals to predict future unavailability of particular food items, rather than merely informing about general hunger state or food scarcity. This possibility is analogous to suggestions that conditioned inhibitors form inhibitory associations with particular sensory aspects of the foods whose absence they signal (Delamater, LoLordo, & Sosa, 2003; Holland, 1989). The formation of such associations might be mediated by CS-activated representations of those foods (Holland & Wheeler, 2008) on CS+/IS trials.

In humans, scarcity and abundance interact in ways that alter food consumption. For instance, prior caloric deprivation can enhance subsequent appetitive and consummatory behaviors towards food (Fisher & Birch, 1999; Pecoraro et al., 2002; Polivy et al., 1994; Polivy, Coleman & Herman, 2005). Timmerman (1998) coined the term *perceived deprivation* to describe excessive preoccupation with food in people who wrongly believe that they are undergoing some form of physiological deprivation. These people are usually restrained eaters, who perceive they eat less than desired, but whose diets and body weight are no less than normal. This type of psychological deprivation also occurs in cyclic dieters who tend to avoid their favorite foods and force themselves to short periods of caloric restriction (Polivy, 1996). The probable consequence of excessive preoccupation with food is the development of binge eating behaviors (Hagan, Whitworth & Moss, 1999). It is possible that during our consumption tests, the IS induces a psychological state similar to what has been described in humans as perceived deprivation.

4. Experimental procedures

4.1. Experiment 1

Subjects—The subjects were 18 male naive Long-Evans strain rats (Charles River Laboratories, Raleigh, NC, USA) which weighed between 300–350g when they arrived in the laboratory vivarium. They had free access to lab chow (2018 Rodent Diet, Harlan Teklad Laboratory, Madison, WI, USA) for a week before their food was restricted to maintain them at 85% of their *ad-libitum* weights. The rats were caged individually in a colony room illuminated from 6:00am to 6:00pm. This research project was approved by the Johns Hopkins University Animal Care and Use committee.

Apparatus—We used 8 training chambers (22.9 × 20.3 × 20.3 cm) with aluminum front and back walls and clear acrylic side walls and top. An infrared activity monitor (Coulbourn Instruments, Allentown, PA, USA) and a panel of infrared lights used to illuminate the chamber for video recording were placed on the top of each chamber. An illuminated clear acrylic food cup, with a capacity of about 1.7 ml, was placed behind a square hole in the center the front wall. A photocell beam in the food cup was used to detect head entries and time spent in the cup. A speaker which was used to present auditory cues was placed on the back wall of a double-walled sound-resistant shell which enclosed each experimental chamber. A video camera was placed 18cm above the speaker to record the rat's behavior, and a second camera was placed under the transparent food cup, to record consummatory responses. Images from each camera were recorded and displayed on video monitors, each of which showed 4 chambers or food cups. Video data are not presented in this article.

Surgical procedures—Surgeries were performed two weeks before behavioral training procedures were initiated. All animals were anaesthetized with isoflurane gas (Abbott Laboratories, Chicago, IL, USA) while their heads were fixed in a stereotaxic frame (Kopf Instruments, Tejunga, CA, USA). Bilateral BLA lesions were performed on eleven rats with two infusions of 17mg/ml N-methyl-D-aspartate (NMDA; Sigma, St. Louis, MO, USA) dissolved in phosphate-buffered saline (PBS) solution. NMDA was infused 2.8mm posterior to Bregma, 5.1mm lateral of the midline, and 8.7mm (0.16µl) and 8.4mm (0.08µl) ventral to the skull surface at the injection site. Seven sham rats were injected with PBS vehicle alone, using identical surgical procedures. All rats were infused with a Hamilton 2.0-mL syringe at a rate of 0.1µl per minute. After surgery all rats were injected 0.01ml buprenorphine hydrochloride (Sigma) to minimize pain.

Pavlovian conditioning- Phase I—Two weeks after surgery, rats first received two 60-min sessions designed to train them to approach the food cup and consume the food reinforcer. Each of these sessions included 16 0.1-ml deliveries of an 8% (w/v) sucrose solution, which served as the reinforcer. This solution was delivered by infusion pumps located outside the double-walled sound-attenuating shells. Next, rats were given six 60-min training sessions intended to establish a Pavlovian association between an auditory cue (CS +) and sucrose. Each of these sessions included 10 CS+ trials. The CS+ consisted of either a 78-dB, 1500-hz intermittent tone or an ~78-dB white noise, and was 2 min in duration. Four reinforcers were delivered at random times (VT30) within each CS+ presentation.

Pavlovian conditioning- Phase II—In the second phase of training, a second 10-s long auditory cue was introduced to serve as an interruption signal (IS). This signal was presented during 10 CS+ trials and its appearance interrupted both the presentation of the CS+ and delivery of reinforcers (see Galarce & Holland, 2009). With IS onset, the CS+ terminated and no further reinforcers were delivered on that trial. The IS occurred at random times between 30-s and 90-s after CS+ trial onset. During interrupted trials, reinforcer delivery

density during CS+ was maintained (VT30s). Thus, in every interrupted trial, rats had access to one, two or three reinforcer deliveries. The IS was always presented 10-s after the last reinforcer delivery. This form of training continued for 15 sessions.

Cue-potentiated feeding tests—At the end of training, the rats were given free access to lab chow in their home cages for 7 days. Then, on consecutive days, sated rats received three 9-min potentiated feeding tests in the experimental chambers. In these tests, consumption of sucrose was examined in the presence of one cue (CS+ or IS; cued sessions) and in the absence of any cue (empty session). Because our primary interest was in the role of BLA during the IS test, all rats were tested with that stimulus in the first test session, unconfounded by prior test experience. In test 2, consumption was examined in the absence of any discrete cue, to provide an index of spontaneous feeding in the absence of explicit Pavlovian cues. Finally, in test 3, consumption was examined during CS+ presentations.

In the first two min of each test session, rats had unlimited access to the sucrose solution without any cue presentation. The purpose of this pretest was to reduce overall sucrose consumption to permit a more sensitive assessment of the effects of the cue test conditions later. In the second portion of each consumption session (test), the rats had free access to sucrose while seven 20-s cues were presented, separated by 40-s intervals. During consumption test 1, each rat was presented with only the IS. In the empty session, the test part consisted of 7 min of sucrose access with no discrete-cue presentations. However, consumption was recorded in 20-s dummy “cue” and 40-s “ITI” periods, as in the cued tests. The timing of these periods was yoked to the cued test's cue and ITI periods. The third test was identical to the IS test, with the difference that the CS+ was presented instead of IS.

In all tests, the food cups were filled with 1.7 ml of sucrose solution before the rats were placed in the experimental chambers. Consumption of the rats was monitored on the video monitors by an experimenter. When the liquid in a cup was nearly depleted, another 0.2 ml was delivered by the experimenter, using a computer program that activated the appropriate infusion pump. The time and number of these deliveries was recorded by the computer, providing a record of the pattern and amount of liquid consumed by each rat. The experimenter delivered the sucrose from a different room and was blind to test periods and group assignment.

Histological procedures—At the end of the experiment, the rats were first deeply anesthetized with a 100mg/kg pentobarbital injection. Then, they were perfused transcardially with a 0.9% (w/v) saline, followed by a 10% (v/v) formalin. Brains were removed and stored in a 25% (w/v) sucrose-formalin solution at 4°C for 48hrs. Brains were sliced with a microtome and 1 in 3 40µm slices were stored and Nissl-stained. Lesions were microscopically examined and damage was determined using imaging software. In order to determine total BLA - and central nucleus of the amygdala (CeA)- damage, these areas were superimposed to sections at interaural 6.70, 6.44, 6.20 and 5.40 of the Paxinos and Watson atlas.

4.2. Experiment 2

Subjects—Twenty-four male naive Long-Evans strain rats (Charles River Laboratories, Raleigh, NC, USA), which weighed between 300-350g when they arrived in the laboratory vivarium, were used in this experiment. They had free access to lab chow (2018 Rodent Diet, Harlan Teklad Laboratory, Madison, WI, USA) for a week before their food was restricted to maintain them at 85% of their ad-libitum weights. The rats were caged individually in a colony room illuminated from 6:00am to 6:00pm.

Apparatus—The training chambers were the same as those used in *Experiment 1*.

Pavlovian conditioning: Phase I—Rats first received two 60-minute sessions designed to train them to approach the food cup and consume each of the 2 food reinforcers. Each of these sessions included 16 0.1-ml deliveries of either 4% (w/v) sucrose (1 session) or 4% (w/v) maltodextrin solution (1 session), which served as the 2 reinforcers. It is noteworthy that the foods used in this experiment differ mostly in taste, but not in their post-ingestive consequences. Each US was administered in a 4% (w/v) solution because pilot data from our laboratory indicated that at this concentration Long-Evans rats consume equal amounts of these foods.

Next, rats were given 4 60-min training sessions intended to establish two Pavlovian associations, CS+1→reinforcer 1 (2 sessions) and CS+2→reinforcer 2 (2 sessions). The CS+s were an intermittent 78-dB, 1500-hz tone and a 78-dB, 4-hz clicker, and were each 2 min in duration. Four reinforcers were delivered at random times within each CS+ presentation. The identities of the CS+s, reinforcers, and their combinations were completely counterbalanced. Each of these training sessions included 10 presentations of only one of the CS+reinforcer combinations.

Pavlovian conditioning- Phase II—After completing the Pavlovian training Phase I, all rats received four 60 min sessions, each of which contained 10 2-min CS+ trials. Each of these training sessions included 5 CS+1→reinforcer 1 trials and 5 CS+2→reinforcer 2 trials, with an inter-trial interval (ITI) of 266 s. Trial presentation was randomly intermixed.

Pavlovian conditioning- Phase III—In the third phase of training, rats were separated into 2 groups (*experimental: n=16, control: n=8*), matched for body weight. During this stage a new auditory cue, a ~78db white noise, was introduced as an interruption signal (IS) or control cue (CS-).

In each session, rats in the experimental group were presented with one 2-min CS+1-reinforcer 1 trial, identical to those delivered in the previous training phases. During the four other CS+1→reinforcer 1 trials, however, the IS was presented. These interrupted trials were identical to those described in *Experiment 1* and elsewhere (Galarce & Holland, 2009). Thus, in every interrupted trial, rats had access to one, two or three reinforcer 1 deliveries. Rats in this group were also presented with five uninterrupted CS+2→reinforcer 2 trials

The control group was presented with 5 CS+1→reinforcer 1 trials and 5 CS+2→reinforcer 2 trials, as in the Pavlovian phase II. In addition, four 10-sec CS- nonreinforced trials were presented in each session in this group. For both groups, Phase 3 training continued for 10 sessions, and trial order presentation was intermixed.

IS-potentiated feeding test—Immediately after the last Phase III Pavlovian training session, rats were given free access to lab chow in their home cages for a week. Next, sated rats received one 9-min potentiated feeding test, in which consumption of either sucrose or maltodextrin was examined in the presence of IS or CS-. Cue presentation was identical to that described in *Experiment 1*. That is, cues were presented seven times for 40sec separated by 20sec ITIs.

Each rat was tested with only one food. Half of the rats were tested with reinforcer 1, while the other half were tested with reinforcer 2. Thus, in this test, rats from the experimental group were divided into two groups: the *consistent* group was tested with reinforcer 1 (i.e. food previously interrupted by IS); and, the *inconsistent* group was tested with reinforcer 2

(i.e. food never interrupted by IS). Finally, rats in the control group, which were also tested with reinforcers 1 or 2, were presented with the same auditory cue that had served as a CS-.

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Figure 1. Photomicrographs of representative (a) sham and (b) basolateral amygdala (BLA) lesions. In both cases, the amygdala central nucleus (CEA) was mostly spared (see text). Arrows depict lesion borders.

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Figure 3.

shows appetitive responding in Experiment 1 Phase II of Pavlovian training sessions during: a) 10-s prior to CS+ trials (ITI); b) first 10-s of CS+ presentations (CS+); and c) 10-s duration of the interruption stimulus (IS). Data sets reflect mean responding for sham (grey circles) and BLA rats (dark circles). Error bars represent SEM.

Figure 4. shows food consumption during the second half of Experiment 1 consumption tests: a) depicts consumption during the IS test; b) during the empty test, and c) during the CS+ test. Dark bars reflect consumption during cues (dummy cues during the empty test), and light bars reflect consumption during ITIs. All graphs display the mean rates of food deliveries needed to replenish the liquid cups (see text). Error bars represent SEM.

Figure 5. shows food consumption during the second half of Experiment 2 consumption test. Dark bars reflect consumption during cues, and light bars reflect consumption during ITIs. All bars reflect the mean rates of food deliveries needed to replenish the liquid cups (see text). Error bars represent SEM.

Table 1

shows the results of Experiment 1 consumption tests: a) IS test; b) during empty test; and c) CS+ test. Data are collapsed in test halves (first and second) by test event (cue or iti). Entries are the mean rates of food deliveries needed to replenish the liquid cups (see text).

(a) IS test:		1 st half		2 nd half	
		pretest	cue	iti	iti
Sham	mean	20.8	18.3	17.1	24.2
	<i>sem</i>	1.14	2.41	2.50	3.89
BLA	mean	18.5	12.7	16.9	14.4
	<i>sem</i>	0.93	1.97	2.05	3.18
(b) empty test:					
Sham	mean	17.2	13.7	15.8	10.0
	<i>sem</i>	1.51	2.69	1.90	2.09
BLA	mean	17.8	13.9	13.2	9.7
	<i>sem</i>	1.24	2.20	1.55	1.71
(c) CS+ test:					
Sham	mean	18.1	22.7	12.0*	26.0
	<i>sem</i>	1.72	2.11	1.57	2.28
BLA	mean	15.6	13.3	14.1	13.8
	<i>sem</i>	1.40	1.72	1.28	1.86

Table 2

shows the results of Experiment 2 consumption tests. Data are collapsed in test halves (first and second) by test event (cue or iti). Entries are the mean rates of food deliveries needed to replenish the liquid cups (see text).

(a) CS-	1 st half			2 nd half		
	pretest	cue	iti	cue	iti	
mean	17.0	10.1	12.9	8.5	11.3	
sem	0.62	1.04	1.22	1.34	1.50	
(b) inconsistent						
mean	13.2	11.1	10.4	4.1	8.1	
sem	1.05	1.51	1.64	1.33	1.42	
(c) consistent						
mean	14.6	10.9	11.0	13.3	6.5*	
sem	0.55	1.63	1.34	2.08	1.26	