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Effects of cues associated with meal interruption on feeding behavior

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Abstract

Food consumption is controlled by both internal and external factors. Environmental signals associated with food may prepare an animal to forage, consume and digest more effectively. Furthermore, environmental cues that provide information about food availability enable animals to make predictions about future food resources and act upon that knowledge in appropriate fashion. For example, when exposed to a cue that signals the presence of food, animals can eat beyond their present needs to cope with predicted future famine. Interestingly, cues previously paired with meal interruption have a similar effect. In two experiments, food-deprived rats learned to associate one conditioned stimulus (CS+) with delivery of a food unconditioned stimulus (US), and another stimulus (IS) with an unexpected termination of CS-US trials. Subsequently, both CS+ and IS enhanced consumption of the US food by sated rats. The results of Experiment 2 indicated that IS's ability to potentiate feeding of sated rats in test depended more on its accompanying CS+ termination in training than on its signaling reductions in US frequency. These experiments may provide a novel animal model of binge-like behaviors in sated rats induced by external cues paired with meal interruption.

Keywords

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

Introduction

Binge-eating is characterized by discrete episodes of eating large amounts of food at a faster pace than usual. These events are not only a problem *per se*, but most importantly they also constitute a serious risk factor for obesity and type 2 diabetes (Herpertz *et al.*, 2000; Herpertz *et al.*, 1998; Mannucci *et al.*, 1997). Risk factors for bingeing include restrictive eating-habits and a complex array of psychosocial variables that are yet to be clearly delineated (Laessle *et al.*, 1996; Stice *et al.*, 2000; Womble *et al.*, 2001). Less is known about the specific environmental triggers for these episodes. There is considerable evidence that cues for food can provoke substantial eating even in sated laboratory animals. For example, after food-deprived rats have learned to associate a cue with food presentation, later presentation of that cue when those animals are sated will increase their food consumption in short time intervals. (Galarce, Crombag & Holland, 2007; Holland & Gallagher, 2003; Weingarten, 1983; Zamble,

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1973). Similar findings have been reported in human subjects (Birch *et al.*, 1989; Cornell, Rodin & Weingarten, 1989; Fedoroff, Polivy & Herman, 2003).

Here, we examined the effects on consumption of food-related cues and of stimuli that signaled the interruption of those cues and the termination of food availability. From a perspective of simple excitation-inhibition theories of learning (Rescorla, 1969; Sergeev, 1961), if a stimulus associated with the delivery of a food meal provokes eating in sated rats, then a signal for termination of that meal might be expected to suppress consumption. However, from an ecological perspective, cues that predict meal termination and subsequent food-specific scarcity, or general caloric deficits, might be expected to especially encourage rapid food consumption, to compensate for the imminent disappearance of food (*see* Urbszat, Herman & Polivy, 2002).

Over generations, the ability to prepare for food-scarce times or environments by anticipatory increases in food consumption near the ends of periods of abundance is likely to be a highly adaptive response, which would contribute to survival in subsequent periods of scarcity. In the life of an individual, repeated experiences of scarcity may put in place allostatic (Schulkin, 2003; Sterling, 2004), feed-forward mechanisms that take into account future negative energy balances (*e.g.* Schaller, 1972, *also see* Woods, 1991). Thus, current motivation to eat would reflect the integration of both current energy status and projected metabolic needs.

Experiment 1 tested the role of predicted meal termination on food consumption in sated rats. Several studies have shown that surprising reward omission invigorates appetitive behavior (Amsel, 1958; Stout *et al.*, 2002; *but see* Daly, 1974) but to our knowledge its effects on consummatory behaviors has not yet been explored. Thus, Experiment 1 was designed to explore the effects of cues paired with the surprising interruption of a “meal” (*i.e.*, 2-min food trials) while rats were food-deprived, on their food consumption later, when they were sated. In this study, during the training phase, rats received food while an auditory cue (CS+) was presented. Some of these CS+ trials included another cue (IS) that interrupted both CS+ and food presentation until the next trial. Testing consisted of presenting these cues while sated rats were eating. Both CS+ and IS were found to enhance food consumption. In Experiment 2 we replicated the basic findings of Experiment 1, and evaluated the relative importance of food termination and CS+ termination on the development of the consumption-enhancing capacity of IS.

Experiment 1

Materials and methods

Subjects—The subjects were 16 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 eight 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 approximately 1.7 ml, was placed in a recessed food receptacle in the center the front wall. A photocell beam in the food receptacle 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 18 cm 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 digitized, and were recorded and displayed in real time on four video monitors, each of which showed 4 chambers or food cups.

Pavlovian conditioning – Phase I—Rats first received two 60-min sessions designed to train them to approach the food cup and consume the food US. Each of these sessions included 16 0.1-ml deliveries of an 8% sucrose solution, which served as the US. 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 the CS-US Pavlovian association. Each of these included 10 CS+ trials. The CS+ consisted of either a 78-dB, 1500-hz intermittent tone or an 80-dB white noise, and was 2 min in duration. Four USs were delivered at random times (VT30) within each CS+ presentation (top portion of Figure 1).

Pavlovian conditioning – Phase II—In the second phase of training, a second 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 USs. With IS onset, the CS+ terminated and no further USs were delivered on that trial. The IS occurred at random times between 30-s and 90-s after CS+ trial onset, and was always presented 10-s after the last US delivery (see bottom portion of Figure 1 for examples). During interrupted trials, US delivery density during CS+ was maintained (VT30s). Thus, in every interrupted trial, rats had access to one, two or three US deliveries. Phase 2 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 2 9-min potentiated feeding tests in the experimental chambers. In these tests, consumption of food was examined in the presence of one cue (CS+ or IS; cued session) and in the absence of any cue (empty session). In this way, all the rats were tested twice: half of them (CS+ group) were tested with the CS+ and with no cues, and the other half (IS group) were tested with the IS and with no CS. The empty test session provided an index of the timing of spontaneous feeding in the absence of discrete Pavlovian cues. Because our primary interest was in comparing consumption during IS and CS+ presentations with responding in the ITIs, we administered the cued test first and the empty test second in all rats. Thus, the results of the cued test would not be influenced by prior exposure to the test situation.

In the first two min of each test session, rats had unlimited access to the test US solution without any cue presentation. The purpose of this *pretest* was to reduce overall consumption of the test US to permit a more sensitive assessment of the effects of the various cue test conditions later. Previous studies (Holland & Gallagher, 2003; Holland, Petrovich & Gallagher, 2002) showed that chow-sated rats consume substantial amounts of more palatable USs when first returned to the experimental context. In the second, *test*, portion of each consumption session, the rats had free access to the US while seven 20-s cues were presented, separated by 40-s intervals. During consumption test 1, each rat was presented with only one type of stimulus (IS or CS+). The first consumption test was a cued session for all rats; half of the rats received IS presentations during this session and half received CS+ presentations. The second consumption test was an empty test which consisted of 7 min of US presentation with no discrete-cue presentations. However, consumption was recorded in 20-s dummy “cue” and 40-s “ITI” periods, as in the cued test. The timing of these periods was yoked to the cue and ITI periods of the cued test.

In all tests, the food cups were filled with 1.7 ml of 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. To reduce chance of experimenter bias, the experimenter was blind to test periods (cue, ITI). Cues were presented in sound isolated chambers and the experimenter delivered food with a computer from a different room, from which the cues were not audible. In addition, the experimenter wore noise cancelling headphones while listening to music.

Results

Pavlovian conditioning – Phase I—To evaluate learned food-cup responding we examined the time spent in the food cup during the periods 10-s prior to CS+ trials (*ITI*) and during the first 10-s of CS+ presentations, on trials that did not include US delivery in that period. The amount of time spent in the food cup during each such period was divided by the total duration of those periods to form the measure percentage time in food cup. These data were subjected to a 2-way analysis of variance (ANOVA), with period (CS+ or ITI) and sessions (1-6) as variables.

The rats showed normal learning of food-cup responses to the CS+, spending more time in the food cup when the CS+ ($49.9 \pm 1.8\%$) was presented than during the ITIs ($25.8 \pm 1.6\%$; $F(1,15) = 123.12$, $p < 0.001$). This elevation of food cup responding during CS+ became more evident as training progressed (period X sessions $F(5,75) = 9.01$, $p < 0.001$).

Pavlovian conditioning – Phase II—After CS+ training, the rats received 15 sessions of training with both CS+ and the IS. Data was grouped in five 3-session blocks. ANOVA showed a significant effect of period ($F(2,30) = 79.85$, $p < 0.001$), and subsequent orthogonal contrasts confirmed that ITI ($18.92 \pm 2.9\%$) responding was lower than responding during CS+ ($62.64 \pm 4.7\%$) or IS ($56.93 \pm 5.1\%$) presentations ($F(1,15) = 131.48$, $p < 0.001$), which did not differ significantly ($F(1,15) = 2.87$, $p > 0.110$). The failure to find a CS+/IS difference was not surprising, since generally when IS was presented rats' heads were already in the food cup. Thus, during training, behavior during IS presentations was not particularly informative.

Cue-potentiated feeding tests—Figure 2 shows the primary results of Experiment 1, sucrose consumption in the cue (top two panels) and empty (bottom panel) consumption test sessions. The left portion of each panel shows consumption rates during the initial pretest periods, and during each cue and ITI period in the test segment of the session. The bars on the right portion of each panel show consumption during the cue and ITI periods, averaged over the last half of the test session. Consumption rates were evaluated with a 4-way ANOVA, with variables of group (tested with CS+ or IS), test type (cued or empty session), period within the session (cue or ITI), and half-session blocks.

Not surprisingly, consumption declined over the course of testing ($F(1,14) = 72.19$, $p < 0.001$). More important, consumption was greater during the cue tests (top two panels) than during the empty test (bottom panel; $F(1,14) = 23.14$; $p < 0.001$). Furthermore, during cue tests, the rats ate more during cue presentations than during ITIs, whereas during the empty session, there was no such pattern, when corresponding dummy “cue” and “ITI” periods were compared (test type X period $F(1,14) = 7.76$, $p = 0.015$). Thus, both CS+ and IS presentations enhanced sucrose consumption, in a highly phasic manner.

Although the CS and IS groups did not differ in their total test consumption ($F(1,14) = 2.09$; $p = 0.170$), the distribution of consumption across cue and ITI periods in the CS+ and IS test conditions differed as the test session progressed. Specifically, although in the group tested with CS+, consumption was greater during the cue periods than during ITI periods throughout

the session, the rats tested with IS showed a more complex consumption pattern. For those rats, consumption was initially slightly greater during ITIs than during IS presentations, but by the second half of the session it was greater during IS periods than during the ITIs (group X test type X period X test half $F(1,14)=11.24$, $p=0.005$). Because four-way interactions are often difficult to interpret, we then analyzed the data from each group separately. The test type $\times$ period $\times$ half session block interaction was significant for the group tested with IS ($F(1,7)=12.39$, $p=0.010$), but not for the group tested with CS+ ($F(1,7)=0.94$, $p=0.364$). Furthermore, during the cued test the period X test half interaction was significant for the IS group ($F(1,7)=5.795$, $p=0.047$), but not for the CS+ group ($F(1,7)=0.145$, $p=0.715$). Thus, these additional analyses confirm our original impression.

Discussion

Sated rats showed elevated food consumption when they were exposed to a cue (CS+) that had previously been paired with food, and to one associated with CS+ trial interruption (IS). Moreover, this increase was temporally dependent on cue presentations, that is, the rats consumed more food when the cues were present than during ITIs. With CS+, this pattern was evident from the beginning of the test, whereas with IS, it was not apparent until the second half of the test session. Thus, consumption was enhanced by cues correlated with either the availability or impending meal termination.

By contrast, other evidence suggested that only CS+ evoked conditioned food cup approach and entry in the absence of food. Although in Phase 2 Pavlovian training, the rats spent comparable amounts of time in the food cup during CS+ and IS, in that phase the rats were already in the food cup whenever the brief IS was presented, thus biasing measures of responding to IS. After this experiment was completed, we examined food cup responding in these rats during CS+ and IS when each was presented independently, in the context of a Pavlovian-to-Instrumental (PIT) experiment. After the rats were trained to lever press for the same food reward, they were given a test in which the CS and IS were presented while the rats were lever-pressing in extinction. Although we found no significant evidence for enhanced lever pressing (PIT) with either stimulus, the rats spent increased time in the food cup during CS+ ($57.9 \pm 5.7\%$), but not during IS ($18.6 \pm 3.4\%$), compared to the time spent in the food cup during no-stimulus periods ($14.4 \pm 2.2\%$).

In Experiment 1, the effects of CS+ and IS on feeding were assessed by comparing consumption in the presence of the cue with consumption in the absence of the cue. It is possible that this cue-enhanced feeding did not in fact depend on learning about those cues, but their mere presence. Although in previous studies of cue-potentiated feeding (Holland & Gallagher, 2003; Holland, Petrovich & Gallagher, 2002) we found little evidence of potentiation by cues that were not previously paired with food, the training and testing methods of this experiment were different from those of past studies. Thus, Experiment 2 included a control stimulus in a design similar to that of Experiment 1.

Experiment 2

This study was designed to replicate Experiment 1 and to provide better understanding of the critical IS/CS+ and IS/US relationships needed in the acquisition of IS's capacity to enhance food consumption. In Experiment 1, IS signaled both the interruption of the CS+ and the cancellation of subsequent food USs. Experiment 2 examined the effects of each of these contingencies separately, and together, by using a design reminiscent of that used by Kamin (1956) to examine the contributions of warning signal termination and shock cancellation to avoidance learning. In four separate groups, the IS interrupted both CS+ and US presentations (as in Experiment 1), only the CS+, only the US, or neither (in that case, the IS was presented separately, as a CS-.) After the consumption tests, food-cup responding to IS and other cues

was examined in the absence of food in an extinction test and a reinforcement test, to explore further the nature of learning about the IS in each training condition. Both these tests evaluated the excitatory and/or inhibitory nature of learning about the IS.

Materials and methods

Subjects and apparatus—Thirty-two 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 for this experiment. Housing, lighting and feeding of the rats, and the apparatus used were the same as in Experiment 1.

Pavlovian conditioning – Phase I—All rats received two initial 60-min food-cup training sessions followed by 7 Pavlovian CS+ training sessions, identical to those described in the *Pavlovian conditioning – Phase I* section of Experiment 1 (see the top portion of Figure 3 for a schematic of CS+ trials.)

Pavlovian conditioning – Phase II—In the second phase of training, rats were separated into 4 groups (ns=8), matched for body weight. In this training stage, all groups received fourteen sessions, each of which contained 8 CS+ trials with an inter-trial interval (ITI) of 266 s. During this stage a new auditory cue, either the tone or white noise, was introduced as an interruption signal (IS) or as a control cue (CS-). The bottom portion of Figure 3 shows a schematic example of IS or CS- trials for each group. In each session, rats in group CsUs-I (CS and US interrupted) were presented with two 2-min CS+ trials, as in phase I. During six other CS+ trials, however, the IS was presented. These interrupted trials were identical to those used in Experiment 1: IS interrupted both the presentation of the CS+ and delivery of USs. With IS onset, the CS+ terminated and no further USs were delivered on that trial. The IS occurred at random times between 30-s and 90-s after CS+ trial onset, and was always presented 10-s after the last US delivery. During interrupted trials, US delivery density during CS+ was maintained (VT30s). Thus, in every interrupted trial, rats had access to one, two or three US deliveries. Training conditions for group Cs-I (only CS interrupted) were identical to those of group CsUs-I except that during the interrupted trials, reward density was modified in order to always deliver 4 USs on every trial. In this way, US density ranged from VT17 to VT30. The rats in Group Us-I (only US interrupted) were exposed to training procedures similar to those of group CsUs-I, except that after IS offset, CS+ presentation resumed, so that there was a total of 2 min of CS+ presentation on each trial. However, during this second CS+ interval no USs were delivered. Finally, CS- (unpaired control) rats were exposed to eight 2-min CS+ trials during each of which they received 4 USs. Additionally, eight 10-sec CS- unreinforced trials were presented in the ITI after each CS+ trial. CS- onset always occurred at each ITI's midpoint (~133s). Finally, note that the differing contingencies of each group resulted in variations in the numbers of USs and the total duration of the CS+ presentation (see table 1).

Cue-potentiated feeding tests—Immediately after the last Phase II Pavlovian training session, rats were given free access to lab chow in their home cages for a week. Next, on consecutive days, sated rats received three 9-min tests in which sucrose consumption was examined. Because our primary interest was in the effects of IS, all rats were tested with that stimulus (or, in Group CS-, the CS-) 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. Each of these tests was identical to the comparable tests of Experiment 1.

Extinction test—The rats were then re-deprived and given two Phase II Pavlovian retraining sessions, followed by an extinction test. This test was designed to determine if IS had excitatory

or inhibitory powers over conditioned food cup responding. After a 160-s habituation period, all rats were given 3 20-s presentations each of CS+, IS/CS-, and the compound stimulus, in counterbalanced order, separated by 40-s intervals. If IS enhanced consumption because it had excitatory associations with sucrose, then it should control substantial responding when presented alone or in compound with CS+. If IS developed inhibitory associations with sucrose, then it should reduce responding to the CS+ when presented in compound with that cue. No food was presented in this test.

Reinforcement test—Finally, all rats received three 60-min 16-trial Pavlovian sessions during which 10-s presentations of the cue previously used as IS (or CS-) was paired with the US. The US was presented during the last second of each cue presentation. Reinforcement tests are frequently used to assess prior excitatory or inhibitory learning about cue (e.g., Rescorla, 1969). Prior excitatory or inhibitory learning would be reflected as savings or retardation, respectively, of IS-sucrose learning.

Results

Pavlovian conditioning – Phase I—All rats learned to respond to the CS+. As in Experiment 1, we evaluated learned responding by examining responding during CSs prior to the delivery of the first US on any trial. Times spent during 10-s CS and ITI periods were analyzed using a 3-way ANOVA which included groups, periods, and sessions as variables. Overall, the rats spent more time in the food cup when the CS+ was presented than during ITIs ($40.6 \pm 1.0\%$, and $15.0 \pm 1.0\%$, respectively; $F(1,28)=324.94$, $p<0.001$). Responding to the CS+ increased over sessions (+23.6%) while ITI behavior remained relatively constant (-3.1%; period X sessions $F(6,168)=18.02$, $p<0.001$). There were no significant main effects or interactions involving groups ($F_s<1.91$, $p_s>0.152$).

Pavlovian conditioning – Phase II—Rats in all groups maintained high levels of responding during the CS+ and IS cues, relative to the ITI periods, whereas responding during the control cue in group CS- was substantially lower than responding to the IS in the other groups (Figure 4). A 3-way ANOVA with variables of group, period (CS+, IS or ITI), and session block showed a main effect of period, ($F(2,56)=159.70$, $p<0.001$), and a significant group X period interaction, ($F(6,56)=7.90$, $p<0.001$). Only responding to IS/CS- differed significantly across groups (other $F_s<1.10$, $p_s>0.359$); a post-hoc Scheffé test showed that responding to the control cue in Group CS- was significantly less than responding to the IS in the other 3 groups ($p_s<0.002$), which did not differ ($p_s>0.839$).

Consumption tests—Figure 5 and Table 2 show the primary data of Experiment 2, the results of the three consumption tests. Figure 5 summarizes responding during cue and ITI periods for each group during the last half of each consumption test, as in the bars in the right portions of each panel in Figure 2. Table 2 provides the data from the first half of each test. Consumption rates were analyzed in a separate 3-way ANOVA for each test session, with variables of group, period (cue or ITI) and test half (first or second), followed by separate 2-way ANOVAs for each group.

Consider first consumption in the first, IS/CS- test session (Figure 5a; top portion of Table 2). The IS enhanced food consumption only in the groups in which IS was associated with CS+ termination (Groups CsUs-I and CS-I). As in Experiment 1, the pattern of greater consumption during IS than in the ITI emerged over the course of the session; the period X test half interaction was significant overall ($F(1,28)=7.55$, $p = 0.010$) and in Group CsUs-I alone ($F(1,7)=20.328$, $p=0.003$), but not Groups CS- or Groups US-I, $F_s<1.845$, $p_s>0.217$. Notably, that interaction was not significant in Group Cs-I ($F(1,7)=1.307$, $p=0.29$) because the IS began enhancing eating earlier in the session in that Group (Table 2).

In the second half of the test session (Figure 5a), the ability of the IS (or CS-) to enhance responding over ITI levels differed substantially across groups; the period X groups interaction was significant, $F(3,28) = 4.06$, $p = 0.016$. Post-hoc comparisons showed that the IS significantly enhanced consumption over ITI levels in Groups CsUs-I (6.25 ± 0.83 deliveries/min, $p < 0.0009$) and Cs-I (7.15 ± 2.45 deliveries/min, $p < 0.023$) but not in Groups CS- (0.56 ± 0.83 deliveries/min, $p = 0.52$) or Us-I (2.94 ± 1.30 deliveries/min, $p = 0.058$). A contrast of the elevation produced by IS in the former two groups vs. that in the latter two groups was likewise significant ($F(1,28)=10.76$, $p < 0.003$); the remaining contrasts orthogonal to that one were all nonsignificant ($ps > 0.273$).

This pattern of consumption in the IS test across the groups was even more obvious when eating during IS/CS- was considered independently of ITI consumption. In this case, the main effect of groups was significant ($F(3,28)=6.92$, $p=0.001$), as was the contrast of Groups CsUs-I and CS-I vs. CS- and US-I, ($F(1,28)=18.12$, $p<0.001$). Consumption during the ITIs did not differ significantly across the groups ($F(3,28)=1.96$, $p=0.143$), although it followed the same pattern as consumption during IS/CS- presentations.

Next, consider consumption during the third test, which included alternating CS+ and ITI periods (Figure 5c). The rats in all groups consumed substantially more during CS+ periods than during ITI periods, throughout the test session. Furthermore, unlike in the IS/CS- test, there was no evidence for between-group differences in the ability of the CS+ to enhance eating. The main effect of periods was significant ($F(1,28)=162.13$, $p<0.001$), whereas the main effect of group ($F(3,28)=0.84$, $p=0.484$), and the group X period ($F(1,28)=0.54$, $p=0.659$) and period X test half interactions ($F(1,7)=0.26$, $p=0.611$) were all insignificant. Consumption declined over the course of the CS+ session ($F(1,28)=31.62$, $p<0.001$).

Finally, Figure 5b shows consumption during the second, empty, test, in which no cues were presented. There were no significant effects or interactions involving the dummy period variable, ($F_s < 1.04$, $ps > 0.319$), nor were there any significant effects or interactions involving group ($F_s < 1.28$, $ps > 0.300$). As in the other tests, consumption declined across the two halves of the session ($F(1,28)=119.09$, $p<0.001$).

Pre-test consumption did not differ across groups in any test session, $F_s < 1$.

Extinction test—Although CS+ and IS both enhanced consumption in Groups CsUs-I and Cs-I, those stimuli had very different effects on food cup approach and entry in the extinction test. Whereas CS+ produced substantial food cup entry, IS did not (Figure 6); indeed, in Group Cs-I, IS suppressed food cup responding relative to baseline (ITI) responding. A groups X stimulus (ITI, CS+, CS-/IS, and compound stimulus) ANOVA of food cup responding during the extinction test showed a significant effect of stimulus ($F(3,84)=106.28$, $p<0.001$), and a marginal group X stimulus interaction ($F(9, 84) = 1.82$, $p = 0.077$). Subsequent comparisons showed that responding to CS+ was greater than during the ITI in all groups, $F_s(1,7) > 23.49$, $ps < 0.002$. By contrast, IS/CS- responding did not differ from ITI responding in any but Group Cs-I; indeed, in that group, responding to IS was significantly lower than ITI responding, $F(1, 7) = 69.70$, $p < 0.001$; other $F_s < 1.32$, $ps > 0.289$. Similarly, presentation of IS/CS in compound with CS+ reduced food cup responding, relative to CS+ alone responding, in all groups, $F_s > 9.16$, $ps < 0.016$.

Reinforcement test—Figure 7 shows the results of the reinforcement test, in which the IS or CS- was paired directly with food. Acquisition of food cup responding was more rapid in the two groups in which the IS had been correlated with a reduction in the density of food delivery (groups CsUs-I and Us-I). A groups X half-session blocks sessions ANOVA showed a significant main effect of blocks ($F(5,140)=71.51$, $p<0.001$), and a significant interaction of

groups with the quadratic trend over blocks, $F(3,28) = 3.03$, $p = 0.046$. A post-hoc contrast of this trend in Groups CsUs-I and Us-I versus that in Groups Cs- and Cs-I (the two groups in which the IS/CS had not signaled a reduction in US density) was also significant, $F(1,30) = 7.95$, $p = 0.008$.

Discussion

As in Experiment 1, CS+ elevated consumption relative to ITI levels in all groups. By contrast, the IS significantly enhanced consumption only in Groups CsUs-I and Cs-I. In both of these groups, IS signaled termination of CS+. Thus, IS augmented eating in test only when it had been linked to a reduction in CS+ duration in training. Although the observation that the control cue in Group Cs- had no effect on consumption was expected, it was surprising that IS also had no significant effect on consumption in Group Us-I. In that group, although IS reduced the number of US deliveries in the same way as in Group CsUs-I, it did not interrupt CS+. Although this lack of a significant enhancement of consumption by IS in Group Us-I might be viewed with some caution because that group also showed (nonsignificantly) less enhancement by CS+, it is notable that the enhancement of eating by IS was no greater in Group CsUs-I, in which both CS and US presentations were interrupted, than in Group Cs-I, in which only CS presentations were interrupted. These results indicate that the IS's orexigenic powers were derived more from its relationship with the CS+ than from its linkage to the reduction in frequency or amount of the food US itself. It is also possible that the IS's powers depended on its ability to signal both the imminent termination of CS+ and the cancellation of future USs; although the IS in Group Cs-I did not signal an overall reduction in the frequency or number of food USs in any trial, it did indicate that no additional USs would be presented on a trial. However, because reductions in overall US frequency or number were irrelevant to the ability of the IS to instigate food consumption, we suspect that it is the IS's signaling of CS termination alone that is critical.

It is noteworthy that in the present study, the consummatory powers of IS derived from CS+ could not be attributed to simple acquisition of excitatory strength, comparable to that acquired to CS+. First, it is unlikely that direct excitatory IS-food associations were formed in any group, because the IS was presented at least 10-s after the last food presentation in all groups; except after small amounts of training, backwards excitatory conditioning is uncommon. Second, IS's powers to enhance consumption were unrelated to any opportunity for it to acquire excitatory strength via second-order associations with CS+. In the two groups in which IS showed orexigenic powers, IS occurred only in a backward relation with CS+, and IS failed to significantly enhance consumption in the only group that included forward pairings of IS and CS+ (Group Us-I). Third, and most important, the results of the extinction test, in which the various cues were presented in the absence of any foods, show no evidence that IS possessed excitatory powers. If anything, those results showed inhibition: all groups showed less responding to a compound of CS+ and IS, and in Group Cs-I, IS responding was even less than baseline ITI responding. Finally, if IS were especially excitatory in Groups CsUs-I and Cs-I, one would expect faster acquisition in the final reinforcement tests in those groups. Instead, we observed faster acquisition in Groups CsUs-I and Us-I. Thus, the effects of the IS/CS- signals on food consumption in the various groups were not correlated with those groups' food cup responding in either the extinction or reinforcement tests.

From the perspective of theories such as that of Rescorla and Wagner (1972), it is puzzling that Groups CsUs-I and Us-I showed faster, rather than slower acquisition than the other groups. Within that model, signals for the reduction of US frequency should acquire inhibitory tendencies, and hence be slow to acquire excitation (Rescorla, 1969). By contrast, another model of associative learning, that of Pearce and Hall (1980), posits that unpredictable reinforcer contingencies maintain attention to CSs at higher levels, and hence permit more

rapid subsequent learning, despite prior acquisition of inhibitory tendencies. Notably, in both Groups CsUs-I and Us-I, expectations about US delivery during training were violated on trials that contained IS; whereas CS+ was accompanied by 4 US presentations on CS+ alone trials, fewer US presentations occurred on trials that included both CS+ and IS. By contrast, in Groups CS- and Cs-I, no such violations of expectancy occurred. In Group CS-, the IS/CS- was presented alone, in the absence of any CS+ to generate a US expectancy, and in Group CS-I, 4 USs were presented on both CS+ alone and CS+/IS trials.

Although Experiment 2 distinguished training procedures that established orexigenic powers to an IS and those that did not, much remains to be learned about the conditions for establishing such powers to an IS. For example, although Experiment 2 showed that an IS's orexigenic powers are not related to its acquisition of excitatory appetitive CRs (e.g., food cup entry), might they be related to the acquisition of conditioned inhibition? Would a standard conditioned inhibition procedure, in which on some trials CS+ alone is paired with food and on other trials, a compound of CS+ and another stimulus is presented entirely in the absence of food, establish orexigenic properties to that stimulus? Or is the presentation of food itself on CS+/IS trials (as in the present experiments) critical to an IS's acquisition of orexigenic powers? It might be argued that it is the termination of consummatory responding during CS+ rather than the simple termination of CS+ in the absence of consumption that endows IS with the ability to enhance eating later. To what extent are the IS effects observed here dependent on our use of a CS+ training procedure in which food USs were presented randomly throughout the CS period, rather than solely at the end of a CS (e.g., Weingarten, 1983; Zamble, 1973)? Although this training procedure has been widely used in the study of the role of CS-US contingency or correlation (Rescorla, 1968) and in the acquisition of motivational properties to CS+s in Pavlovian-instrumental transfer (Blundell, Hall & Killcross, 2001; Crombag, Galarce & Holland, 2008; Dickinson, Smith & Mireniewicz, 2000; Galarce, Crombag & Holland, 2007; Hall *et al.*, 2001) and a previous study showed that both training methods endow a CS+ with the ability to potentiate feeding (Holland & Gallagher, 2003), we have only begun the investigation of the conditions under which an IS may acquire such powers.

General Discussion

In two experiments, food-sated rats consumed more of a food US when it was accompanied by either a cue (CS+) that previously predicted delivery of that food or a cue (IS) that interrupted CS+ trials. Both cues exhibited strong stimulus control over eating within test sessions, with consumption significantly higher during cue periods than during ITI periods. However, in both experiments, although the CS+ enhanced eating from the beginning of the consumption test, IS's control over eating emerged over the course of that test. Although in Experiment 2, it might be argued that this difference was confounded with test order (IS testing always preceded CS+ testing), it is notable that the same difference occurred in Experiment 1, in which separate groups of rats were tested with IS and CS+. This emergence of feeding control by IS may reflect the combination of IS's facilitatory effects on consumption and its inhibitory effects on anticipatory appetitive behaviors such as rate of food cup entries, percentage of time spent in the food cup and latency to approach the food cup. To the extent the training procedures established IS as a signal of no food, that cue would initially reduce the likelihood of food cup approach and perhaps even encourage withdrawal from the food cup. However, as the rats discovered food was constantly available in the food cup, they consumed it in fast and short bouts, as if attempting to maximize intake before the predicted CS+ trial interruption took place. Finally, the results of Experiment 2 revealed important training variables that influenced an interruption cue's ability to potentiate feeding. First, a cue that occurred apart from CS+ trials in training (CS-) had no effects on test consumption, showing that IS's ability to enhance eating depended on its prior relationship with CS+ trials. Second, with the training parameters

used here, IS's interruption of the food cue (CS+) was more important than its relation to reductions in the frequency/amount of food in determining IS-potentiated eating.

Why does a cue paired with meal interruption elicit food consumption? Within a simple excitation-inhibition learning theory framework, one might expect that if a signal for food availability enhances feeding, then a signal for meal termination might suppress feeding. However, a more adaptive, allostatic, response to foreknowledge of impending meal termination, while in the presence of food, would be to consume more. Thus, the IS may affect food consumption by triggering anticipatory behaviors that minimize the impact of future scarcity. At the broadest level, this claim is consistent with findings showing the development of anticipatory intake of foods that are repeatedly ingested before long fasting intervals (Jarvandi, Booth & Thibault, 2007), and observations of animals augmenting their feeding behaviors in preparation for seasonal scarcity (Stalmaster & Gessaman, 1984).

At first glance, such a view seems incompatible with our observation in Experiment 2 that the orexigenic effects of IS were more related to its relation with CS+ termination than to reductions in the frequency or amount of food itself. However, the local mechanism by which an IS acquires orexigenic powers need not reflect the ultimate adaptive consequences of that mechanism. In natural situations, food- and food-related cues are often confounded. This confound is especially obvious when considering the distal properties of food itself (e.g., sights, smells, and perhaps sounds), which are often essential to guiding appetitive and consummatory behaviors. A food is fully appreciated as such if it is accompanied by the usual conditioned cues, and not others. We are more inclined to eat a piece of meat that is red and not green, or a peach that emanates its characteristic perfume over an odorless one. Thus, interruption of food cues may often be confounded with interruptions in the availability of food itself. Indeed, under the conditions of food delivery used in training in our studies, in which food cup licking responses may continue between sucrose deliveries during CS presentations, CS+ duration may have served as a convenient stand-in for meal size, thus linking IS to a reduction in perceived meal size in both Groups Cs-Us-I and Cs-I, but not Us-I or CS-.

It may be instructive to consider our results within models from other disciplines. For example, commodity theory provides accounts for how messages that convey predicted unavailability, in the form of scarcity, effort, restriction or delay, can increase the perceived value of a determined item (Brock, 1968). Consistent with this theory are Friedman *et al's* (1968) findings which showed that rats preferred foods originally found in a hard-to-get location rather than a comparable food that was situated in an easily-accessible place. Our results might also be described within Brehm's (1966) reactance theory, which states that unavailability of an object is perceived as a threat, and the value of the object when present increases due to an emotional reaction of "survival value". Although these conceptual models were posed in the context of human communication and economics, many of their empirical predictions are supported by studies that examined effects of the unavailability of food (*i.e.* West, 1975; Worchel, Lee & Adewole, 1975, *also see* Collier, 1989; Marsh, Schuck-Paim & Kacelnik, 2004; Rowland *et al.*, 2008).

Scarcity and abundance may interact in many ways to alter food consumption. Prior caloric deprivation can enhance subsequent and posterior increased appetitive and consummatory behaviors towards food. For example, Polivy *et al* (1994) showed that war veterans who faced extensive food deprivation in prison camps were more likely to engage in binge eating later than veterans who were never kept captive. Furthermore, food-related behaviors may be augmented by scarcity even without such general caloric restriction. Restricting access to a single palatable food increases normal children's responses and intake of that food item (Fisher & Birch, 1999). Similarly, Polivy *et al* (2005) deprived undergraduate students from chocolate and found that restrained eaters increased chocolate consumption when compared to non-

restrained eaters. Lastly, the mere threat of unavailability of a food item can induce increased consumption of that item (Urbszat, Herman & Polivy, 2002). All in all, external cues that signal future scarcity have the power to induce binge eating under many conditions.

Given the increasing prevalence of eating disorders, it is of utmost importance to identify the critical risk factors for the development of overconsumption. The experiments described in this paper provide a novel animal model of binge-like behaviors in sated rats induced by external cues paired with meal interruption. We believe that this line of work will further the understanding of the psychological processes involved in binge-like behaviors.

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Figure 1. schematizes Experiment 1 training procedures. The upper panel shows a typical CS+ trial, which were presented throughout training phases I and II. CS+ trials consisted of a 2-min auditory stimulus during which four US deliveries of 0.1ml where presented at random times (VT30). The lower panel depicts interruption signal (IS) trials, presented only in phase II. During these trials, a new 10-s auditory stimulus, IS, interrupted both the presentation of the CS+ and delivery of USs. With IS onset, the CS+ terminated and no further USs were delivered on that trial. During IS trials, US delivery density (VT30) was maintained.

Figure 2.

shows food consumption during Experiment 1 consumption tests. Figure 3a) and 3b) depict consumption during the cue test for IS and CS+ groups, respectively. Figure 3c) shows consumption data during the empty test, collapsed across both groups. On the left, line graphs show food consumption (y-axis) during the different test events (x-axis; cued intervals=dark markers; ITIs=white markers). On the right, bar graphs condense consumption test data from the second half of the test (cued intervals=dark bars; ITIs=white bars). Dark bars reflect consumption during cues (dummy cues during the empty test), while white bars during ITIs. All graphs display the mean rates of food deliveries needed to replenish the liquid cups (*see text*). Error bars represent SEM (*: $p < 0.05$; *n.s.*: no statistical significance).

Training: CS+ trials

Training: IS/CS- trials

Figure 3.

schematizes Experiment 2 training procedures. The upper panel shows a typical CS+ trial, which were presented throughout training (both during phase I and II). CS+ trials consisted of a 2-min auditory stimulus during which four US deliveries of 0.1ml were presented at random times (VT30). The lower panel depicts interruption signal (IS) trials for the different groups: **1)** the CsUs-I group received IS trials identical to those used in Experiment 1. That is, IS interrupted both the presentation of the CS+ and delivery of USs. In this group US delivery density (VT30) was maintained; **2)** stimuli for rats in the CS-I group were identical to those in the CsUs-I group. In this group, however, rats always received 4 US deliveries per trial, regardless of CS+ length; **3)** rats in Group Us-I were exposed to training procedures similar to those of group CsUs-I, except that after IS offset, CS+ presentation resumed, so that there was a total of 2 min of CS+ presentation on each trial. However, during this second CS+ interval no USs were delivered; **4)** CS- rats were exposed to eight 2-min CS+ trials during each of which they received 4 USs. Additionally, eight 10-sec CS- unreinforced trials were presented in the ITI after each CS+ trial.

Figure 4.

shows appetitive responding in Experiment 2 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 CS- or the interruption stimulus (IS). Data sets reflect mean responding for each group: CsUs-I (grey circles); Cs-I (dark circles); Us-I (grey squares), and Cs- (dark squares). Error bars represent $\pm$ SEM.

Figure 5. shows food consumption during the second half of Experiment 2 consumption tests: **a)** depicts consumption during the IS or CS- 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 (*: $p < 0.05$; †: $p = 0.058$; n.s.: no statistical significance).

Figure 6. reflects appetitive behavior during the extinction test. Mean percentages of time spent in the food cup were measured during 20-s corresponding to ITIs (plain dark bars), CS+ (plain white bars), CS-/IS (striped dark bars), and during presentation of a CS+/CS- or CS+/IS compound (striped white bars). Error bars represent SEM.

Figure 7.

represents food cup behavior during the reinforcement test IS/CS- trials. Each data point shows mean responding over eight consecutive trials: CsUs-I (grey circles); Cs-I (dark circles); Us-I (grey squares), and CS- (dark squares). Error bars represent $\pm$ SEM.

shows Experiment 2 Phase II training parameters. Columns represent groups and rows different conditions or parameters. The design of the *normal trials* (i.e. uninterrupted) trials did not differ between groups. All groups received 2 normal trials except the CS- group, which received 8. The other groups were also presented with interrupted trials which were different for each group. Note that animals in the CsUs-I group received the same amount of reward as the Us-I group, but were exposed to the same CS+ time as the Cs-I group. US density refers to the average time elapsed between reward deliveries.

Table 1

	CsUs-I		Cs-I	Us-I	Cs-
Normal trials	# trials	2	2	2	8
	duration	120s	120s	120s	120s
	US density	30s	30s	30s	30s
	Σ time	240s	240s	240s	960s
	Σ rewards	8	8	8	32
Interrupted trials	# trials	6	6	6	0
	duration	30-90s	30-90s	120s	0s
	US density	30s	17.5s	30s	0s
	Σ time	420s	420s	720s	0s
	Σ rewards	14	24	14	0
Totals	Σ trials	8	8	8	8
	Σ time	660s	660s	960s	960s
	Σ rewards	22	32	22	32

Table 2
shows the results of Experiment 2 consumption tests: *a*) IS test; *b*) during empty test; and *c*) CS+ test. Data is 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*).

<i>a</i>) IS test:		1 st half		2 nd half	
group	CsUs-I	cue	iti	cue	iti
	mean	11.6	11.6	11.8	5.5
	sem	1.23	1.02	1.67	1.1
	Cs-I	mean	16.9	14.9	7.8
	sem	1.49	1.46	1.89	1.19
group	Us-I	mean	12.8	7.4	4.4
	sem	1.24	1.02	1.76	0.87
	Cs-	mean	10.3	5.3	4.7
	sem	1.13	1.42	1.27	0.87
<i>b</i>) Empty test:		1 st half		2 nd half	
group	CsUs-I	cue	iti	cue	iti
	mean	14.8	14.4	7.1	5.8
	sem	1.5	1.42	1.3	1.02
	Cs-I	mean	13.1	8.5	5.6
	sem	1.21	0.95	1.66	0.91
group	Us-I	mean	12.8	8.1	7.8
	sem	1.42	1.37	1.62	1.09
	Cs-	mean	12.3	6.4	6.6
	sem	0.99	1.34	1.26	1.08
<i>c</i>) CS+ test:		1 st half		2 nd half	
group	CsUs-I	cue	iti	cue	iti
	mean	17.1	10	16.4	3.3
	sem	1.75	1.63	2.6	0.94
	Cs-I	mean	20.3	13.8	5
	sem	1.17	1.34	1.81	1.07
group	Us-I	mean	19	10.9	6.7
	sem	2.08	1.47	1.96	1.25

<i>a</i>) IS test:	1 st half		2 nd half	
	cue	iti	cue	iti
Cs-	mean	6.6	11.4	2.8
	<i>sem</i>	<i>1.32</i>	<i>2.63</i>	<i>0.9</i>