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The effects of amygdala lesions on conditioned stimulus-potentiated eating in rats

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Abstract

Both control rats and rats with neurotoxic lesions of the amygdala central nucleus ate more food during presentations of a conditioned stimulus (CS) previously paired with food than during an unpaired CS. This potentiation occurred regardless of whether the food was presented in its usual place or in a different location. By contrast, rats with neurotoxic lesions of basolateral amygdala showed no evidence for conditioned potentiation of eating. These results are considered in the context of anatomical projections from these amygdalar areas to other brain regions involved in feeding, and the role of amygdala subregions in the acquisition of motivational value in conditioning. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

Eating behavior is often modulated by social and cognitive variables that bear little immediate relation to current energy needs [52]. Both the initiation and termination of eating can be modulated by external sensory events that have acquired their powers by associative learning [5,64]. For example, external cues previously associated with feeding enhance eating in rats [67], even if the rats are food-sated at the time of test [61]. The control of eating by sensory cues in food-sated subjects has been of special interest, in part because of its relation to natural circumstances that are often thought to induce overeating [50,52,53].

Weingarten [61] first exposed food-deprived rats to pairings of a 4.5-min light + buzzer compound conditioned stimulus (CS^+) with meal delivery to a food cup, and presentations of an intermittent pure tone unpaired (CS^-) with meals. Later, the rats were tested for food consumption while food-sated, when either the CS^+ or CS^- was pre-

sented. The rats approached the food cup more rapidly and spent more time in contact with that cup during CS^+ presentations than during CS^- presentations.

The observation of enhanced feeding-related behavior in the presence of Pavlovian CSs for food delivery is consistent with a common view of Pavlovian conditioning by which the CS gains control over incentive motivational functions [4,39,49]. By this view, a CS associated with meal delivery while the rat is food-deprived may come to induce a motivational state akin to hunger, activate food-related responses directly, or provoke a craving for the food US.

Considerable evidence implicates the basolateral amygdala (BLA) in the acquisition of motivational value by CSs paired with food delivery in Pavlovian appetitive conditioning experiments. Although rats with BLA lesions are often unimpaired in their display of simple conditioned responses (CRs) elicited by those CSs, including orienting to the CS and approach to the source of food delivery [21,24,44], they show profound deficits in their sensitivity to the acquired motivational value of those CSs. For example, a first-order CS paired with food fails to serve as a reinforcer for second-order conditioning of another CS [21], or as an effective secondary reinforcer of an operant response when the occurrence of the first-order CS is made contingent on that response [14].

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Interestingly, these acquired motivational functions do not seem to involve BLA efferents to amygdala central nucleus (CN), which are critical to the expression of aversive motivational significance by CSs in Pavlovian fear conditioning [33]. Lesions of CN have no effect on the acquisition of either second-order conditioning [21] or secondary reinforcement [51] when food reinforcers are used.

In the experiments reported here, we examined the effects of BLA (Experiment 1) and CN (Experiment 2) lesions on the ability of Pavlovian CSs to enhance feeding in rats. If the ability of food-paired CSs to enhance feeding depends on the same acquired motivational functions as second-order conditioning and conditioned reinforcement, then BLA lesions, but not CN lesions, would also be expected to disrupt CS-potentiated feeding. Preliminary investigations in our laboratories yielded results consistent with these predictions. We found that rats with BLA lesions failed to show enhanced feeding in the presence of a CS previously paired with food [24], whereas in another study, CN lesions had no effect on that potentiation [17]. However, neither of those studies was designed to provide a controlled demonstration of potentiated feeding, or to explore its basis. The experiments reported here extended those preliminary findings in a number of ways to provide a more systematic examination of the effects of CN and BLA lesions on the potentiation of feeding by Pavlovian CSs.

First, in these experiments, we used a discriminative conditioning procedure to permit the assessment of the effects of both a CS^+ and a CS^- on feeding behavior. As in many potentiated feeding studies, our earlier studies [17,24] used nondiscriminative conditioning procedures, in which food consumption in the presence of a CS^+ in one consumption test was contrasted with food consumption in the absence of that CS^+ in another test. It is possible that in these earlier studies, the CSs enhanced eating by a more general arousal function not dependent on associative learning (for an example, see Ref. [50]), rather than via an explicitly conditioned motivational state.

Second, we examined the influence of food location on CS-potentiated feeding. In all other published studies of conditioned potentiation of feeding in rats [11,62–65], in the consumption test, the food was placed in the same cup as was used in the Pavlovian conditioning phase of the experiment. In those studies, the CS^+ may have enhanced the intact rats' eating simply by eliciting appetitive CRs that get the rat to the food cup more quickly, or that direct its consummatory responses more effectively to the food site, rather than by inducing "hunger" or some other motivational process. Indeed, Weingarten [63] (p. 157) reported in passing that "eating initiated by conditioned cues is directed specifically to the expected food source." Therefore, in these experiments, we tested potentiated feeding both when the food was placed in the original food cup and when it was placed in a very different (but familiar) receptacle at the opposite end of the conditioning chamber. If the potentiating effects of Pavlovian CSs are confined to directing the rat to the food

source, then a CS^+ would only enhance consumption of food placed in the original location. Moreover, if the CS^+ acted only by directing the rat to the original food cup, then it might interfere with consumption of food from the alternate food cup. By contrast, if the CS^+ acted by enhancing a more general motivational process, then it would enhance food consumption regardless of food location.

Third, we recorded several aspects of the rats' behavior during the conditioning and consumption test phases. During the training phase, we measured both food cup entry CRs and auditory conditioned orienting responses (ORs). In intact rats, the auditory CSs used in this study initially elicit unconditioned ORs, which habituate rapidly when the CSs are presented by themselves. Subsequent CS–food pairings result in the acquisition of high levels of conditioned ORs [22]. Previous studies showed that the acquisition of conditioned ORs to CSs paired with food (but not the display of unconditioned ORs to those CSs) is impaired by CN lesions [16], but not by BLA lesions [21,24]. During the potentiated feeding consumption tests, we measured not only the amount of food consumed, but also other aspects of overt behavior during the CSs, including the latency to approach the food cup after a CS was presented, the time spent in the food cup, and other more qualitative aspects of behavior obtained from video tapes. Assessment of food consumption itself and indirect measures of feeding, such as the latency to contact the food cup or the time spent in contact with the food cup, are often used interchangeably in studies of feeding. However, we found that both the behavioral treatment conditions and the lesions affected the various measures differently.

2. Experiment 1

2.1. Method

2.1.1. Subjects

The subjects were 24 male Long–Evans rats (Charles River Laboratories, Raleigh, NC), which weighed 300–325 g when they arrived in the laboratory vivarium. After 1 week with ad libitum access to food and water in individual cages, the rats were reduced to 85% of their ad libitum weights by restricting their access to food. They then participated in another experiment, which examined the effect of BLA lesions on second-order conditioning. In that study, the rats first received pairings of a visual CS, followed by BLA neurotoxic or sham lesion surgery. After 2 weeks of recovery from the surgery, the rats received pairings of another visual CS with food pellets, and second-order conditioning training, in which a noise CS was paired with one visual CS and a tone CS was paired with the other CS. A week after the completion of that study, the rats began the experiment reported here. Throughout both studies, the rats lived in individual cages, with free access to water, in a colony room illuminated from 6 a.m. to 8 p.m.

2.1.2. Surgical procedures

Surgery was performed under Nembutal (50 mg/kg) anesthesia with aseptic conditions. Fourteen rats received bilateral lesions of BLA, using stereotaxic coordinates [43] 2.8 mm posterior of bregma and 5.0 mm from the midline, with infusions at 8.7 and 8.4 mm ventral from the skull surface. The BLA lesions were made using 12.5 mg/ml NMDA (Sigma, St. Louis, MO) in phosphate-buffered saline (PBS) solution, infused with a Hamilton 2.0-μl syringe at a rate of 0.1 μl/15–30 s; 0.2 μl at the deeper site and 0.1 μl at the shallower site. We used NMDA to make the BLA lesions because that agent tends to spare neurons in the neighboring CN. Ten vehicle BLA control rats received injections of the PBS vehicle alone to these sites, in a similar manner. All rats were allowed to recover from surgery for 2 weeks prior to experimental participation.

2.1.3. Apparatus

The behavioral training apparatus consisted of four individual chambers (22.9 × 20.3 × 20.3 cm) with aluminum front and back walls, clear acrylic sides and top, and a floor made of 0.48-cm stainless steel rods spaced 1.9 cm apart. A dimly illuminated food cup was recessed in the center of one end wall. An infrared photocell placed just inside the food cup was polled (1 kHz) by computer circuitry. Each chamber was enclosed in a sound-resistant shell. A speaker, used to present the auditory CSs, was mounted on the inside wall of the inner shell, 10 cm above the experimental chamber and even with the end wall opposite the food cup. Ventilation fans provided masking noise (70 dB). Constant dim illumination was provided by a 6-W lamp behind a dense red lens, mounted next to the speaker. A TV camera was mounted within each shell to provide a view of the chamber. Television images were recorded in selected sessions. This apparatus was similar, but not identical to, the apparatus used in the previous training of these rats.

2.1.4. Behavioral training procedures

Table 1 provides an outline of the behavioral training procedures of this experiment. The rats first received twelve 32-min training sessions, one each day, at approximately the same time every day. In each of the first two conditioning sessions, half of the rats received eight 10-s presentations of an 80-dB white noise, followed by the delivery of two 45-mg food pellets (PJ Noyes, Lancaster, NH), and the other half received similar pairings of an 80-dB, 1500-Hz tone with food. These two auditory cues had been used as second-order CSs in the previous study, but had never been paired with food. By the end of the previous study, second-order CRs to those cues were extinguished.

In these and all remaining training sessions, the intertrial intervals were variable (mean = 4 min) within a range of 2–6 min. In each of the next 10 discrimination training sessions, the rats received four reinforced presentations of the CS they had received in the first 2 days of training (CS$^+$), randomly intermixed with four nonreinforced pre-

Table 1
Outline of experimental procedures

CS$^+$ training (two sessions)	Discrimination training (10 sessions)	Satiation (7 days)	Consumption testing (four sessions)
CS$^+$ → food	CS$^+$ → food CS$^-$ → no food	free food in home cage	CS$^+$; food in bowls CS$^-$; food in cups CS$^+$; food in cups CS$^-$; food in bowls

Rats received excitotoxic or sham lesions of BLA (Experiment 1) or of amygdala CN (Experiment 2) prior to behavioral training. The identities of CS$^+$ and CS$^-$ (noise and tone) were counterbalanced across all lesion and test conditions. Consumption tests included both a 10-min pretest with no CS presentations and a 10-min test period with 10 presentations of either CS$^+$ or CS$^-$. The order of the four kinds of consumption tests was counterbalanced within each lesion condition (see text). All consumption tests were conducted while rats were food-sated. CS = conditioned stimulus.

sentations of the other auditory stimulus (CS$^-$). In the final four discrimination training sessions, an opaque glass bowl, 9 cm in diameter and 7 cm high, was placed (empty) in each chamber, wired to the wall opposite the food cup. Behavior during the conditioning trials in these last four sessions was recorded on video tape.

The rats were then given 11 days of free access to food in their home cages. On each of the last four of those days, the rats received a consumption test in the experimental chambers. In two "bowl" tests, food pellets were placed in the glass food bowls, and in two "cup" tests, the food pellets were placed in the food cups, with the bowls removed from the chambers. Half of the rats received the bowl tests on the first and fourth test days and cup tests on the second and third test days, and the other half received the opposite sequence of cup and bowl tests.

Each consumption test began with a 10-min pretest of pellet consumption in the chambers before the presentation of CSs. In previous studies of potentiated feeding, we found that when satiated rats were placed in the experimental chambers, they often ran immediately to the food cup and ate any food there. The pretest was intended to reduce the contribution of this effect to feeding during the CS presentations. In the pretest, 50 pellets were present in the appropriate food receptacles when the rats were placed in the chambers. After 5 min, the rats were removed, placed in transport cages, and then returned to the chambers, as quickly as possible, to provide another handling and chamber placement experience. After 10 min had elapsed from the initial placing of the rats in the chambers, the rats were again removed and placed in transport cages. Food pellets were suctioned from the food cup and trays beneath the chamber floor and saved for counting.

The food receptacles were then quickly refilled with 50 new pellets, and the rats were again returned to the chambers for a 10-min test of consumption in the presence of CS$^+$ or CS$^-$. During that test, ten 10-s CS$^+$ or CS$^-$ were presented (but no additional pellets were delivered after CS$^+$). Both the reinforcement contingency in training (CS$^+$ or CS$^-$) and identity of the CS (tone or noise) were counterbalanced

within each pair of tests (cup or bowl). Behavior was videorecorded throughout each test. Finally, at the end of the 10-min CS consumption test, the rats were quickly removed, and the remaining pellets suctioned and reserved for counting.

The experiment was conducted in two replications. In the first replication ($n = 16$), opaque glass food bowls identical to those used in the chambers were partly filled with pellets and placed in the home cages for 1 h prior to each test session. This procedure seemed to excessively suppress consumption from the bowls in the chamber, and was omitted in the second replication ($n = 8$) in an effort to enhance in-chamber food consumption from the bowls. Otherwise, the replications were identical.

2.1.5. Response measures

Three measures of conditioning to the auditory CSs during the Pavlovian training phases were reported. The first, percentage time in food cup, was the percentage of time during which the food cup photobeam was broken (presumably indicating that a rat's head was in the food cup) during the last 5 s of each CS interval. The second measure, latency of food cup entry, was the time between the onset of an auditory CS and the first food cup entry (breaking of the photobeam). The third measure of conditioning was the percentage of trials on which an OR occurred. The OR was defined as a jump or sudden change in position within 1 s of CS onset. Two observers, both unaware of the rats' lesion conditions, independently scored ORs from the video tapes of performance in the final four discrimination training sessions. The two observers agreed on over 90% of their judgments.

Three behavioral measures were also recorded in the food consumption tests. The primary measure was food consumption itself, which was measured by counting the number of whole pellets remaining in the food cups or bowls. In the rare case of a rat leaving fractional pellets uneaten, a fractional pellet was arbitrarily defined as 0.5 pellet. In no case did a rat leave more than two fractional pellets. For both the cup and bowl tests, we reported the number of pellets consumed by rats during the separate CS^+ and CS^- tests, and the difference between the number of pellets consumed in CS^+ and CS^- tests. The difference scores measure the effect of the CS's past conditioning relation with food on consumption in each rat, and thus serve as a direct, within-subject measure of the potentiated feeding effect.

Two nonconsummatory measures were also recorded: the percentage of time in food cup and the latency of cup entry (both as described for the Pavlovian training phases.)

2.1.6. Histological procedures

After completion of behavioral testing, the rats were deeply anaesthesized with Nembutal (150 mg/kg) and perfused with 0.1 M PBS, followed by 10% (vol/vol) formalin. The brains were removed and stored in 0.1 M PBS with 20% (wt/vol) sucrose and 1% (wt/vol) DMSO at 4 °C for 24–48 h. Sections (60 μm) were taken from each brain, and al-

ternate sections were mounted on slides and Nissl-stained to verify the lesions.

2.2. Results and discussion

2.2.1. Histological results

Nine BLA-lesioned brains were judged as having acceptable lesions of the basolateral region, including the lateral, basal, and accessory basal nuclei. Lesions were rejected ($n = 5$) if there was less than 50% damage to BLA on either side, or if there was more than minimal bilateral damage to the adjoining CN or cortical regions. The acceptable brains averaged 90% damage on one side and 80% on the other. Fig. 1 shows Nissl-stained cells in BLA-lesioned (panel B) and control (panel C) brains, along with diagrams (panel A) of the extent of the largest and smallest acceptable lesions at several rostral–caudal levels. Except around the injector tracks, no cellular damage was evident in any of the vehicle control brains.

2.2.2. Behavioral results: Pavlovian discrimination training

The BLA lesion did not affect any measure of auditory Pavlovian discrimination learning. Both BLA-lesioned and sham rats acquired the discrimination rapidly, although that performance was disrupted in Session 9, when the empty food bowls were first introduced into the experimental chambers. The left side of Table 2 shows the mean ($\pm$ S.E.M.) percentage time in food cup, latency to food cup entry, and OR (startle) scores, collapsed over the final two training sessions. For all three measures, ANOVA with the variables of replication, lesion type (neurotoxic or sham), identity of the reinforced CS (noise or tone), and CS contingency (reinforced or nonreinforced) showed only one significant effect, that of CS contingency [$Fs(1,11) \leq 35.59$, $Ps < .001$; remaining $Ps > .10$]. The mean percentages of time spent in the food cup in the pre-CS periods were less than 10% in all groups, and did not differ as a function of any of the variables in the ANOVA ($Fs < 1$).

2.2.3. Weight gain in satiation phase

The lesions had no effect on the rats' weight gain during the satiation phase (Table 3). ANOVAs showed no effect of lesion for the presatiation weights, the test weights, or the absolute or relative weight increases, within Experiment 1 ($Fs < 1$).

2.2.4. Pellet consumption in CS^+ and CS^- tests

The primary data of this experiment are the results of the consumption tests with CS^+ and CS^-, shown in Fig. 2. The left panel shows the consumption difference scores (CS^+ minus CS^-), and the middle and right panels show consumption during CS^+ and CS^- individually, during the cup and bowl tests, respectively. Sham-lesioned rats ate more pellets in tests in which the previously reinforced CS^+ was presented than in tests with the nonreinforced CS^-. Thus, the ability of the noise and tone stimuli to potentiate eating
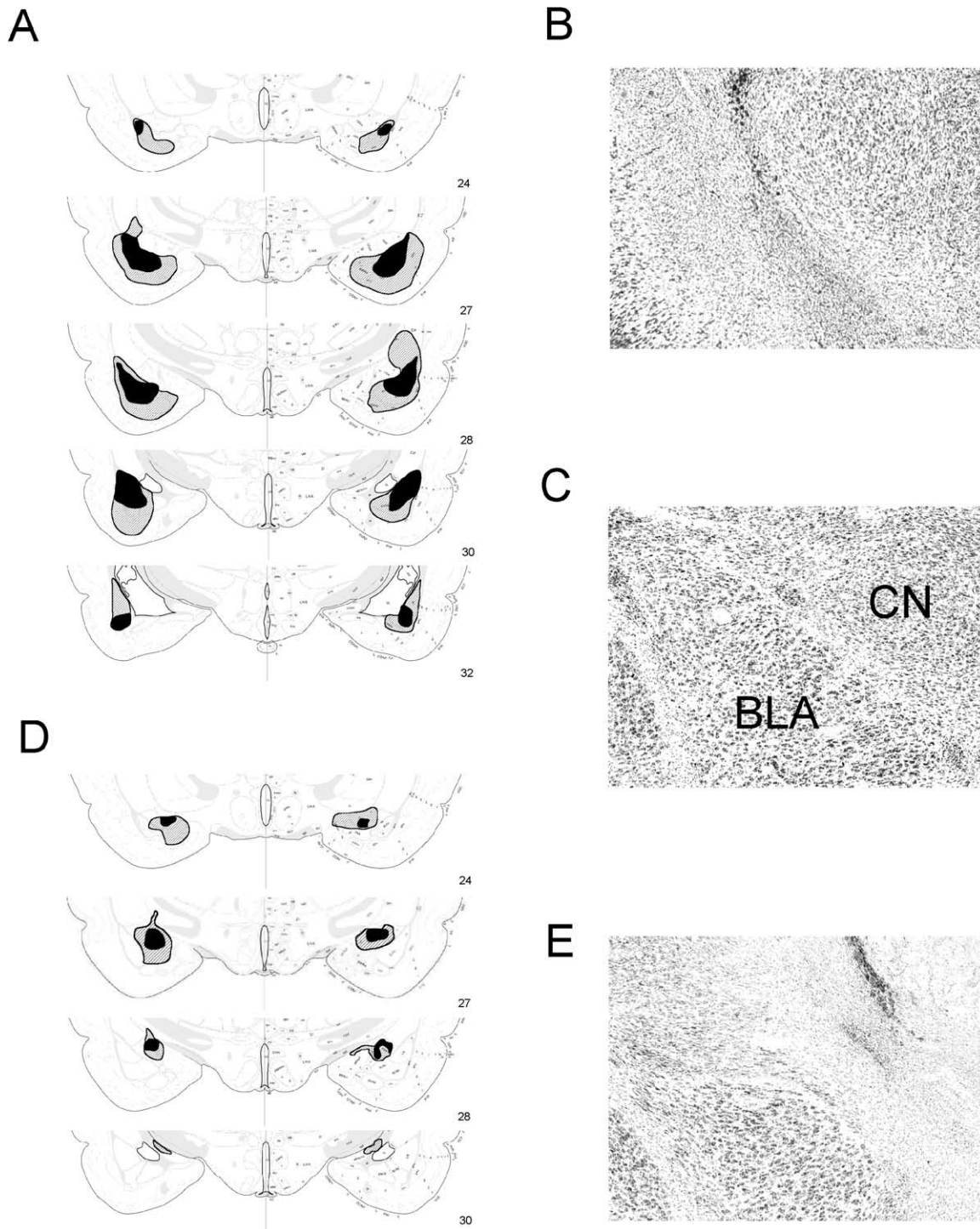
Fig. 1. Surgical results. (A) Largest (hatched area) and smallest (black-shaded area) lesions of the BLA in various coronal sections. The plates are adapted from the atlas of Swanson [58], and are numbered accordingly. (B) Representative lesion of BLA. (C) Representative sham lesion. (D) Largest (hatched area) and smallest (black-shaded area) lesions of the amygdala central nucleus in various coronal sections, as in (A). (E) Representative lesion of the central nucleus. Note the sparse cells and gliosis, especially along the injector tracks, in the lesioned areas. BLA = basolateral amygdala; CN = central nucleus.

depended on their associative history, i.e., their prior pairing with food. Furthermore, this potentiation of feeding by the CS^+ was evident both when the food was available in the usual food cups, and when it was presented in the bowls on the opposite side of the chambers. Therefore, the potentiated

feeding by the CS^+ was not an artifact of greater opportunity to consume food because the explicit CR guided the rat to the food cup. Instead, in the sham rats, the CS^+ appeared to enhance the normal control of feeding by properties of the food itself or by internal, "hunger" cues.

Table 2
Conditioned responding in the final two discrimination training sessions

	Experiment 1		Experiment 2	
	BLA lesion	BLA sham	CN lesion	CN sham
Percentage time in food cup				
CS⁺	66.6 ± 6.7*	66.9 ± 6.3*	67.1 ± 4.7*	69.7 ± 3.8*
CS⁻	8.8 ± 5.0	11.2 ± 8.4	2.8 ± 2.9	7.8 ± 2.9
Latency of food cup entry				
CS⁺	2.76 ± 0.27*	2.37 ± 0.38*	2.71 ± 0.81*	2.95 ± 1.11*
CS⁻	5.71 ± 0.76	5.23 ± 0.62	5.71 ± 0.64	5.71 ± 2.18
Orienting responding				
CS⁺	83.3 ± 5.9*	85.0 ± 5.5*	31.3 ± 4.1* ,§	81.3 ± 5.8*
CS⁻	14.0 ± 6.0	17.6 ± 5.6	12.5 ± 4.1	9.4 ± 3.1

Percentage time in food cup is expressed as the mean ± S.E.M. percentage of time during which the rat's head was in the food cup during the last 5 s of each CS presentation. Latency of food cup entry is expressed as the mean ± S.E.M. seconds between conditioned stimulus (CS) onset and the first food cup entry. Orienting responding is expressed as the mean ± S.E.M. percentage of trials on which an orienting response (startle) occurred. CS^+ = CS paired with food in training; CS^- = CS not paired with food in training.

　* Indicates significance, $P < .001$, over corresponding CS^-.

　§ Indicates significance, $P < .001$, compared to CS^+ in each of the other groups.

By contrast, rats with BLA lesions failed to show potentiated feeding under any condition. Consumption during both CS^+ and CS^- presentations occurred at low levels, comparable to the sham rats' consumption during CS^- tests. Thus, unlike shams, rats with BLA lesions were unable to acquire or use learned motivational properties of the CS to modulate feeding.

These claims are supported statistically. First, an ANOVA of the consumption difference scores, with replication, lesion, identity of the reinforced CS (noise or tone), and test type (cup or bowl) as variables, showed only a reliable effect of lesion (potentiated feeding was greater in shams than in BLA-lesioned rats) $[F(1,11) = 15.83, P = .002$; other $Ps > .100]$. Second, a comparable ANOVA of individual test consumption scores with the additional factor of CS contingency (CS^+ or CS^-) showed a reliable Lesion × CS type interaction (the BLA lesion affected consumption during CS^+ more than consumption during CS^-) $[F(1,11) = 15.83, P = .002]$, a marginally reliable effect of test type (the rats ate more in the cup tests than in the bowl tests) $[F(1,11) = 4.79, P = .051]$, and a reliable effect of replication (rats ate more pellets in the second replication, in which food pellets were not available in the home cages, than in the first replication) $[F(1,11) = 12.25, P = .005]$. No other effects or interactions were reliable ($Ps > .100$).

Separate Lesion × CS type ANOVAs for both the cup and bowl tests also showed reliable Lesion × CS type interactions $[Fs(1,17) = 12.69$ and 10.53, respectively, $Ps = .002$ and $.005]$. In the cup tests, consumption in the CS^+ test was greater than consumption in the CS^- test in the sham rats $[F(1,17) = 13.31, P = .002]$, but not in the BLA-lesioned rats $[F(1,17) = 2.10, P = .166]$. Consumption in the CS^+ test was

greater in the sham rats than in the lesioned rats $[F(1,17) = 7.23, P = .016]$, but CS^- test consumption of lesioned and sham rats did not differ ($F < 1$). Likewise, in the bowl tests, consumption in the CS^+ test was greater than consumption in the CS^- test in the sham rats $[F(1,17) = 11.68, P = .003]$, but not in the lesioned rats, which showed the opposite (but insignificant) tendency $[F(1,17) = 1.51, P = .235]$.

2.2.5. Pellet consumption in pretests

We also recorded the number of pellets consumed in the experimental chambers during the 10 min prior to the 10-min CS consumption tests (bottom left portion of Table 3). There was more pretest food consumption in cup tests than in bowl tests $[F(1,11) = 9.42, P = .011]$. Furthermore, the immediately prior presentation of food pellets in the home cage in the first replication reduced (relative to the second replication) pretest consumption in the bowl tests $[F(1,11) = 140.09, P < .001]$, but not in the cup tests ($F < 1$;

Table 3
Results of satiation and consumption test phases

	Experiment 1		Experiment 2	
	BLA lesion	BLA sham	CN lesion	CN sham
Satiation phase				
Weight (g) at start	281 ± 5**	283 ± 6**	384 ± 10	381 ± 8
Weight (g) at finish	388 ± 7**	394 ± 10**	497 ± 15	493 ± 10
Absolute weight gain (g)	107 ± 5	111 ± 6	113 ± 5	112 ± 3
Relative weight gain (%)	38 ± 2**	39 ± 2**	29 ± 1	29 ± 1
Consumption test phase				
Pretest pellets eaten (cup)	21.0 ± 4.3*	29.0 ± 5.4*	14.8 ± 3.6	15.3 ± 4.9
Pretest pellets eaten (bowl)	18.2 ± 7.2*	14.5 ± 7.1*	8.2 ± 2.4	8.0 ± 3.8
Latency to first CS^+ cup entry (s)	6.76 ± 0.46§	7.74 ± 0.56	8.46 ± 0.36	8.32 ± 0.33
Latency to first CS^- cup entry (s)	8.12 ± 0.41	7.64 ± 0.54	8.99 ± 0.38	8.37 ± 0.26

All entries are mean ± S.E.M. Start weights were obtained from the last 4 days of discrimination training and finish weights were from the four consumption test days. Pretest consumption refers to consumption in the first 10 min of each consumption test, prior to the delivery of CS^+ or CS^-; the table entries are the average number of pellets consumed over the CS^+ pretest and CS^- pretest. Cup entry latencies refer to the average latency to the first cup entry in the CS^+ or CS^- sessions, averaged over the eight 10-s CS presentations. The primary data of these experiments, food pellet consumption in the CS^+ and CS^- consumption tests, are shown in Figs. 2 and 3. CS^+ = conditioned stimulus that had been paired with food; CS^- = conditioned stimulus that had not been paired with food.

　* Significantly different from CN Lesion and CN sham, $P < .05$.

　** Significantly different from CN Lesion and CN sham, $P < .001$.

　§ Significantly different from corresponding CS^- and from all other groups CS^+, $P < .05$.
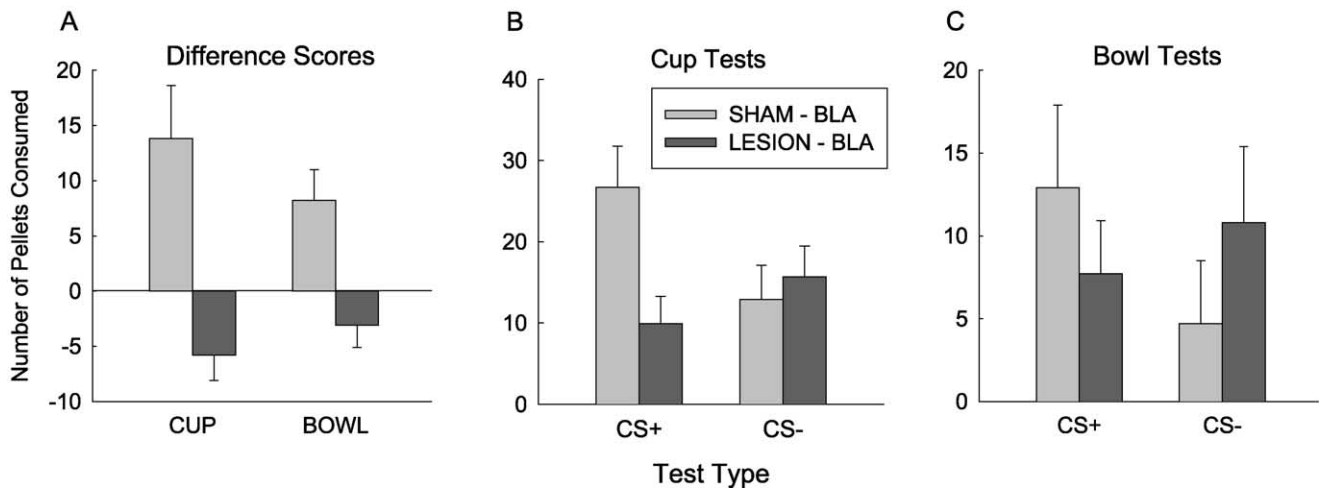
Fig. 2. Food pellet consumption of sham rats and rats with BLA lesions during the potentiated eating tests of Experiment 1. (A) Differences between consumption during the tests with CS^+ and consumption during the tests with CS^-. (B) Consumption during the tests in which food was placed in the food cup used in Pavlovian conditioning. (C) Consumption during the tests in which food was placed in a bowl on the side of the chamber opposite from the food cup used in training. CS^+ = conditioned stimulus paired with food in training; CS^- = conditioned stimulus not paired with food in training. The error bars indicate the S.E.M.

data not shown). Unlike consumption during CS^+, mean pretest consumption did not differ as a function of BLA lesions in either the cup tests or the bowl tests ($Fs < 1$). Thus, the lesions did not affect any enhancement of eating that may have occurred as a result of simple placement in a context previously associated with food.

2.2.6. Nonconsummatory CRs in consumption tests

Unsystematic observations of the videotapes of the CS^+ and CS^- consumption tests suggested that the effects of the auditory CS on food consumption were mostly delayed. That is, CS onset did not provoke either a rapid move to the food receptacle or sustained presence in that receptacle. Typically, a rat would approach the food receptacle only after a substantial delay, remove a pellet, and consume it outside the receptacle, although that last tendency was less evident in the bowl tests. Thus, most eating occurred soon after CS termination. Unfortunately, our use of brief (10 min) consumption tests with frequent CS presentations made it difficult to separate the contributions of the CS to the initiation of feeding or its maintenance, which are often thought to be controlled by different mechanisms [56].

More important, the observations suggested that differences in food consumption across lesion condition, test type (bowl or cup), and CS type (CS^+ or CS^-) were not attributable to differences in approach to the food cup used in training. These impressions were supported by automated measures of time spent in the food cup during the CS in the consumption test sessions. First, the percentages of time in food cup were less than 10% in all conditions. ANOVAs of these scores (identical to the ANOVAs used for the pellet consumption data) showed no effects or interactions ($Fs < 1$). This result is consistent with the video observation that the rats did not typically consume pellets with their heads in the food cups during CS presentations.

Second, the latencies to the first food cup entry (bottom left portion of Table 3) were clearly dissociated from food consumption. Whereas BLA-lesioned rats failed to eat more during CS^+ tests than during CS^- tests, they showed a shorter latency response to CS^+ than to CS^-. Similarly, although BLA sham rats ate more during CS^+ tests than during CS^- tests, they showed no difference in their cup entry latencies between those tests. This longer latency response to CS^+, relative to the training phase, probably reflects in part the effects of food satiation on food-based CRs in sham rats. We discuss other implications of the reduced sensitivity of food cup approach responding to satiation in BLA-lesioned rats in the General Discussion. ANOVA showed a reliable Lesion × CS contingency interaction [$F(1,11) = 8.62$, $P = .014$]. Interestingly, this observation of more rapid response to the food cup on CS^+ trials among BLA lesion rats was equally true for both bowl and cup tests; the Lesion × Test Type × CS type interaction was not significant ($F < 1$).

3. Experiment 2

Experiment 2 was identical to Experiment 1 except that lesions were placed in the CN rather than the BLA and the rats had experienced different previous behavioral training.

3.1. Method

3.1.1. Subjects

The subjects were 24 male Long–Evans rats (Charles River Laboratories), which weighed 300–325 g when they arrived in the laboratory vivarium. After 1 week with ad libitum access to food and water in individual cages, the rats were given CN neurotoxic or CN sham lesions and were

allowed to recover for 2 weeks. They were then gradually reduced to 85% of their ad libitum weights, after which they participated in another unpublished experiment. That experiment was conducted in experimental chambers similar (but not identical) to those used in the present experiment, and involved the pairing of two visual CSs with the delivery of food pellets, and the presentation of the tone and noise CSs to be used in Experiment 2, in compound with those visual CSs for a brief period, in a blocking (e.g., Ref. [26]) experiment. A week after the completion of that study, the rats began the experiment reported here. Throughout both studies, the rats lived in individual cages, with free access to water, in a colony room illuminated from 6 a.m. to 8 p.m.

3.1.2. Surgical procedures

Surgery was performed under Nembutal (50 mg/kg) anesthesia with aseptic conditions. Sixteen rats received bilateral lesions of CN, using stereotaxic coordinates 2.3 mm posterior to bregma and 4.2 mm from the midline, with infusions at a depth of 7.9 mm from the skull surface. The CN lesions were made using 0.25 μl of 10 μg/μl ibotenic acid (Sigma) in PBS solution, infused with a Hamilton 2.0-μl syringe over a 2-min period. Eight vehicle control rats received injections of the PBS vehicle alone in a comparable manner. All rats were allowed to recover from surgery for 2 weeks prior to experimental participation.

The apparatus, behavioral training procedures (Table 1), response measures, and histological procedures were identical to those used in Experiment 1. The first replication ($n = 16$) was conducted immediately after the first replication of Experiment 1, and the second replication ($n = 8$) was conducted concurrently with the second replication of Experiment 1.

3.2. Results and discussion

3.2.1. Histological results

Eight CN-lesioned brains were judged as having acceptable lesions. Lesions were rejected ($n = 8$) if there was less than 30% damage to CN on either side, or if there was more than minimal damage to adjoining regions. The acceptable brains averaged 50% damage on one side and 45% on the other. All had substantial damage in the anterior portions of CN, which show the heaviest projections to lateral hypothalamus (LH) [42]. Fig. 1 shows Nissl-stained cells in CN-lesioned (panel E) and control (panel C) brains, along with diagrams (panel D) of the extent of the largest and smallest acceptable lesions at several rostral–caudal levels. Except around the injector tracks, no cellular damage was evident in any of the vehicle control brains.

3.2.2. Behavioral results: Pavlovian discrimination training

The CN lesion did not affect the acquisition of anticipatory food cup behaviors during auditory Pavlovian discrimination learning, but as in previous studies [16], the lesioned rats failed to acquire the auditory OR, startle, over the course of training. The right side of Table 2 shows the mean ($\pm$S.E.M.) percentage time in food cup, latency to food cup entry, and OR (startle) scores, collapsed over the final two training sessions. For the two food cup measures, ANOVAs with the variables of replication, lesion type (neurotoxic or sham), identity of the reinforced CS (noise or tone), and CS contingency (reinforced or nonreinforced) showed only one significant effect, that of CS contingency [$Fs(1,8) = 370.19$, $P < .001$, and 11.46, $P = .010$, respectively; remaining $Ps > .100$]. The mean percentages of time in the food cup in the pre-CS periods were less than 10% in all groups, and did not differ as a function of any of the variables in the ANOVA ($Fs < 1$).

A comparable ANOVA of startle responding showed reliable effects of lesion (lesioned rats showed less startle than shams) [$F(1,8) = 72.9$, $P < .001$], identity of CS (the noise elicited more startle than the tone) [$F(1,8) = 8.10$, $P = .022$], CS contingency (CS^+ elicited more startle than CS^-) [$F(1,8) = 115.56$, $P < .001$], and a significant CS identity $\times$ CS contingency interaction (although the noise CS^+ elicited more startle than the tone CS^+, the two CS^- elicited comparable amounts of startle) [$F(1,8) = 10.56$, $P = .012$]. Most important, there was a significant Lesion $\times$ CS contingency interaction (the CS^+ elicited more startle responding in the sham rats than in the CN-lesioned rats, but startle to the CS^- was not affected by the lesion) [$F(1,8) = 39.06$, $P < .001$].

3.2.3. Weight gain in satiation phase

As in Experiment 1, the lesions had no effect on the rats' weight gain during the satiation period (top right portion of Table 3). ANOVAs showed no lesion effect for either the training or test weights, or the absolute or relative weight increases ($Fs < 1$).

3.2.4. Pellet consumption in CS^+ and CS^- tests

The primary data of this experiment are the results of the consumption tests with CS^+ and CS^-, shown in Fig. 3. The left panel shows the consumption difference scores (CS^+ minus CS^-), and the middle and right panels show consumption during CS^+ and CS^- individually, during the cup and bowl tests, respectively. The CN lesions had no effects on potentiated eating; both CN- and sham-lesioned rats' performance was comparable to that of the sham-lesioned rats in Experiment 1. Both sham-lesioned and CN-lesioned rats ate more pellets in tests in which the previously reinforced CS^+ was presented than in tests with the non-reinforced CS^-. Furthermore, this potentiation of feeding by the CS^+ was evident both when the food was available in the usual food cups, and when it was presented in the bowls on the opposite side of the chambers.

These claims are supported statistically. An ANOVA of the difference scores showed no reliable effects or interactions ($Ps > .10$). An ANOVA of the individual test consumption scores, like that described for the BLA-lesioned rats, showed a reliable effect of CS type (greater consump-
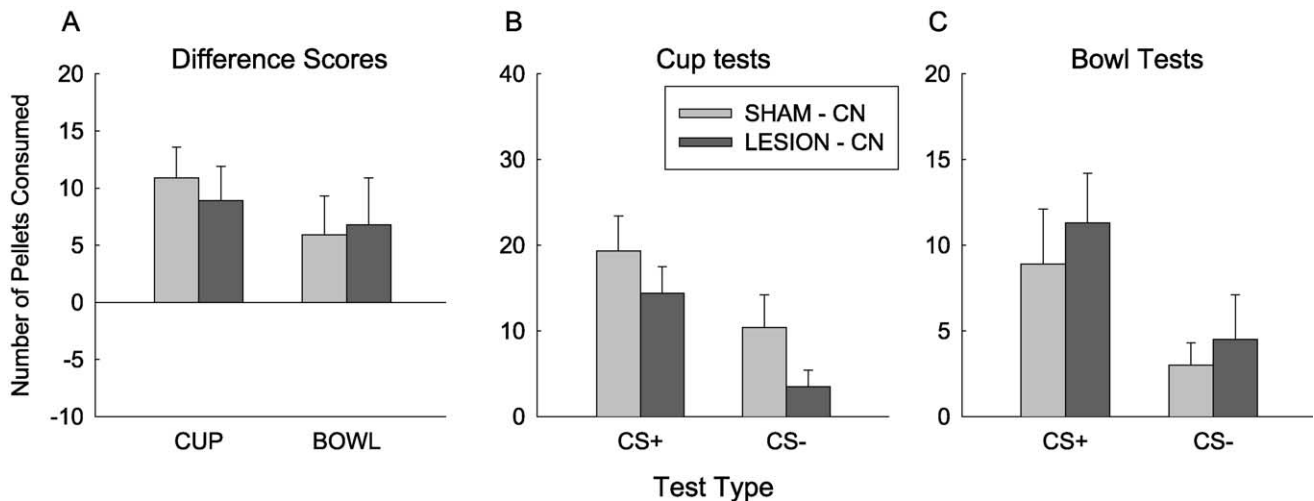
Fig. 3. Food pellet consumption of sham rats and rats with amygdala CN lesions during the potentiated eating tests of Experiment 2. (A) Differences between consumption during the tests with CS^+ and consumption during the tests with CS^-. (B) Consumption during the tests in which food was placed in the food cup used in Pavlovian conditioning. (C) Consumption during the tests in which food was placed in a bowl on the side of the chamber opposite from the food cup used in training. CS^+ = conditioned stimulus paired with food in training; CS^- = conditioned stimulus not paired with food in training. The error bars indicate the S.E.M.

tion in the CS^+ tests than in the CS^- tests) [$F(1,8) = 37.71$, $P < .001$], and an effect of replication (rats ate fewer pellets in the first replication than in the second replication, in which food pellets were not available in the home cages) [$F(1,8) = 37.75$, $P < .001$]. No other effect or interaction was reliable ($Ps > .100$); most important, the Lesion × CS contingency (CS^+ or CS^-) interaction was not reliable [$F(1,8) = 1.92$, $P = .203$]. Separate analyses for both the cup and bowl tests also showed only a reliable effect of CS contingency [$Fs(1,14) = 23.96$ and 5.54, respectively, $P < .001$ and $P = .034$; other $Ps > .100$].

3.2.5. Pellet consumption in pretests

There was marginally more pretest food consumption in cup tests than in bowl tests [$F(1,8) = 4.00$, $P = .080$] (bottom right portion of Table 3), and there was reliably less pretest food consumption in the initial replication (in which pellets had also been presented in the home cages prior to testing) than in the second replication [$F(1,8) = 15.14$, $P = .005$] (data not shown). However, the lesions had no effect on pretest food consumption; the effect of lesion condition was not reliable and lesion condition did not interact with any other variable ($Fs < 1$).

3.2.6. Nonconsummatory CRs in consumption tests

As in Experiment 1, observations made from the videotapes of the CS^+ and CS^- consumption tests suggested that the effects of the auditory CSs on food consumption were mostly delayed until some time after CS onset, with most eating occurring after CS termination.

As in Experiment 1, the percentages of time in the food cup during CS presentations were less than 10% in all conditions; ANOVA showed no reliable effects or interac-

tions ($Fs < 1$). Unlike in Experiment 1, ANOVA of the latency to food cup entry scores (bottom right portion of Table 3) failed to show a reliable Lesion × CS contingency interaction ($F < 1$). Both sham and CN-lesioned rats showed uniformly long latencies to both CS^+ and CS^-, as would be anticipated if posttraining food satiation reduced CRs to CS^+, and if this effect was not affected by CN lesions.

4. General discussion

As in earlier studies, presentation of a Pavlovian CS previously paired with food potentiated feeding behavior of food-sated, intact rats. The present data extended these earlier findings in several ways, and supported the claim that the potentiated feeding was mediated by the CS's learned ability to control a motivational state or process that promotes food consumption. First, the potentiated feeding effect was discriminative, in that the rats consumed more food in test sessions in which CS^+ was presented than in sessions in which CS^- was presented. Thus, the effect of the CS^+ on eating was the result of its previously learned relation with food, rather than some nonspecific activation, such as waking or arousing the rats. Second, this discriminative control over food consumption occurred despite the lack of a discriminative effect of the CS^+ and CS^- on any measure of approach to the food cup during the consumption tests. Third, the CS-potentiated eating effect occurred regardless of whether the food was presented in the recessed food cup used in conditioning or in a bowl placed in a different location in the conditioning chamber, and which had never before contained food. Both of these last two observations are important because they show that the CS-potentiated

eating was not merely the consequence of food cup approach responses conditioned to CS⁺, which in turn might increase the opportunity to eat. Rather, the potentiation was specific to consummatory behavior. Finally, video observations and recordings of the latency to enter the food cup supported the view that the potentiated eating did not occur immediately after CS onset and was not confined to CS presentations but rather occurred throughout the CS⁺ tests after delivery of the first CS⁺. This observation is consistent with observations that other effects of appetitive CSs ascribed to motivational processes are more evident with longer duration signals [35,64].

Our observation of general dissociations between food consumption and food cup approach and contact measures in the consumption tests of both experiments is also important methodologically. So-called feeding behavior is often indexed by the latency to approach a food cup or the amount of time spent in the food cup [63], rather than by the amount of food actually consumed. Our results suggest caution in relying entirely on these convenient but perhaps quite differently determined measures of feeding, especially when assessing the effects of brain lesions.

In Experiment 1, BLA lesions interfered with the CS-potentiated eating effects found in sham-lesioned rats. Coupled with the previous indications that in sham rats these effects reflect CS-induced motivational processes rather than simple conditioned food cup approach CRs, this lesion deficit supports our general claims that BLA is involved in the acquisition of (or access to) the motivational significance of Pavlovian CSs [18]. The observation that during the feeding tests, BLA-lesioned rats approached the food cup on CS⁺ trials with a shorter latency than sham rats is especially noteworthy. First, it provides a powerful demonstration that the potentiated feeding deficits of BLA-lesioned rats were not secondary to deficits in appetitive food cup approach behavior, either while the rats were food-deprived or food-sated. Note that although the lesioned rats arrived at the food cup on CS⁺ consumption test trials sooner than the sham rats, they ate less food. Thus, the CS⁺'s control of appetitive and consummatory behavior was differentially affected by the BLA lesions.

Second, it indicates that the BLA-lesioned rats' appetitive approach CRs to the CS⁺ were less sensitive to the posttraining devaluation of the food US by satiation than comparable responding of sham rats. This observation is consistent with previous findings [21] that conditioned responding of BLA-lesioned rats is less sensitive than that of shams to posttraining devaluation of a food US by flavor aversion training. In that previous experiment [21], rats first received light–food pairings. Then, the food was paired with an illness-inducing toxin, lithium chloride, in the absence of the light, producing an aversion to the taste of the food. Both lesioned and unlesioned control rats acquired equivalent aversions to the food. In a final test of responding to the light CS, control rats showed a spontaneous reduction in CRs, but BLA-lesioned rats did not. Likewise, monkeys with BLA lesions are less sensitive to the effects of posttraining selective food satiation on choice performance than intact monkeys [36].

Thus, although BLA is not critical to the acquisition of simple food cup approach responses to Pavlovian CSs, it is important for a range of acquired motivational functions of those CSs. In addition to its role in the potentiation of consummatory responses by signals of food, BLA is important for the sensitivity of conditioned appetitive behavior to posttraining changes in the motivational value of the US [21].

By contrast, in Experiment 2, CN lesions had no effect on any aspect of performance during the consumption tests. At the same time, as in previous studies [16,24], the CN lesions interfered with the acquisition of conditioned ORs to the auditory CSs. This result is important because it shows that the CN lesions were behaviorally effective in these rats, despite having no effects on potentiated feeding.

Statistical comparisons across the two studies (ANOVAs followed by posthoc Newman–Keuls comparisons, $Ps < .05$) confirmed the differential effects of these two lesions. First, the feeding difference scores were significantly smaller in the BLA-lesioned rats than in any of the other three groups. Second, the latency to approach the food cup on CS⁺ trials was reliably shorter in the BLA-lesioned rats than in any of the other groups. Third, the frequency of ORs in training was reliably less in the CN-lesioned rats than in any of the other groups.

Some caution should be exercised when comparing the results of the two experiments. Although the training and testing procedures of the two studies were identical, the second replications of each study were conducted concurrently, and both involved similar (but not identical) prior experience; the rats in Experiment 1 were considerably younger when they were first food-deprived than the rats in Experiment 2. As a result, both the 85% weights during training and the ad libitum weights during testing of the rats in Experiment 1 were considerably lower than those of the rats in Experiment 2 ($Ps < .001$). Perhaps as a consequence of these different deprivation histories and body weights, the rats in Experiment 1 ate more pellets in the consumption pretests than the rats in Experiment 2 ($Ps < .05$). Some evidence suggests that rats deprived of food earlier in life may eat faster at the beginning of a feeding bout, even after free access to food for extended periods [37].

Nevertheless, we do not think that these differences in history of the rats in the two experiments affected the display of potentiated eating. First, of all the behavioral measures recorded, the sham-lesioned rats of Experiments 1 and 2 differed only in pretest pellet consumption. Both pellet consumption during the CS⁺ and CS⁻ consumption tests, and the performance of appetitive CRs during Pavlovian discrimination training were similar in the two experiments. Second, within each experiment, the pretest consumption was identical for both lesioned and sham rats, and thus whatever factors produced greater pretest consumption in Experiment 1 than in Experiment 2 did not

contribute to the differential lesion effects on consumption during CS^+. Third, in our previous research with simpler potentiated feeding procedures, we found the same pattern of lesion effects on potentiated feeding, despite the fact that the lesions and the weights of those rats were confounded in the opposite way. That is, the CN lesion and CN sham rats of Gallagher and Holland [17] weighed *less* (382 ± 6 g) than the BLA lesion and BLA sham rats (473 ± 7 g) of Holland et al. [24]. Indeed, the weights of the BLA rats in the present study were similar those of the CN rats in the previous study of Gallagher and Holland [17], and those of the CN rats in this study were similar to those of the BLA rats in the study of Holland et al. [24].

The lack of effects of CN lesions on any aspect of potentiated eating shows that, as in other cases of acquired motivational function in appetitive conditioning [21,51], BLA's role in potentiated feeding is mediated by BLA efferents other than those to CN. Instead, we suggest that BLA's role in CS-potentiated feeding may be mediated by its connections with regions often implicated in the initiation of feeding, e.g., the LH. First, although projections from BLA to LH have often been characterized as sparse [42,47], recent studies provide evidence for more substantial projections. In a PHAL tracing study, Petrovich et al. [45] identified a moderate BLA projection to the ventromedial portions of LH. Likewise, the results of a recent viral labeling study [9] indicate that BLA projects (directly or indirectly) to hypothalamic systems that express leptin receptor and neuropeptide Y (NPY), two peptides known to regulate feeding [15,34]. NPY activity is of special interest because it is known to induce the induction of eating in food-satiated rats [57]. DeFalco et al. [9] injected a pseudorabies virus, constructed to infect neurons that express either NPY or leptin receptor genes (in separate studies), into the arcuate hypothalamic nucleus (AHN) of mice. AHN projects to the two hypothalamic regions most sensitive to NPY-induced eating, the perifornical area and the periventricular nucleus (PVN) [57], as well as several leptin receptor-rich hypothalamic nuclei, including PVN and LH. In both of the studies of DeFalco et al. [9], viral labeling appeared first in LH and later in BLA, suggesting connections between BLA and LH that modulate AHN and other hypothalamic neurons that express NPY and leptin receptors. Interestingly, no label was observed in CN, which suggests that the major projections from CN to LH [32,42,47] are not involved with action of hypothalamic neurons that express these two regulatory peptides. This last observation is consistent with the lack of a CN lesion effect on potentiated feeding in our study.

Second, in another experiment, we [46] showed that communication between BLA and LH is critical to the potentiated feeding effect. Rats received unilateral lesions of BLA and LH, either in the same hemisphere or in opposite hemispheres. Because interhemispheric projections are sparse at this level, rats with contralateral lesions lack communication between BLA and LH in either hemisphere, whereas those with ipsilateral lesions have BLA–LH com-

munication intact in one hemisphere. Using a behavioral procedure similar to that used in the present studies, we [46] found potentiated feeding in sham-lesioned controls and rats with ipsilateral lesions, but not in rats with contralateral lesions that disconnected BLA and LH.

That study [46] did not distinguish between the action of direct and indirect connections between BLA and LH. For example, BLA has major projections to nucleus accumbens (ACB) [29,32], which in turn sends substantial projections to LH [30]; moreover, an ACB–LH system has been implicated in feeding behavior [27,28]. Mediation of potentiated feeding by an BLA–ACB pathway is consistent with evidence that other behavioral manifestations of CS value, such as second-order conditioning [55] and secondary reinforcement [14], depend on the integrity of connections between those brain regions. Finally, BLA may influence LH by other routes as well; it projects heavily to several other brain regions that project to LH, including medial and lateral prefrontal cortical areas, the substantia innominata, and the bed nuclei of the stria terminalis [1,19,25,29,31,32,54].

Further specification of the functional neuroanatomy of these different behavioral aspects of CS value may help clarify some apparent inconsistencies in the effects of BLA and CN lesions on phenomena attributed to conditioned incentive motivation. Hall et al. [20] found that CN, but not BLA, lesions interfered with the ability of a Pavlovian CS previously paired with food to enhance operant responding for that same food reinforcer. This Pavlovian-to-instrumental transfer (PIT) effect is often attributed to the expression of a Pavlovian-conditioned motivational or incentive process, which in turn enhances operant behavior [12]. Given our current and previous findings, we would have anticipated the opposite results—that PIT would have been disrupted by BLA, but not by CN, lesions. Of course, if BLA control of potentiated feeding is mediated by its action on hypothalamic systems specifically involved in consummatory aspects of feeding, it would not be surprising that BLA lesions do not affect PIT. Many researchers, from Craig [7] to the present day [40,41], have argued that control of appetitive and consummatory aspects of motivated behavior can be quite independent, and involve different brain systems. Alternately, PIT effects are known to be determined by a number of factors, and are not always described as reflecting motivational processes [6,48,60]. For example, Rescorla [48] attributed PIT to mediation by sensory, rather than affective, aspects of reinforcers.

Unfortunately, although our data support the idea that CS-potentiated feeding in this preparation reflects the control of some incentive motivational process by Pavlovian CSs beyond simple approach CRs, they do not permit precise specification of that process. For example, as a result of associative learning, the CS might activate physiological signals that normally produce reports of hunger or otherwise engage eating [11], they might facilitate the performance of consummatory responses elicited by oral stimulation [59], or

they might induce a "craving" for a particular foodstuff by activating a representation of that food [2,8,23]. Amygdala function has been implicated in each of these processes [18].

From a broader perspective, it is important to note that a variety of conditioning manipulations, drugs, and lesions have been noted to have diverse effects on different components of food-related behavior [2,3,13,23,40,41,59]. For example, under some circumstances, sated rats will perform operant or Pavlovian CRs anticipatory to food delivery, but fail to consume that food ("resistance to satiation" [38,64]). Similarly, Weingarten and Martin [65] showed that systemic administration of the opiate antagonist naloxone reduced food consumption but not appetitive CRs anticipatory to the delivery of that food. By contrast, those investigators also found that systemic injection of the dopamine antagonist α-flupentixol reduced conditioned food cup responses to a CS but did not affect its ability to potentiate eating. Likewise, Wyvell and Berridge [66] found that intraaccumbens injections of amphetamine enhanced Pavlovian to instrumental transfer, but left orofacial responses to food itself unaffected. And Dickinson et al. [13] found different effects of dopamine antagonists on Pavlovian and instrumental expressions of incentive motivation.

On the other hand, a variety of behavioral, pharmacological, and surgical interventions have been observed to have consistent, rather than dissociated, effects on other subsets of feeding behaviors [2,10,28]. Although the many reported dissociations indicate that "incentive motivation" is not a unitary psychological process, further examination of the neural systems involved in phenomena attributed to "hunger" or "incentive motivation" will be useful for a more complete specification of the organization of the behavioral systems engaged in feeding and appetitive learning.

Acknowledgments

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