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Effects of reward timing information on cue associability are mediated by amygdala central nucleus

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Abstract

The central nucleus of the amygdala (CeA) has been implicated in a range of associative learning phenomena often attributed to changes in attentional processing of events. Experiments using a number of behavioral tasks have shown that rats with lesions of CeA fail to show the enhancements of stimulus associability that are normally induced by the surprising omission of expected events. By contrast, in other tasks, rats with lesions of CeA show normal enhancements of associability when events are presented unexpectedly. In this experiment, we examined the effects of CeA lesions on changes in cue associability in a reward timing task. In sham-lesioned rats, the associability of cues that were followed by stimuli that provided reward timing information was maintained at higher levels than that of cues that were followed by uninformative stimuli. Rats with lesions of CeA failed to show this advantage. These results indicate that the role of CeA in the modulation of associability is not limited to cases of event omission.

Keywords

amygdala; associability; attention; information; Pearce-Hall model; reward timing

The central nucleus of the amygdala (CeA) has been implicated in a range of associative learning phenomena often attributed to attentional processes, including the acquisition of approach or orienting responses to food-reinforced conditioned stimuli (CSs; Gallagher, Graham, & Holland, 1990; Parkinson, Robbins, & Everitt, 2000), the occurrence of surprise-induced enhancements in learning (e.g., Holland & Gallagher, 1993a,b; 2006) and performance in a spatial multiple-choice task thought to reflect sustained or selective attention (Holland, Han, & Gallagher, 2000; Robbins, 2002.)

Recently, our laboratory has been especially concerned with CeA's role in producing the enhanced learning normally seen after the surprising omission of an expected appetitive event. Within the context of the Pearce-Hall (1980) model of associative learning, the associability of a CS (the rate at which it can enter into new associations) declines if the consequences of that CS are well-predicted, but increases if they are unexpected. For example, if after extensive exposure to a light→tone serial compound, the tone is omitted on a portion of the trials, rats subsequently learn light→food associations more rapidly than if only light→tone trials were presented (Wilson, Boumphrey, & Pearce 1992). Within the

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Pearce-Hall model, extended exposure to the light→tone sequence reduces the associability of the light, but violating the light→tone expectancy by presenting the light alone restores that associability, allowing the light to acquire associations with the food more rapidly later. Holland and Gallagher (1993b) replicated Wilson et al.'s finding, but showed that rats with CeA lesions failed to display such enhancements in learning after surprising omission of the expected tone. Similarly, in unblocking experiments in which one CS is first paired with a serial reinforcer comprising two deliveries of food separated by a few seconds, the surprising omission of the second food delivery can yield more learning about a new CS added at that time than occurs if the serial reinforcer is delivered as usual (e.g., Holland, 1984, 1988; Dickinson, Hall, & Mackintosh, 1976). However, Holland & Gallagher (1993a) found that rats with CeA lesions failed to display this unblocking after downshifts in the number of food pellets delivered. Finally, Maddux et al. (2007) found that overshadowing of conditioning to auditory cues in visual+auditory compounds was greater after partial reinforcement of the visual cues than after consistent reinforcement in control rats, but that no such enhanced overshadowing was noted after partial reinforcement in CeA-lesioned rats. In each of these examples, omission of expected events after delivery of a cue enhanced the subsequent associability of that cue in control rats, but not in rats with CeA lesions.

These and other findings (e.g., Holland, Ciall, & Thornton, 2000; Holland & Kenmuir, 2005) led us to propose that CeA is critical for the enhancement of associability after the surprising omission of important events (Holland & Gallagher, 1999; Holland & Maddux, 2010). Interestingly, evidence also suggests that CeA is not critical for associability enhancements that occur after the surprising presentation, rather than omission, of a reinforcer in unblocking procedures (e.g. Holland & Gallagher, 1993b, Holland, 2006).

The present experiment was designed to determine whether the CeA is important for a different example of an attention-driven enhancement of learning that may occur independently of the surprising omission of an outcome. Specifically, the associability of a cue is enhanced if that cue is followed by stimuli that are informative about the delay to reinforcement, relative to the associability of a cue that is followed by the same stimuli, but which provide less information about reinforcement delay. We used a serial compound conditioning task originally developed by Swan and Pearce (1988, Experiment 2) to manipulate the information value of an immediate outcome, while keeping the rate of reinforcement and all other characteristics of the outcomes constant with regard to the target cue. We trained half of our rats with one short (10 s) and one long (30 s) CS (tone and noise, counterbalanced). The other half of the rats were also trained with both noise and tone CSs but each of those CSs was 10 s in duration on half of the trials, and 30 s on the other half. Thus, in the former, informative, training procedure, at their onsets, the identity (tone or noise) of the auditory CSs provided information about the time to reinforcer delivery, whereas in the latter, uninformative, condition, the CS onsets provided no such information. After these CSs were extensively trained, a visual cue (light) was introduced that preceded the long and short CSs on every trial. In both groups, the light itself provided no information regarding the timing of the ultimate reward, but the light in the informative condition was followed by useful information, because the physical identity of the subsequent CS informed the subjects about the time of reward. After this serial conditioning, changes in the associability of the light were assessed by examining the rate of acquisition of light→food associations after direct pairing of those two events. As in Swan and Pearce's (1988) experiment, light→food learning was faster after exposure to the informative treatment. Notably, rats with lesions of CeA failed to show this enhancement of learning.

Method

Subjects

The subjects were 37 male Long-Evans rats (Charles River laboratories, Raleigh, NC) housed individually in plastic cages with metal-grid tops and wood-chip bedding. Two weeks after surgery and one week prior to any behavioral training and assessment, the rats were reduced to and maintained at 85% of their free-feeding body weights. The laboratory vivarium was illuminated from 7 a.m. to 7 p.m., and sessions were conducted in the light phase.

Surgical Procedure

Approximately three weeks before behavioral training, 23 rats received bilateral neurotoxic lesions of the CeA under isoflurane (Isovet; Mallinckrodt, Mundelein, IL) anesthesia. CeA lesions were made using ibotenic acid at a concentration of 10.0 mg/ml, in phosphate buffered saline (Sigma-Aldrich, St. Louis, MO) to which sodium hydroxide was added to yield a pH of 7.4. Injections (0.20 μ l) were made 2.3 mm posterior to bregma, 4.2 mm from the midline, and at 7.7 mm ventral from the skull surface at the injection site. Fourteen other rats received sham lesions, which involved the same process with only vehicle injections. Neurotoxins and vehicle were infused at a rate of 0.1 μ l/min with a Hamilton microsyringe equipped with a 27-gauge needle.

Apparatus

The behavioral training apparatus consisted of four individual chambers ($22.9 \times 20.3 \times 20.3$ 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. Ventilation fans provided masking noise (70 dB). A 6-W lamp behind a dense red lens, mounted next to the speaker, provided constant dim illumination. A solenoid was mounted outside of the box, which could deliver a 0.2-M sucrose solution into a food cup recessed in the center of one end wall. A 0.1-ml delivery of sucrose served as the reinforcer in this experiment. An infrared photocell placed just inside the food cup was polled (1 kHz) by computer circuitry to measure head entries and time spent in the food cup. A 6-W lamp was mounted behind a jeweled lens on the wall above the food cup; illumination of this lamp served as the target, light CS. Each chamber was enclosed in a sound-resistant shell. A speaker, used to present a white noise and a 1,500-Hz tone (both 8 dB above background), was mounted on the inside wall of the inner shell.

Behavioral training procedures

All rats first received a single 64-min session in which each rat received 16 unsignalled reinforcers, intended to habituate the rats to the chambers, and train them to consume sucrose from the food cup when the solenoid was operated. Table 1 shows an outline of the remaining procedures of the experiment. First, all rats received twenty 64-min Phase 1 training sessions, each with 8 reinforced noise and 8 reinforced tone trials, randomly intermixed. In the informative (I) group, the identity of auditory cue was informative concerning its duration and delay to reinforcement, with one cue (counterbalanced) always 30 s in duration and the other always 10 s. In the uninformative (U) group, cue identity provided no information about the delay to reinforcement; half of the noise and tone presentations were 30 s, whereas the other half of the presentations were 10 s in duration.

Phase 2 training continued as in Phase 1, except that a 10-s light preceded the auditory stimuli on every trial. Thus, in Group I, the light was followed by auditory cues whose onsets signaled delay to reinforcement, whereas in Group U, the light was followed by cues whose onsets were uninformative about reinforcement delay. This training was scheduled to

occur over 10 sessions. Due to an experimenter error, 3 sham and 8 lesion subjects received 2 extra sessions of Phase 2 training; data from these extra sessions are not presented.

Finally, assessment of the associability of the light occurred across eight 64-min test sessions. In each session, subjects received 16 reinforced presentations of the 10-s light CS.

Data analysis

Conditioned responding was measured as the percentage of the time that the subjects spent in the food cup during pre-CS and CS periods. To reduce within-group variance in responding observed during the CSs, elevation scores were constructed by subtracting the average pre-CS responding on a given day for each rat from the CS responding on that day for that rat. Pre-CS responding did not differ among the groups. Preliminary analyses showed no differences in responding to the noise and tone cues, and no interactions of cue identity with the other experimental variables, and so for this report, the data were collapsed across cue identity. The primary measure of responding to the auditory cues during Phases 1 and 2 was the elevation scores during the first 10-s of each cue. Presumably, rats in Group I should exhibit greater responding during the 10-s cues than during the first 10 s of the 30-s cues, whereas rats in Group U should respond indiscriminately during the first 10 s of each cue. We also reported elevation scores for each 10-s interval of the 30-s cues. Finally, the elevation scores for the light cue were calculated with the last 5 s of the 10-s cue, because previous studies have shown that food-seeking conditioned responses are most frequent in that time interval, and are less likely to be contaminated by visual orienting responses (Holland, 1977). Although Swan and Pearce (1988) determined that the augmented conditioning in the informative group was preceded by increased orienting to the light in Phase 2, we did not record the orienting response here, because in the past we have not found changes in orienting to be correlated with changes in associability in our lab

The data from each phase were subjected to mixed-design analyses of variance (ANOVAs), with variables of lesion (CeA vs. sham), group (informative vs. uninformative), cue (10 s vs. 30 s; for auditory cues only), and session. Separate ANOVAs were conducted on the elevation scores for the auditory cues and the visual cue, and on pre-CS responding. For the test phase, ANOVAs were followed by planned comparisons to evaluate the hypotheses that sham-lesioned rats in Group I should show more test responding to the light than sham-lesioned rats in Group U, whereas no such enhancement should be observed in CeA-lesioned rats.

Histological procedures

All rats were euthanized with an overdose of sodium pentobarbital (100 mg/kg) and perfused with 4% formalin. The brains were removed and post-fixed in 4% formalin with 20% sucrose as a cryoprotectant for 24 hrs. The brains were then sliced on a freezing microtome into 40- μ m sections. These sections were then mounted on slides and Nissl-stained with thionin to assess lesion damage. Each lesion was drawn on plates (shown in Figure 1) from Paxinos and Watson's (1998) atlas, and the areas of the lesions were compared to the areas of the CeA regions outlined on those plates.

Results

Histology

Six lesioned rats were discarded from the study because of insufficient damage to the CeA. Acceptable lesions destroyed at least 40% of the medial division of CeA. The mean ($\pm$ sem) damage was $55\pm 8\%$ in Group Lesion-I and $63\pm 8\%$ in Group Lesion-U. Except for minimal

damage at the injector tips, no tissue loss was observed in the CeA of sham-lesioned rats. Figure 1 shows drawings of minimum and maximum lesion extents.

Behavior

Two CeA-lesioned and two sham-lesioned rats failed to show appreciable acquisition of food cup responding to the auditory cues in the first two phases, and were eliminated from the analyses. The resulting numbers of subjects in each group were 7, 5, 8, and 7 for Groups Sham-I, Sham-U, Lesion-I, and Lesion-U, respectively.

Phase 1—Pre-CS responding declined over the course of Phase 1 in all groups, $F(19, 437) = 5.37$, $p < .001$. There were no effects of group or lesion, $F_s < 1$, $p_s > .37$; overall mean $\pm$ sem percentage times in the food cup during the pre-CS periods were $12.0 \pm 2.2\%$, $10.7 \pm 2.2\%$, $13.3 \pm 2.2\%$, and $14.8 \pm 3.9\%$ in groups Sham-U, Sham-I, Lesion-U, and Lesion-I.

Responding to the auditory cues was acquired over the course of Phase 1, but differed substantially as a function of group. The left portions of Figures 2A and 2B show responding during the first 10 s of the 10-s and 30-s cues in the informative (I) and uninformative (U) groups, respectively. As would be expected, rats in the informative condition responded more during the 10-s cue than during the 30s cue, whereas rats in the uninformative condition (in which there was no way to distinguish the two cue durations until the end of the first 10-s interval) did not. Cue responding was not significantly affected by lesion, although there was a trend toward greater responding by lesioned rats than shams in the uninformative condition.

A lesion X group X cue X session ANOVA of cue elevation scores for the first 10-s cue periods showed significant main effects of cue, $F(1, 23) = 13.67$, $p = .001$, and session, $F(19, 437) = 7.97$, $p < .001$, and significant interactions of group X cue, $F(1, 23) = 18.83$, $p < .001$, cue X session, $F(19, 437) = 3.92$, $p < .001$, and group X cue X session, $F(19, 437) = 2.52$, $p < .001$. Neither lesion nor any of its interactions was significant, $F_s < 1.76$, $p_s > .19$. A separate ANOVA of elevation scores in the informative groups only, showed significant main effects of cue, $F(1, 13) = 21.56$, $p < .001$, and session, $F(19, 247) = 8.22$, $p < .001$, and a significant cue X session interaction, $F(19, 247) = 5.76$, $p < .001$. Neither lesion nor any of its interactions was significant, $F_s < 1$, $p_s > .63$. By contrast, a comparable ANOVA of uninformative groups only, showed no significant main effects or interactions, $F_s < 2.27$, $p_s > .163$, except for sessions, $F(19, 190) = 2.10$, $p = .006$.

Rats in the informative groups showed timing during the 30s cue, displaying increasing responding over each 10-s bin, whereas rats in the uninformative groups did not (Table 2). Timing did not differ significantly as a function of lesion. A lesion X group X bin X session ANOVA showed significant group X bin, $F(2, 46) = 4.50$, $p = .016$, and bin X session, $F(38, 874) = 1.93$, $p < .001$, interactions. No other effects or interactions were significant, $F_s < 2.15$, $p_s > .128$. A subsequent lesion X interval ANOVA for informative rats only, over the last 10 sessions, showed only a significant effect of bin, $F(2, 26) = 8.06$, $p = .002$; no other effects or interactions were significant, $F_s < 1.07$, $p_s > .390$. By contrast, a comparable ANOVA for the uninformative rats showed no significant main effects or interactions, $F_s < 1.62$, $p_s > .224$.

Phase 2—Pre-CS responding remained stable over the course of Phase 2 in all groups. Overall mean $\pm$ sem percentage times in the food cup were $10.4 \pm 2.5\%$, $9.0 \pm 1.6\%$, $11.4 \pm 1.8\%$, and $15.0 \pm 3.8\%$ in groups Sham-U, Sham-I, Lesion-U, and Lesion-I, respectively. A lesion X group X sessions ANOVA showed no significant main effects or interactions, $F_s < 1.60$, $p_s > .21$.

Figure 1C shows elevation scores to the added light cue, and the right portions of Figures 2A and 2B show responding during the first 10s of the auditory cues in the informative and uninformative groups, respectively. Presentation of the light initially suppressed food cup responding relative to baseline, but that tendency habituated over the course of Phase 2. In addition, introduction of the light temporarily disrupted responding to the auditory cues, especially in the CeA-lesioned rats, but by the end of Phase 2, the patterns of responding observed during the auditory cues were similar to those observed at the end of Phase 1. However, in the uninformative condition, by the end of Phase 2 the lesioned rats showed significantly more responding during the first 10 s of the auditory cues than the sham-lesioned rats.

A lesion X group X session ANOVA of elevation scores to the light showed only a main effect of sessions, $F(9, 207) = 10.97$, $p < .001$; remaining $F_s < 1.55$, $ps > .227$. A lesion X group X cue X session ANOVA of cue elevation scores for the first 10-s period of the auditory cues showed significant main effects of cue, $F(1, 23) = 20.16$, $p < .001$, and session, $F(9, 207) = 6.81$, $p < .001$, and significant group X cue $F(1, 23) = 17.93$, $p < .001$, and lesion X sessions, $F(9, 207) = 1.96$, $p < .050$, interactions. No other effects or interactions were significant, $F_s < 2.74$, $ps > .111$. A separate ANOVA of elevation scores in the informative groups only, showed significant main effects of cue, $F(1, 13) = 24.81$, $p < .001$, and session, $F(9, 117) = 5.36$, $p < .001$, and a significant lesion X session interaction, $F(9, 117) = 2.43$, $p = .014$. Neither the main effect of lesion nor any other interaction was significant, $F_s < 1.83$, $ps > .199$. By contrast, a comparable ANOVA of uninformative groups only, showed no significant main effects or interactions, $F_s < 2.95$, $ps > .116$, except for sessions, $F(9, 90) = 2.46$, $p = .015$. An identical analysis of these rats' performance confined to the last 2 sessions of Phase 2 showed a significant effect of lesion, $F(1, 10) = 5.485$, $p = .041$. Interestingly, whereas among sham-lesioned rats, cue responding in the uninformative condition was similar to responding to the 30-s cue in the informative condition, among CeA-lesioned rats, cue responding in the uninformative condition was similar to responding to the 10-s cue in the informative condition. Post-hoc comparisons using the Tukey HSD procedure showed that the sham-lesioned rats' responding during the 10-s cues and the initial 10-s period of the 30-s cues in the uninformative condition each was significantly ($ps < .001$) lower than responding to the 10-s cue in the informative condition, but did not differ from responding during the first 10-s period of the 30s cue in the informative condition ($ps > .953$), whereas the opposite pattern held among CeA-lesioned rats ($ps > .999$ and $ps < .049$, respectively).

As in Phase 1, rats in the informative groups showed timing during the 30-s cue, displaying increasing responding over each 10-s bin, whereas rats in the uninformative groups did not (Table 2). Timing did not differ significantly as a function of lesion. A lesion X group X bin ANOVA of elevation scores during each 10-s bin of the 30s cues showed a significant effect of bin, $F(2, 46) = 9.29$, $p < .001$, and a significant group X bin interaction, $F(2, 46) = 3.95$, $p = .026$. No other effects or interactions were significant, largest $F(2, 46) = 2.55$, $p > .124$ (lesion). A subsequent lesion X bin ANOVA for informative rats only, showed a significant effect of bin, $F(2, 26) = 9.80$, $p < .001$, but no other effects or interactions were significant, $F_s < 1$, $ps > .504$. By contrast, a comparable ANOVA for the uninformative rats showed no significant main effects or interactions, $F_s < 1.71$, $ps > .220$.

Test—Pre-CS responding declined over the course of the test phase in all groups. Overall mean $\pm$ sem percentage times in the food cup were $8.0 \pm 2.1\%$, $7.4 \pm 1.5\%$, $8.6 \pm 1.3\%$, and $10.2 \pm 1.8\%$ in groups Sham-U, Sham-I, Lesion-U, and Lesion-I, respectively. A lesion X group X sessions ANOVA showed only a significant main effect of session, $F(7, 161) = 4.86$, $p < .001$; remaining $F_s < 1.21$, $ps > .283$.

Figure 1D shows the primary data of this experiment, the elevation scores to the light cue in the test phase. Among sham-lesioned rats, acquisition of responding was faster in the informative condition than in the uninformative condition, but no such superiority was observed among lesioned rats. Thus, the CeA lesion prevented the normal enhancement of the light's associability when it had preceded informative cues in Phase 2. A lesion X group X session ANOVA for all sessions but the first (prior to the beginning of acquisition) showed a significant effect of session, $F(6,138) = 14.10$, $p < .001$, and a significant lesion X group interaction, $F(1, 23) = 4.84$, $p = .038$. Planned comparisons showed that responding in the informative condition was greater than responding in the uninformative condition in sham-lesioned rats, $F(1, 23) = 7.79$, $p = .010$, but not in lesioned rats, $F(1, 23) = 0.026$, $p = .872$, and that responding was greater in group Sham-I than in group Lesion-I, $F(1, 23) = 5.36$, $p = .030$.

It is important to note that because there were no differences in the test performances of CeA- and sham-lesioned rats in the uninformative condition, it is unlikely that the CeA lesion effects observed in the informative condition were due to more general deficits in the ability to detect or process the various cues. Similarly, it is unlikely that the low levels of test performance observed in the CeA-lesioned rats in the informative group were due simply to deficits in their ability to perform at such high levels of responding. Although because we ended the test prior to asymptotic performance in any group except Sham-informative we have no evidence in this experiment that CeA-lesioned rats can display high (45–60%) levels of responding to the visual cue, in other procedures involving simple light-food pairings, we have never observed differences in responding of CeA- and sham-lesioned rats even at high (elevation scores of 55–70%) asymptotic levels (e.g., Holland & Gallagher, 1993a, b).

Discussion

Sham-lesioned rats performed in a manner similar to that reported by Swan and Pearce (1988, Exp. 2). Acquisition of conditioned responding to the light in test was more rapid in rats for which that light had previously been followed by auditory cues whose onsets provided information about the timing of food delivery than in rats for which the light had been followed by relatively uninformative auditory cues. One account for this result is that the provision of information about reward timing by the auditory cues contiguous with the light enhanced its associability, relative to the effects of the uninformative treatment. Notably, the temporal relations between light and food itself were identical in the two training conditions: in each group, termination of the light was followed by food 10 s later on half the trials and 30 s later on the other half. Thus, the predictive value of the auditory cues, and not the ability of the light itself to predict the time of reinforcer delivery, was critical to the observed effect.

By contrast, rats with lesions of CeA failed to show more rapid acquisition after pairings of the light with temporally-informative auditory cues. Lesioned rats in both the informative and uninformative treatment conditions acquired light-food conditioning at rates comparable to that observed with sham-lesioned rats in the uninformative condition. Thus, the lesion appeared to interfere specifically with the enhancement of associability consequent to information about reward timing. Although our data do not distinguish between enhancement of the light's associability over its initial (novel) level in the informative condition, versus simple maintenance of that associability relative to a decline in the light's associability consequent to its presentation without informative consequences in the uninformative condition, it is notable that test performance of lesioned and control rats in the uninformative condition did not differ. If the CeA lesions had disrupted any losses in associability that might manifest in Group U, then one would expect greater conditioning in

both CeA lesioned groups relative to the group Sham-U. Thus, consistent with the results of previous studies that used other procedures to assess associability losses (e.g., Holland & Gallagher, 1993a, b), the CeA lesion did not interfere with losses in associability that might accrue when the light was presented without informative consequences.

These results add to our knowledge of the circumstances under which the CeA is critical to the modulation of attention in conditioning. In previous experiments, we found that rats with CeA lesions failed to display the associability enhancements normally observed when expected cue or reinforcement events were omitted (e.g., Holland & Gallagher, 1993a,b; Maddux et al., 2007). By contrast, Holland (2006) found no evidence that CeA lesions impaired associability enhancements observed when an unexpected reinforcer was added in an unblocking 'upshift' design. That outcome led us (Holland, 2006; Holland & Maddux, 2010) to suggest that CeA function might be especially critical for processing the consequences of omission of expected events. However, in the present experiment, the rats in the informative and uninformative conditions received the same auditory and food events and the same timing of food delivery; only the temporal information about food delivery provided by the auditory cues differed across conditions. Thus, CeA-dependent enhancements in associability are not limited to cases of event omission.

Swan and Pearce (1988, Exp. 2) proposed a more detailed, mechanistic analysis of their observation of greater associability in (what we call) the informative than uninformative conditions. They suggested that in the informative condition, the two auditory cues acquired quite different conditioned reinforcement values, because of their different temporal relations with food. Because the light did not predict which of these two valued outcomes would be presented, the rats would be surprised by the outcome's value on each trial, and hence the light's associability would remain, or be driven, high. By contrast, in the uninformative case, both auditory cues had the same, although variable, temporal relation with food, and hence the light was followed by a cue with comparable conditioned reinforcement value on each trial. This consistent relation between the light and the value of the subsequently-presented auditory cues would lead to reductions in the light's associability. Consequently, subsequent learning about the light would be more rapid in the informative than uninformative condition. An advantage of this account is that it recasts the present observation as another case of overexpectation, similar to others that have been found affected by CeA lesions (e.g., Holland & Gallagher, 1993a, b). However, his more detailed explanation requires the assumption that rats in the uninformative condition would indeed abstract a single value for the 4 types of trials presented after the light. If instead, the values of the auditory cues were dynamic throughout training due to their variable duration, or if the rats coded the individual events themselves rather than only a single abstracted conditioned reinforcement value, then rats in the uninformative condition might be at least as surprised by each of those four outcomes as the rats in the informative condition, which received only two possible outcomes. Either way, the fact remains that in the present experiments, CeA lesions interfered with associability enhancements under circumstances that did not involve event omission, unlike in previous studies.

Although few significant differences between CeA-lesioned and sham-lesioned rats were observed prior to the test phase, several features of the data from Phases 1 and 2 deserve comment. As one would expect, in the informative condition, in which one auditory cue was always 10 s long and the other always 30 s in duration, sham-lesioned rats showed evidence of reinforcement timing in two ways. First, responding during the 10-s cue was significantly greater than responding during the first 10 s of the 30-s cue. Second, within the 30-s cue, responding was greater during at the end of the cue, near the time of food delivery, than near the beginning of the CS. Notably, neither of these aspects of timing was affected by the CeA

lesions. Thus, the lack of enhanced associability of the lesioned rats in the informative condition cannot be attributed to lesion-induced deficiencies in timing.

By contrast, sham-lesioned rats in the uninformative condition showed little evidence of timing. Not only was responding during the 10-s cues and during the first 10 s of the 30-s cues identical (recall that these could not be distinguished), but also responding did not increase significantly in frequency over the three 10-s bins on 30-s trials. CeA-lesioned rats in the uninformative condition also failed to show these manifestations of timing. Interestingly, whereas among sham-lesioned rats, cue responding in the uninformative condition was similar to responding to the 30-s cue in the informative condition, among CeA-lesioned rats, cue responding in the uninformative condition was similar to responding to the 10-s cue in the informative condition. We do not have a ready explanation for this lesion effect, which implies different temporal averaging rules for lesioned and nonlesioned rats in the uninformative condition, but note that it is unlikely to have affected the associability of the light, because the lesions did not affect test performance of rats in the uninformative condition.

These findings join many others in describing a role for the CeA in modulating attention in associative learning. However, evidence suggests that CeA functions within distinct brain circuitry for different aspects of this attention. First, the acquisition and expression of conditioned orienting responses (ORs) to cues paired with food depends on a circuit that includes the CeA, the substantia nigra pars compacta (SNc), and the dorsolateral striatum (DLS; Han et al., 1997; Lee et al., 2005). Second, the surprise-induced enhancement of cue associability depends on circuitry that includes the CeA, magnocellular cholinergic neurons in the substantia innominata (SI; Chiba et al., 1995; Han, Holland, & Gallagher, 1999), the posterior parietal cortex (PPC; Bucci, Holland, & Gallagher, 1998), and the SNc (Lee et al., 2006, 2008). Third, performance in a spatial five-choice (5CRT) task depends on the integrity of a circuit that includes CeA, SI, and the medial prefrontal cortex (Holland et al., 2000; Holland, 2007; Maddux et al., 2007). There are multiple dissociations among these functions. For example, SI damage interferes with performance in the 5CRT task and surprise-induced enhancements of cue associability, but has no effect on conditioned ORs (Chiba et al., 1995; Han et al., 1999; Holland, 2007). Similarly, PPC damage interferes with surprise-induced enhancements of cue associability but spares both 5CRT performance and conditioned ORs (Bucci et al., 1998; Maddux et al., 2007; Bucci & Chess, 2005). It remains to be seen how enhancements in cue associability produced by pairings with informative events, as in the present study, is processed within these systems.

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Figure 1.

Drawings of the smallest and largest lesions of amygdala central nucleus at each of several coronal planes (mm caudal to bregma). Brain drawings from Paxinos & Watson, 1998; used by permission of Elsevier.

Figure 2.

Panel A shows the mean ($\pm$ sem) elevation scores during the first 10-s period of the 10-s and 30-s auditory cues in Groups Sham-informative and Lesion-informative in Phases 1 and 2. Panel B shows comparable elevation scores in Groups Sham-uninformative and Lesion-uninformative. Panel C shows elevation scores for the light cue in Phase 2, and Panel D shows elevation scores for the light cue in the eight test sessions.

Table 1

Experimental design

Group	Phase 1	Phase 2	Test
informative (I)	160 T10→S / 160 N30→S or 160 N10→S / 160 T30→S	80 L10→T10→S / 80 L10→N30→S or 80 L10→N10→S / 80 L10→T30→S	128 L→S
uninformative (U)	80 T10→S / 80 N10→S/ and 80 T30→S / 80 N30→S	40 L10→T10→S / 40 L10→N10→S/ and 40 L10→T30→S / 40 L10→N30→S	128 L→S

Notes. T = tone; N = white noise; L = light, S = sucrose; 10 denotes a cue duration of 10 s; 30 denotes a cue duration of 30 s; 40, 80, 128, and 160 refer to the total number of trials of a given type that were delivered in a phase.

Table 2
Temporal distribution of elevation scores during the 30-s cues in Phases 1 and 2 (mean $\pm$ sem)

Group	Phase 1			Phase 2		
	bin 1	bin 2	bin 3	bin 1	bin 2	bin 3
Sham-I	20.4 $\pm$ 3.0	26.1 $\pm$ 4.9	29.0 $\pm$ 6.6	17.2 $\pm$ 2.6	21.4 $\pm$ 4.6	28.3 $\pm$ 6.7
Lesion-I	13.4 $\pm$ 2.8	16.4 $\pm$ 3.2	17.5 $\pm$ 3.7	19.4 $\pm$ 2.0	27.9 $\pm$ 2.9	29.3 $\pm$ 3.6
Sham-U	13.3 $\pm$ 8.6	14.5 $\pm$ 9.1	14.5 $\pm$ 9.3	14.0 $\pm$ 7.6	17.2 $\pm$ 10.0	17.6 $\pm$ 9.5
Lesion-U	24.2 $\pm$ 7.8	24.0 $\pm$ 9.2	23.6 $\pm$ 8.4	33.2 $\pm$ 8.6	34.6 $\pm$ 10.0	33.8 $\pm$ 9.1

Notes. Elevation scores were constructed by subtracting the percentage of time in the food cup during pre-CS periods from the percentage of time in the food cup during cue periods. The bins refer to the first, second and third 10-s intervals after cue onset. I = informative condition, U = uninformative condition (see Table 1).