Published in final edited form as:

Behav Neurosci. 2005 February ; 119(1): 202–212.

Amygdala central nucleus function is necessary for learning but not expression of conditioned visual orienting

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Abstract

When exposed to pairings of a visual stimulus with food delivery, rats normally acquire both conditioned orienting responses directed toward the visual stimulus and conditioned food-related responses. Consistent with the results of previous lesion studies, reversible inactivation of amygdala central nucleus function before each conditioning session prevented the acquisition of conditioned orienting responses, whereas food-related behaviors were acquired normally. By contrast, neither inactivation nor neurotoxic lesions of central nucleus affected the expression of previously-acquired conditioned orienting responses. Thus, the central nucleus is apparently not critical to the maintenance of information required for conditioned orienting, but instead is necessary for memory storage elsewhere. Specialized roles for components of a circuit for conditioned orienting, which includes the central nucleus, the substantia nigra, and dorsolateral striatum, are discussed.

Keywords

amygdala central nucleus; attention; conditioning; NBQX; orienting; rat

Keywords

ANOVA Analysis of variance; CN central nucleus of amygdala; CS conditioned stimulus; DLS dorsolateral striatum; NBQX 1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide; OR orienting response; SNC substantia nigra pars compacta

Novel stimuli often elicit orienting responses (ORs), including both diffuse and directed movements, autonomic responses, and changes in brain activity that may indicate arousal or vigilance (e.g., Holland, 1984; Kapp et al., 1992; Sokolov, 1963). Although these ORs typically habituate rapidly when a stimulus is presented without consequence, they may reemerge if the stimulus signals the delivery of a biologically significant event in associative learning procedures (e.g., Dykman, 1967; Holland, 1984; Kapp et al., 1979; Maltzman, 1977; Pavlov, 1927; Sokolov, 1963). For example, Holland (1977) found that rats normally acquire conditioned behavioral ORs to visual or auditory conditioned stimuli (CSs) that signal food delivery. Gallagher, Graham, and Holland (1990) found that the emergence of these conditioning-dependent ORs (rearing and moving toward a visual stimulus and startle to an auditory stimulus) depended on intact function of the amygdala central nucleus (CN). Rats with bilateral neurotoxic lesions of CN showed normal unconditioned ORs to those stimuli when they were initially presented alone, but failed to acquire conditioned ORs when those stimuli were paired with food. Notably, that learning deficit was confined to conditioned ORs;

lesioned rats were unimpaired in other aspects of learned behavior, including the acquisition of responding directed toward the site of food delivery.

Han et al. (1997) found that CN exerted its influence on conditioned orienting through the nigrostriatal system. Neurons in the medial division of the CN project to dopaminergic neurons of the substantia nigra pars compacta (SNc) that innervate the dorsolateral striatum (DLS; Gonzalez & Chesselet, 1990; Lee, Cantalini, Gallagher, & Holland, 2003). Elevated activity in the nigrostriatal dopamine system has frequently been linked to enhanced responsiveness to sensory stimuli (e.g., Chevalier & Deniau, 1990; Schultz, 1992). Furthermore, damage to this system, including both lesions of the DLS and of ascending dopamine neurons (e.g., Carli et al, 1985, 1989; Ljungberg & Ungerstedt, 1976) impairs even unconditioned orienting to sensory cues.

Han et al (1997) demonstrated that disconnection of CN and DLS, accomplished by a unilateral neurotoxic lesion of CN in one hemisphere combined with depletion of dopaminergic innervation of the DLS by 6-hydroxydopamine (6-OHDA) in the contralateral hemisphere, produced the same pattern of deficits in conditioned orienting as bilateral CN lesions. Because unilateral damage to either CN or DLS alone had no effects (Han et al., 1997, 1999), the deficits found with contralateral damage to these two structures suggest that normal acquisition and expression of conditioned ORs involves interaction of CN and DLS.

Using similar procedures, Han et al (1997) also found that transient inactivation of DLS by lidocaine infusion during training interfered with the display of conditioned ORs during the periods of inactivation, but those ORs emerged when the inactivation procedures were terminated. They concluded that intact DLS function was necessary for the expression of conditioned ORs, but was not critical for OR acquisition. Instead, the substrate for conditioned ORs is likely to be afferent to DLS, for example, in SNc or CN itself. The present studies used transient inactivation and lesion procedures to determine the role of CN in the acquisition and expression of conditioned orienting.

Experiment 1

In Experiment 1 we examined the effects of transient inactivation of CN on the acquisition and expression of conditioned orienting. Half of the rats received nonreinforced pretesting and reinforced training of a visual CS while CN function was interrupted by infusions into the CN of NBQX (1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide), a competitive antagonist at non-NMDA ionotropic AMPA receptors. This agent, which has been used in previous studies of amygdala function (e.g., Walker & Davis, 1997), effectively blocks the induction of excitatory post synaptic currents. The remaining rats received those same experiences after infusion of vehicle. Responding to the visual CS was then examined in two test sessions. For all rats, CN function was disrupted by NBQX infusion prior to one session, whereas in the other session function was intact (vehicle infusions).

If CN function, like that of DLS, is critical only for the expression of conditioned ORs, then rats should display those learned behaviors when tested in the absence of NBQX, regardless of CN status in training. Alternately, if CN function is required for both acquisition and expression of conditioned orienting, for example, if CN is a site responsible for maintenance of information needed for conditioned potentiation of the OR, then rats that receive NBQX infusions during either training or testing should fail to display conditioned ORs during the tests. Finally, if CN function is necessary for the acquisition of conditioned ORs, but not for their expression, then rats trained with CN inactivated by NBQX should fail to display that learned responding in the post-training tests, regardless of whether those tests occur with CN inactivated or with CN function intact. Also in that case, rats trained with CN function intact should express conditioned ORs, regardless of CN status in the post-training tests. This pattern

of results would indicate that CN is critical for encoding the light-food relation, but that information is available for expression elsewhere in the brain.

After the conclusion of testing of responding to the visual CS, we examined the acquisition of conditioned startle responding to a noise paired with food, without any infusions. This test was designed to assess any persistent effects of prior NBQX administration on CN function. If NBQX infusions during visual CS training produced long-term damage or dysfunction in CN, then rats trained with those infusions should be impaired in the acquisition of the conditioned OR to the auditory cue (Gallagher et al., 1990).

Methods

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 one week with ad lib access to food and water, the rats received surgery, followed by two weeks of recovery. The rats were then reduced to 85% of their *ad libitum* weights by restricting their access to food. Throughout the study, the rats lived in individual cages in a colony room illuminated from 6 a.m. to 8 p.m. The experiment was conducted in six procedurally identical replications, each with 4 rats.

Apparatus—The behavioral training apparatus consisted of four individual chambers (22.9 X 20.3 X 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 to record the time each rat spent with its head in the food cup. Each chamber was enclosed in a sound-resistant shell. A speaker, used to present 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. A 6-w lamp was mounted behind a jeweled lens on the front panel, 10 cm above the food cup; illumination of this lamp served as the light CS. 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; the output from each camera was digitized, merged into a single image of all four chambers, and recorded on videotape.

Surgical procedures—All rats received unilateral ibotenic acid lesions of CN and a contralateral placement of a cannula dorsal to the CN. We used this combination of lesion and inactivation, rather than a bilateral inactivation procedure, to provide better localization of orienting function to CN. Although we can not readily specify the boundaries of the inactivating effects of NBQX, the placement and boundaries of the lesion are readily defined. Previous studies (Han et al, 1997, 1999) showed no effects of CN lesions on the acquisition of conditioned orienting unless the medial regions of CN (targeted in the present study) were damaged bilaterally; unilateral lesions had no effect, even when they were very large, encompassing neighboring regions as well as CN. Thus, even if the effects of NBQX spread widely on the cannula side, the observation of limited, specifiable damage on the lesioned side constrains the localization of conditioned orienting function. Because unilateral CN lesions do not affect conditioned orienting, rats that receive infusions of NBQX into the CN contralateral to a lesion should display impairments in that behavior, but rats that receive infusions of vehicle should not.

Surgery was performed under isoflurane anesthesia with aseptic conditions. All 24 rats received unilateral lesions of CN, with contralateral implantation of a cannula just above CN. For half of the rats, the lesions were on the left side and for the other half they were on the right side. The CN lesions were made using stereotaxic coordinates 2.4 mm posterior to bregma and 4.3

mm from the midline, with infusions at a depth of 7.9 mm from the skull surface. Each lesion was made using 0.25 μ l of 10 μ g/ μ l ibotenic acid (Sigma, St. Louis, MO) in PBS solution, infused with a Hamilton 2.0 μ l syringe over a 6-min period. A 31 gauge guide cannula, fitted with a dummy injector (Plastics One, Roanoke Rapids, Virginia) was chronically implanted in each rat using stereotaxic coordinates 2.4 mm posterior to bregma and 4.3 mm from the midline, with its tip at a depth of 5.9 mm from the skull surface.

After surgery, all rats received a single 0.02 ml subcutaneous injection of buprenorphine hydrochloride (Sigma, St. Louis, MO) for amelioration of pain, and were allowed to recover from surgery for 10–14 days before further behavioral testing. To prepare the rats for infusions, they were handled extensively for the last 7 days of the recovery period, including acclimation to removal and insertion of the dummy injector, for the final 5 days.

Histological procedures—After completion of behavioral testing, the rats were deeply anaesthetized with Nembutal (150 mg/kg), and perfused with 0.1-M phosphate-buffered saline (PBS), followed by 10% (v/v) formalin. After removal of the cannulas, the brains were removed and stored in 0.1-M PBS with 20% (w/v) sucrose at 4 C° for 24–48 hr. Sections (40- μ m) were taken from each brain, and alternate sections were mounted on slides and Nissl-stained to verify the lesions.

Behavioral observation procedures—Conditioned orienting was evaluated by scoring behavior from the videotaped recordings of the sessions. An instantaneous sampling procedure was used in which ongoing behavior was recorded at 1.25-s intervals (signaled by a click recorded on the videotape) during the 10 s prior to each CS presentation and during each CS presentation. At each interval the nature of ongoing behavior was recorded; these behaviors were chosen from a number of categories including behaviors directed toward the food cup, head jerk, walking, and rearing (see Holland, 1977). Only rearing was reported here; this behavior was scored when a rat's front feet were both off the floor, excluding when the rat was grooming. Because rearing is more frequent during the first half of 10-s CSs (e.g. Holland, 1977; Gallagher et al., 1990) we used the frequency of rearing in that period as our measure of orienting. The metric "percentage behavior" was constructed by dividing the number of samples in which rearing was observed in a CS or pre-CS interval by the number of sampling intervals and multiplying by 100. Because the number of sampling intervals was constant, this measure is an absolute frequency measure. In addition, on trials with auditory CSs, the occurrence of a startle response, defined as a sudden jump or change in position within the first 1.25 sec of each CS presentation, was recorded. The relation of this rapidly-habituating behavior to the more robust acoustic startle response to high intensity auditory cues, which is often defined more precisely in terms of latency and waveform (e.g. Davis, 1992), is unknown. A single observer scored the data; to assess the objectivity of the scoring, two additional observers scored data from a random selection of sessions from all replications. Each additional observer agreed on 92% of over 1000 joint observations.

Finally, the primary measure of food related behavior was the percentage of time the rat spent in the recessed food cup, as measured by the infrared photocell sampling system. Because food cup entry is more frequent during the second half of 10-s CSs (e.g. Holland 1977; Gallagher et al., 1990), and this interval is less contaminated by orienting behaviors, we reported food cup responding in the last five sec of CS periods.

Infusion procedures—Twenty minutes before each designated training session, the dummy cannula was removed from each rat, and an injector cannula inserted and secured into the guide cannula so that its tip projected 2 mm beyond the guide cannula. The injector cannulas were connected by PE50 tubing to 10 microliter Hamilton syringes placed in a multigang precision syringe pump. The syringe pump delivered 0.2 μ l of 20 mg/ml NBQX (Sigma, St. Louis, MO)

or the PBS vehicle alone over a 1-min interval. The injector was left in place for an additional 1 min. After removal of the injector cannula and replacement of the dummy cannula, each rat was placed in a small (22 x 11 x 11 cm) confinement cage for the time remaining before the behavioral training session.

Behavioral training procedures—Table 1 provides an outline of the procedures of Experiment 1. The rats first received a 32-min pretest session with 8 illuminations of the light CS for 10 s, with no food delivery. In this and all subsequent sessions, the events were presented with intertrial intervals that ranged from 2 to 6 min, mean = 4 min. Twenty min before this session, rats in the NBQX condition received NBQX infusions and rats in the VEH condition received vehicle infusions. Next, two 64-min sessions were used to train the rats to approach and eat from the food cup on food delivery. Each of these sessions included 16 deliveries of two food pellets (45 mg grain pellets; Research Diets, New Brunswick, NJ). No infusions were given prior to these two sessions. Then, the rats received three 64-min conditioning sessions, each of which had 16 light-food pairings. These sessions were also preceded by NBQX or vehicle infusions, as with the pretest session. Over the next two days, the rats received 2 test sessions identical to the pretest session. Half of the rats from each training condition received NBQX infusions prior to test 1 and Vehicle infusions before test 2, and the other half received the two infusions in the opposite order. Finally, in five of the replications, the rats received 3 additional 64-min sessions to assess any lingering effects of NBQX on the acquisition of startle responding to a noise CS. In each of these sessions the rats received 8 reinforced presentations of a 10-s white noise and 8 nonreinforced presentations of a 1,500-hz tone stimulus. Throughout the experiment, one session was administered each day at approximately the same time (10:00 a.m.); the three training and two test sessions were run on consecutive days.

Results

Histological results—Eight rats were discarded from the study because they lost their headsets, or developed obstructed cannulas. Of the remaining 16 rats, 13 were judged as having acceptable lesions. Lesions were rejected ($n = 3$) if there was less than 50% damage to the medial portion of CN (which projects to SNC), or if there was more than minimal damage to regions adjoining CN. The acceptable brains averaged 65% damage to medial CN, and 40% to CN overall. One additional rat was discarded because of damage to CN on the cannulated side. The remaining 12 rats showed accurate placement of the cannula above CN but no obvious damage to CN on that side. Of those rats, 6 had received NBQX infusions in training (3 on the left side and 3 on the right) and 6 had received vehicle (4 on the left side and 2 on the right side). Figure 1 shows a Nissl-stained section of a brain with a CN lesion on the left side and a cannula placement and intact CN on the right side.

Behavioral results—Figure 2 shows conditioned ORs (rearing, Figure 2A) and food cup entry (Figure 2B) elicited by the visual CS during the pretest and training phases of Experiment 1. As in previous studies with intact rats (e.g., Holland 1977,1980), among rats that received infusions of vehicle prior to each session, unconditioned rearing to the light CS habituated rapidly in the pretest, but was reacquired as a conditioned response after light-food pairings. By contrast, rats that received infusions of NBQX during these training phases showed no evidence of acquisition of conditioned rearing, despite normal unconditioned rearing in the pretest. These data are similar to those displayed by rats with pretraining lesions of CN (Gallagher et al, 1990). An infusion type (NBQX or vehicle) X sessions (with half-session blocks, shown in Figure 2, nested within sessions) ANOVA of rearing during training showed significant effects of infusion type, $F(1, 10) = 5.02$, $p = .049$, and sessions, $F(2, 20) = 5.35$, $p = .014$, and an infusion type X sessions interaction, $F(2, 20) = 5.57$, $p = .012$. An infusion type X block ANOVA of responding in the pretest showed only a significant effect of block, $F(1,$

10) = 7.11, $p = .024$, indicating the habituation of rearing over trials, but no significant effect of infusion or infusion X block interaction, $F_s < 1$.

At the same time, food cup responding was unaffected by NBQX infusion; no food cup responding was observed during visual CS presentations in the pretest session, but that responding was rapidly acquired in both groups when the light was paired with food. ANOVAs comparable to those reported for rearing showed no significant effects or interactions involving infusion type for either phase, $F_s < 1.08$. These data also replicate the findings of Gallagher et al (1990), who found no effect of pretraining lesions of CN on the acquisition of conditioned food cup responding.

The primary data of this experiment are those from the two test sessions, in which all rats were tested both after NBQX infusions and after vehicle infusions. Figure 3A shows rearing in these test sessions. The rats trained with vehicle infusions exhibited high levels of rearing, both when tested with NBQX infusions and when tested with vehicle infusions. By contrast, the rats trained with NBQX injections showed low levels of rearing, with either NBQX or vehicle infusions in testing. Thus, CN function was critical only for the acquisition of conditioned ORs. Once conditioned rearing was established, continued function of CN was not essential for its expression. After an initial analysis showed no effect of test order, a training infusion X test infusion ANOVA showed a significant effect of training infusion, $F(1, 10) = 5.97$, $p = .035$, but no effect of test infusion or their interaction, $F_s < 1$.

Figure 3B shows food cup responding in the test sessions. As in training, the display of this behavior was unaffected by CN inactivation or training history. ANOVA showed no significant effects or interactions, $F_s < 1$.

Comparable analyses of baseline responding, in the absence of the visual CSs, showed no significant effects or interactions, $F_s < 1.14$. During the training phase, baseline rearing averaged $4.4 \pm 2.1\%$ and $9.5 \pm 3.2\%$ in NBQX and vehicle rats, respectively, and food cup responding averaged $14.5 \pm 4.1\%$ and $11.1 \pm 3.4\%$. During the test phase, these values were $3.6 \pm 1.4\%$, $3.1 \pm 2.5\%$, $3.8 \pm 3.9\%$, and $3.2 \pm 2.6\%$, respectively. ANOVAs like those performed for responding during the visual CSs showed no significant effects or interactions, $F_s < 2.15$.

At the conclusion of visual CS testing, we examined the acquisition of orienting (startle responding) to a noise CS paired with food (Figure 4) in a subset of the rats (5 NBQX and 5 vehicle rats), without any infusions. The purpose of this test was to determine if prior infusions of NBQX had any long-lasting effects with behavioral consequences. The rats' infusion history did not affect performance in this phase, suggesting that unilateral CN function was intact in rats previously infused with NBQX. A training infusion X cue (reinforced noise vs nonreinforced tone) X session ANOVA showed significant effects of cue, $F(1, 8) = 34.60$, $p < .001$, and a reliable cue X session interaction, $F(2, 16) = 17.69$, $p < .001$ (reflecting the acquisition of the discrimination over sessions), but no effect of infusion or interactions with that variable, $F_s < 1$.

Experiment 2

The result of Experiment 1 showed both that CN function is critical for the acquisition of conditioned ORs and that it not essential for the display of previously acquired ORs. Experiment 2 was designed to confirm this second result using post-training lesions of CN rather than inactivation procedures. Although the substantial effect of NBQX on the acquisition of conditioned orienting removes concerns about the effectiveness of the inactivation procedure, it is notable that some investigators have reported that temporary inactivation and permanent lesions of the amygdala can have different outcomes (e.g., McGaugh, et al, 2000).

Methods

Subjects—The subjects were 20 male Long-Evans rats (Charles River Laboratories, Raleigh, NC), which weighed 300–325 g when they arrived in the laboratory vivarium. The rats were maintained as in Experiment 1. The experiment was conducted in two procedurally identical replications, one with 12 rats and one with 8 rats; the proportions of lesioned and control rats were similar across replications.

The apparatus was that used in Experiment 1.

Surgical and histological procedures—Surgery was performed under isoflurane anesthesia with aseptic conditions, as in Experiment 1. Between one and three days after the final conditioning session, eleven rats received bilateral lesions of CN, using the same procedures and coordinates used in Experiment 1. Nine vehicle sham rats received injections of the PBS vehicle alone in a comparable manner. After surgery, all rats received a single 0.015 ml subcutaneous injection of buprenorphine hydrochloride (Sigma, St. Louis) for amelioration of pain, and were allowed to recover from surgery for 10–14 days before further behavioral testing.

The histological procedures were the same as used in Experiment 1.

Behavioral training procedures—Table 1 provides an outline of the procedures of Experiment 1. The rats first received a single 32-min session that served as a pretest of the visual stimulus to be used as the CS. In that session, there were 8 10-s illuminations of the light CS. In this and all subsequent sessions, the events were presented with intertrial intervals that ranged from 2 to 6 min, mean = 4 min. Next, the rats were trained to eat from the recessed food cups in a single 32-min session, which included 8 deliveries of two 45-mg food pellets. Then, all rats received eight 32-min Pavlovian conditioning sessions, each of which included 8 10-s presentations of the light, followed immediately by the delivery of two food pellets.

After surgery and recovery, the rats received four 32-min sessions to assess the effects of the CN lesions on previously acquired conditioned responding. Each of the first two test sessions was identical to the pretest session, and included 8 nonreinforced presentations of the light CS. The last two test sessions were identical to the original training sessions, and were intended to assess the lesion effects on reacquisition or reinstatement of responding. Finally, the rats received five sessions of training with an auditory CS to determine the effect of the lesions on the establishment of ORs to a new CS. In each of those sessions, the rats received eight 10-s presentations of either an 80-db white noise or an 80-db 1,500-hz tone paired with food delivery.

Results

Histological results—Using the same criteria as in Experiment 1, seven CN-lesioned brains were judged as having acceptable bilateral lesions. Apart from the injector tracks, no cellular damage was evident in any of the vehicle control brains. One vehicle rat died, leaving a total of 8 subjects in that group.

Behavioral results—Figure 5 shows the display of conditioned orienting (rearing, Figure 5A) and food cup (Figure 5B) behaviors during pretesting, training and testing of the visual CS. Although compared to Experiment 1 the levels of rearing attained in this experiment were lower and those of food cup behavior were higher, the general pattern of data was similar. As in Experiment 1, unconditioned rearing to the light CS habituated rapidly in the pretest, but was reacquired with light-food pairings, whereas food cup responding was evident only after light-food pairings. Neither behavior differed between the groups in these two pre-surgical

phases. Groups X sessions ANOVAs showed no significant effects of group nor group X sessions interactions, $F_s < 1$; the sessions effects were significant in training for both responses, $F_s(7, 91) > 6.90$, $p_s < .001$, and in pretraining for rearing, $F(1, 13) = 17.06$, $p = .001$. Comparable analyses of baseline responding, in the absence of the visual CSs, showed no effects or interactions, $F_s < 1.14$; during the training phase, baseline rearing averaged $14.9 \pm 4.6\%$ and $11.3 \pm 3.3\%$ in lesioned and sham rats, respectively, and food cup responding averaged $13.7 \pm 5.6\%$ and $13.7 \pm 4.1\%$.

The primary data of Experiment 2 are the results of the test sessions, conducted after excitotoxic (Group CN) or sham (Group sham) lesions of CN. These results confirmed Experiment 1's finding that post-training disruption of CN function did not interfere with the display of previously established conditioned ORs. The right portions of Figure 5 show that the post-training lesions of CN had no effect on either rearing or food cup entry, $F_s < 1$. Rearing declined across the four test sessions in both groups, whereas food cup responding quickly recovered after the reintroduction of the food reinforcer in the third test session. We have no explanation for the lack of comparable recovery of rearing, but for our present purposes, it is notable that the display of rearing was unaffected by the post-training lesion throughout testing. The levels of baseline rearing ($9.8 \pm 6.6\%$ in sham and $6.8 \pm 4.6\%$ in lesioned rats) and food cup entry ($8.6 \pm 5.5\%$ and $9.8 \pm 3.2\%$, respectively) in testing did not differ across groups, $F_s < 1$.

To assess the behavioral effectiveness of the CN lesion, after testing of the visual CS, we examined the acquisition of orienting (startle responding) to an auditory CS (noise or tone) paired with food. Overall, the sham rats showed more startle responding ($28.8 \pm 10.7\%$ of trials) than the lesioned rats ($8.2 \pm 4.8\%$ of trials). However, inspection of the data showed that only the noise CS elicited conditioned startle behavior. A lesion X cue identity ANOVA indicated that the simple effect of lesion was significant for the noise $F(1,11) = 11.15$, $p < .001$ ($47.5 \pm 14.7\%$ vs $7.0 \pm 6.0\%$), but not for the tone, $F < 1$ ($10.0 \pm 9.6\%$ vs $11.2 \pm 15.9\%$). These data show that the CN lesions in this experiment, which were ineffective in altering previously-established conditioned ORs, were behaviorally effective in disrupting the acquisition of new conditioned ORs.

Discussion

Previous research identified circuitry critical for the establishment of conditioned ORs to Pavlovian CSs signaling a food reinforcer, but not for the production of unconditioned ORs or for the acquisition of food-related CRs. This circuit, comprised of CN and DLS, likely includes CN's projections to SNC. Both bilateral lesions of CN (Gallagher et al, 1990) and functional disconnection of CN and DLS (Han et al, 1997) prior to training selectively interfere with the acquisition of conditioned ORs.

In the present study, CN function was necessary for the acquisition, but not the expression, of conditioned ORs. These results complement those of Han et al (1997), which indicated that DLS is critical for the expression, but not the acquisition, of conditioned ORs. Thus, different components of this circuit have specialized roles in conditioned orienting.

Notably, this pattern of results implies that neither CN nor DLS is critical to the maintenance of information required for conditioned orienting. If this information were maintained solely in CN, then bilateral disruption of CN function at the time of behavioral expression should prevent the display of previously-conditioned ORs. No such effect was observed in the present studies. Likewise, if CN acted at the time of learning to facilitate storage of that information in DLS, then functional disconnection of CN and DLS at that time would prevent such storage. However, Han et al (1997, Experiment 2) found that although transient inactivation of DLS

contralateral to a CN lesion during light-food training prevented the display of conditioned ORs at that time, those ORs emerged when inactivation was discontinued.

Instead, CN appears to be critical for the modulation of memory storage elsewhere in the brain, and DLS is critical for using that information in the generation of these responses, for example, by disinhibiting cortical pathways that control movement (Chevalier & Deniau, 1990). Because intact dopamine function in DLS is critical for the expression of learning, SNC seems a likely candidate for the maintenance of learning, because it both receives CN input (Gonzalez & Chesselet, 1990; Lee et al., 2003) and provides the dopaminergic innervation of DLS (Swanson, 1982). By this view, intact CN function is critical for storage of information in SNC during learning, and intact DLS function is necessary to use that stored information in behavioral expression. However, once the information was stored in SNC, CN would no longer be essential for expression (as observed here). This view is also consistent with the results of Han et al.'s (1997) CN-DLS disconnection studies. Because the connections between CN, SNC, and DLS are almost exclusively ipsilateral, rats with contralateral lesions of CN and DLS made prior to training (Han et al., 1997, Experiment 1) could store conditioned OR information only in the SNC that has little functional connection to the surviving DLS, thus precluding the observation of conditioned ORs. A similar outcome would be expected in rats with transient inactivation of DLS contralateral to CN lesions (Han et al., 1997, Experiment 2); however, once DLS function was regained in the previously inactivated hemisphere, DLS in that hemisphere could access information from the trained SNC, and conditioned ORs should emerge. Han et al. (1997) observed exactly this pattern of data.

Recent data from our laboratory support a role for CN's projections to SNC in conditioned orienting. Lee et al (2003) found that individual CN neurons that project to SNC are selectively activated by CSs that signal food USs. That study examined expression of the immediate-early gene *c-fos* in CN neurons after presentation of a visual CS that had previously been paired with food, or presented unpaired with food. Prior to this training, which was similar to the procedures used in Experiment 1 of the current report, a retrograde tracer (Fluorogold) was injected into SNC. *Fos* labeling in CN was more prevalent in rats trained with paired than with unpaired procedures, and a significant number of *fcs*-labeled neurons in CN were double labeled with retrograde tracer after paired training. Those results demonstrate activation of the CN projection to SNC during acquisition of the conditioned OR. Furthermore, Groshek and Holland (unpublished data) recently found that functional disconnection of CN and SNC by an ibotenic acid lesion of CN in one hemisphere and a contralateral 6-OHDA lesion of SNC in the other, prior to training, had the same effects on conditioned ORs as bilateral CN lesions (Gallagher et al., 1990), or disconnection of CN and DLS (Han et al., 1997). A role for SNC in the maintenance of learned information critical to conditioned orienting, providing an essential interface between CN and DLS, would be demonstrated if SNC lesions made after the acquisition of conditioned orienting also interfered with the expression of that learning. Although it remains to be seen whether the particular SNC targets of these CN neurons themselves project to DLS, SNC nevertheless seems a reasonable candidate locus for the long-term changes that subserve conditioned orienting.

CN lesion effects similar to those reported here have been observed in a discriminative autoshaping preparation (Cardinal et al, 2002; Parkinson, Robbins and Everitt, 2000). In that task, illumination of a screen on one side of a conditioning chamber is followed by food delivery, but illumination of a similar screen on the other side is nonreinforced. As a consequence of learning, intact rats approach both the screen and the food cup when the food-paired screen is illuminated. Many investigators have argued that these learned CS-approach responses reflect the acquisition of incentive motivational significance to the visual CS (e.g., Cardinal et al., 2002; Everitt et al, 2002; Holland, 1977; Tomie, 1996). From this perspective, the visual CS acquires incentive properties as a result of learning, and rats come to approach

and perhaps contact it, just as they would approach food, a natural incentive. Parkinson et al. (2000) found that rats with CN lesions made prior to training approached the food cup normally, but failed to approach the illuminated screen. Reminiscent of the present results, Cardinal et al (2002) found that rats with CN lesions made after training maintained their previously-established screen-directed responses. Because these screen-directed approach responses might be mediated by mesolimbic dopamine circuits often thought to underlie incentive processes, Parkinson et al (2000) suggested that this function is achieved by circuitry that includes CN and ventral striatum, specifically the accumbens core. Modulation of accumbens by CN might be mediated by CN's projections to dopaminergic neurons in the ventral tegmental area (Krettek & Price, 1978; Parkinson et al, 2000) or SNC (Swanson, 1982), or perhaps through its innervation of various nuclei within the midline thalamus (Holland & Gallagher, 2003; Pitkänen, 2000; Rosen, et al., 1991), which in turn project to the striatum. In support of their claim that autoshaped responses reflected incentive learning, Hall et al. (2001) and Parkinson et al. (2000) found that bilateral lesions of either CN or accumbens core impaired both approach responding in the autoshaping task, and Pavlovian-instrumental transfer, another conditioning phenomenon which is often thought to index incentive learning.

Although the conditioned ORs described here might be similarly characterized as autoshaped responses, reflecting an acquired motivational function, we favor an account that relates ORs to attentional processes. This account does not preclude a role for CN in the acquisition of motivational function by CSs, rather, it identifies the origins of the CN-dependent learned responses observed in the present study as functionally related to the initially unconditioned OR, which serves to alert the animal to salient events and prepare it for action through a variety of autonomic and skeletal responses. By this view, CN acts to enhance orienting to predictors of behaviorally significant events such as food by potentiating the activity of nigrostriatal systems that control orienting. We prefer such an account for our data for several reasons. First, previous studies in rats and pigeons have noted that autoshaped responses often comprise several components, distinguishable by their form, temporal sequence, and susceptibility to experimental manipulation (Holland, 1979, 1980; Timberlake, 1994; Wessels, 1974). Rearing occurs as the initial component of responding to a variety of visual CSs, but the approach and contact responses that characterize autoshaping emerge only to more limited sets of visual CSs (e.g. Holland, 1980). Second, relating conditioned ORs to unconditioned ORs has permitted a fuller specification of those CRs in our laboratories. Indeed, previous behavioral studies showed that both the form and the frequency of conditioned ORs to different CSs was correlated with those of the unconditioned ORs to those cues prior to training (Holland, 1977, 1980). In addition, a similar pattern of CN lesion effects has been observed with CSs that come to elicit conditioned ORs but do not elicit any approach to the CS source. For example, auditory CSs with sudden onsets elicit a startle response as an unconditioned OR, which, as with rearing to visual CSs, rapidly habituates when the CS is presented alone, and reemerges when it is paired with food delivery (Holland, 1977). As with conditioned ORs to visual cues, the acquisition of these conditioned auditory ORs depend on CN function (Gallagher et al, 1990; Groshek et al, 2004). Finally, conditioned orienting is readily interpreted within the broader context of top-down attentional processing of Pavlovian CSs, which we have found to be modulated by CN in a variety of tasks.

Top-down attentional control refers to the capture of attention by a stimulus based on the goals and expectations of the observer (e.g., learned expectancies that allow the stimulus to predict other, behaviorally significant events), rather than "bottom-up" control by the intrinsic physical properties of a stimulus (e.g. sudden onset or high intensity). Several kinds of evidence from our laboratories indicate a critical role for CN in top-down control of attention. Holland, Han and Gallagher (2000) found that rats with CN lesions showed deficits in a multiple-choice reaction time task during conditions of elevated attentional load (e.g., reduced processing time, unpredictable cue onset), when maintained attention to visual stimuli was crucial for obtaining

food reinforcers. Likewise, rats with CN lesions made before training fail to show surprise-induced increases in learning rate attributable to enhanced attention to CSs in a number of conditioning tasks (e.g. Holland & Gallagher, 1993a, 1993b, 1999). Within this more general framework, initial unconditioned ORs (not affected by CN lesions) reflect bottom-up attentional control by CSs, but the essential role for CN in acquisition of conditioned ORs (impaired by pre-training CN lesions) reflects enhanced attention to those CSs as they come to predict significant events such as food.

Acknowledgements

This research was supported in part by grant MH53667 from the National Institutes of Health. We thank Mary Keough and Frank Groshek for assistance in behavioral data scoring.

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Figure 1.

Photomicrograph of the amygdalar regions of a representative brain from Experiment 1. This rat had an excitotoxic lesion (outlined) of amygdala central nucleus (CN) in the left hemisphere and a cannula implant in the right hemisphere. Although there was substantial tissue loss along the cannula placement (which could have resulted either from the presence of the cannula during the experiment or from its removal after perfusion), note that neurons in the CN ventral to the cannula were intact. BLA = basolateral amygdala; OT = optic tract.

Figure 2.

Mean ($\pm$ s.e.m.) rearing (orienting) and food cup responding during the visual CS in the pretest (P) and conditioning (C) phases of Experiment 1. Prior to each pretest and conditioning session, some rats were injected with NBQX and some were injected with vehicle (VEH) only.

Figure 3.

Mean ($\pm$ s.e.m.) rearing (orienting) and food cup responding during the visual CS in the test sessions of Experiment 1. The bars on the left side of each panel show responding in the test session with NBQX infusions, and the bars on the right side show responding in the test session with vehicle (VEH) infusions. The filled bars show responding of rats that were trained with vehicle infusions and the open bars show responding of rats trained with NBQX infusions.

Figure 4.

Mean ($\pm$ s.e.m.) startle responding to the food-reinforced noise (N+) and nonreinforced tone (T-) in the final phase of Experiment 1, in which no infusions were given. Filled symbols indicate responding of rats that received vehicle injections during the previous pretest and conditioning phases and open symbols indicate responding of rats that received NBQX injections in those phases.

Figure 5.

Mean ($\pm$ s.e.m.) rearing (orienting) and food cup responding during the visual CS in the pretest (P), conditioning (C) and test (T) sessions of Experiment 2. Immediately after session C8, the rats received either sham or neurotoxic lesions of CN.

Table 1

Outline of behavioral training procedures

Experiment 1 Group	Pretest	Conditioning	Test 1	Test 2	Train new CS
NBQX	(NBQX) light alone	(NBQX) light→food	(NBQX) light alone -or- (Vehicle) light alone	(Vehicle) light alone -or- (NBQX) light alone	noise→ food, tone alone (no infusions)
VEH	(Vehicle) light alone	(Vehicle) light→food	(NBQX) light alone -or- (Vehicle) light alone	(Vehicle) light alone -or- (NBQX) light alone	noise→food, tone alone (no infusions)
Experiment 2 Pretest	Conditioning	Surgery	Test	Retraining	Train New CS
light alone	light→food	CN lesion -or- Sham lesion	light alone	light→food	Noise→food - or- Tone→food