Neurobiology of Learning and Memory **76,** 403–425 (2001)
doi:10.1006/nlme.2001.4019, available online at http://www.idealibrary.com on IDEAL®

Lesions in the Central Nucleus of the Amygdala: Discriminative Avoidance Learning, Discriminative Approach Learning, and Cingulothalamic Training-Induced Neuronal Activity

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The amygdala is critically involved in discriminative avoidance learning. Large lesions of the amygdala block discriminative avoidance learning and abolish cingulothalamic training-induced neuronal activity. These results indicated that amygdalar processing is critical for cingulothalamic plasticity. The larger lesions did not allow differentiation of the specific functioning of various amygdalar nuclei. Anatomical analysis showed that damage in the central (CE) nucleus of the amygdala was correlated with the severity of the behavioral deficit. The present study was carried out to determine whether smaller lesions, centered in the CE nucleus, would impair discriminative avoidance learning and block cingulothalamic plasticity. In addition, the possible role of the CE nucleus in appetitively motivated discriminative approach learning was examined for the first time. New Zealand White rabbits with CE nuclear lesions were first trained in the discriminative approach task. After attaining asymptotic performance, discriminative avoidance training sessions were alternated with continuing approach training sessions, one session each day. The rabbits with lesions were severely impaired in avoidance learning but showed no impairment of approach learning. Surprisingly, the attenuating effects of the lesions on cingulothalamic training-induced neuronal activity were more prevalent during approach learning than during avoidance learning. These results indicated that avoidance learning can be impaired by lesions centered in the CE nucleus that leave cingulothalamic plasticity largely intact and that the CE nucleus is involved in extra-cingulothalamic learning processes.

This work was supported by NIH NS36591, NIMH MH58259, NIDA DA11164, and NSF BIR95-04842 to M.G. and NIMH F31-MH12077 to D.S.

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1074-7427/01 $35.00

Key Words: amygdala; cingulate cortex; limbic thalamus; avoidance learning; approach learning; discrimination.

INTRODUCTION

The present study applied the model systems approach pioneered by Dr. Richard F. Thompson to the analysis of the role of the amygdala in discriminative instrumental learning. The amygdala is critically involved in aversively motivated learning. Lesions of the amygdala disrupt classical aversive conditioning of immobility (Iwata, Chida, & LeDoux, 1987), arterial blood pressure (LeDoux, 1993), heart rate responses (Kapp, Frysinger, Gallagher, & Haselton, 1979), and fear-potentiated startle responses (Hitchcock & Davis, 1986), and they block aversively motivated instrumental learning, including passive (Liang, McGauch, Martinez, Jensen, Vasquez, & Messing, 1982; Nagle & Kemble, 1976; Parent & McGaugh, 1994) and active avoidance learning (Roozendaal, Koolhaas, & Bohus, 1993). The amygdala is critically involved in discriminative avoidance, wherein rabbits learn to produce a locomotor response to an acoustic (pure tone) conditional stimulus (CS^+) in order to avoid a foot shock and they learn to ignore a second tone (CS^-) that is not followed by a foot shock (Poremba & Gabriel, 1997, 1999). Neurons in the basolateral (BL) nucleus of the amygdala exhibited robust training-induced activity, wherein they became more responsive to the CS^+ than to the CS^- (Maren, Poremba, & Gabriel, 1991). Lesions of the amygdala blocked avoidance learning and abolished training-induced neuronal activity in cingulothalamic circuitry, comprised by the cingulate cortex and the interconnected limbic (anterior and medial dorsal) thalamic nuclei (Poremba & Gabriel, 1997). The cingulothalamic circuit is essential for discriminative avoidance learning (Gabriel, Kubota, Sparenborg, Straube, & Vogt, 1991a; Gabriel, Lampert, Foster, Orona, Sparenborg, & Maiorca, 1983; Gabriel & Saltwick, 1977). Reversible inactivation of the amygdala using the $GABA_A$ agonist muscimol early in training temporarily blocked learning and the development of cingulothalamic training-induced neuronal activity, but amygdalar inactivation during performance of the well-learned behavior did not have these effects (Poremba & Gabriel, 1999). These results indicated that amygdalar processing is critical for the development of learning-related plasticity in cingulothalamic circuitry.

The amygdala is not a homogenous structure, but rather is a collection of distinct nuclei, each with different afferent and efferent projections (Amaral, Price, Pitkanen, & Carmichael, 1992; Krettek & Price, 1977a, 1978). A number of authors have proposed that circuitries involving different nuclei of the amygdala mediate different aspects of learning (Amorapanth, Ledoux, & Nader, 2000; Gallagher & Holland, 1994; Killcross, Robbins, & Everitt, 1997; LeDoux, Iwata, Cicchetti, & Reis, 1988; Pitkänen, Savander, & LeDoux, 1997; Swanson & Petrovich, 1998). The BL nucleus projects to the anterior cingulate cortex directly and indirectly, via projections to the medial dorsal (MD) thalamic nucleus (Krettek & Price, 1977a). These projections may account for the loss of cingulothalamic plasticity in rabbits with amygdala lesions (Poremba & Gabriel, 1997, 1999). Less is known about the role of the central (CE) nucleus of the amygdala in discriminative avoidance learning. A previous study (Poremba & Gabriel, 1997) employed large lesions, encompassing much of the lateral (LA), BL, and CE nuclei. However, the avoidance learning deficit was highly correlated with the amount of damage to the CE nucleus,

suggesting that the CE nucleus is critically involved in mediating this form of learning. The CE nucleus receives input from the LA and BL nuclei and sends projections to many brain regions known to influence behavior, including the lateral hypothalamus, the ventral tegmental area, the periaquiductal gray, the reticular formation, and the substantia nigra (Amaral et al., 1992; Krettek & Price, 1978). Numerous studies have demonstrated an involvement of the CE nucleus in aversively motivated learning (Davis, 1990; LeDoux et al., 1988; Roozendaal et al., 1993). Indeed, the CE nucleus has been proposed to be the primary "output" region whereby the amygdala influences learned behavior (LeDoux et al., 1988; Maren & Fanselow, 1996; Pitkänen et al., 1997; but see Smith & Pare, 1994, Collins & Pare, 1999). The present study was carried out to determine whether smaller lesions centered in the CE nucleus would impair discriminative avoidance learning and block the development of cingulothalamic training-induced neuronal activity.

In addition to its well-documented role in aversive learning, various regions of the amygdala have been implicated in phenomena of appetitively motivated learning, including conditioned place preference (Everitt, Morris, O'Brian, & Robbins, 1991), visual object discrimination (Gaffan, Gaffan, & Harrison, 1988; Gaffan & Murray, 1990), unblocking (Holland & Gallgher, 1993), stimulus devaluation, and second-order conditioning (Hatfield, Han, Conley, Gallagher, & Holland, 1996; Cador, Robbins, & Everitt, 1989). Recently, an appetitively motivated discrimination task has been developed, wherein rabbits learn to approach and make oral contact with a drinking spout to obtain a water reward following a CS^+ and they learn to withhold spout contact responses following a CS^- that does not predict the availability of water (Freeman, Cuppernell, Flannery, & Gabriel, 1996). Rabbits in the present study were tested concurrently in both the discriminative approach and avoidance tasks in order to compare directly the effects of CE nuclear lesions on discriminative approach and avoidance learning in the same subjects.

MATERIALS AND METHODS

Subjects and surgical procedures. The subjects were 27 New Zealand White rabbits weighing 1.5–2.0 kg at the time of delivery from the supplier (Myrtle's Rabbitry Inc., Thompson Station, TN). Seven days after arrival in the Beckman Institute Vivarium, the rabbits were placed on a moderately restricted diet (1 cup of Purina rabbit chow daily) to control obesity. After 1–2 weeks for recovery from surgery, the rabbits were placed on a restricted regimen of 100 ml of water daily. They were given at least 1 week to adjust to this regimen before training began. Bilateral electrolytic lesions centered in the CE nucleus of the amygdala were induced in 17 rabbits using stainless steel insect pins insulated with epoxylite. Approximately 0.7–0.8 mm of the insulation was removed from the tip of the pins to provide a conductive surface. Whereas the previous study (Poremba & Gabriel, 1997) employed three bilateral lesion targets, the present study employed a single bilateral target yielding substantially smaller lesions. Lesioning electrodes were positioned 0.0 mm posterior to bregma, 5.5 mm lateral to bregma, and 14.5 mm ventral to the dorsal surface of the brain (Girgis & Shih-Chang, 1981). A 1.5-mA cathodal DC current was passed for 50 s. Control rabbits underwent the same surgical procedures, except that no lesions were made. Instead, recording electrodes were implanted in the amygdala. During surgery six fixed-position stainless steel microelectrodes were implanted in all rabbits for the recording of unit activity during training. The target sites for recording electrodes

were anterior cingulate cortex (AP = 3.5 mm anterior to bregma, ML = 0.8, DV = 3.5), posterior cingulate cortex (AP = 4.0 mm posterior to bregma, ML = 0.8, DV = 1.5), anterior ventral thalamic nucleus (AP = 2.0 mm posterior to bregma, ML = 2.3, DV = 7), medial dorsal thalamic nucleus (AP = 4.6 mm posterior to bregma, ML = 1.5, DV = 8.0), medial division of the medial geniculate nucleus (AP = 7.5 mm posterior to bregma, ML = 5.0, DV = 9.0), and the BL nucleus of the amygdala in control rabbits (AP = 0.5 mm posterior to bregma, ML = 5.5, DV = 13.25). Because insufficient numbers of records were obtained from the medial geniculate nucleus, the anterior cingulate cortex, and the amygdala, data from these regions are not reported.

Training procedure. Each rabbit was first given training in a discriminative approach task. After attaining asymptotic performance, discriminative avoidance training sessions were alternated with continuing approach training sessions, one session each day.

Discriminative approach training was administered while rabbits occupied an apparatus designed for instrumental conditioning of a response consisting of head extension and oral contact with a drinking spout. The chamber provided electrical shielding and sound attenuation. Within the chamber, rabbits occupied a Plexiglass rabbit restrainer that allowed free head movement. A 70-dB re 20 $\mu N/m^2$ masking noise was played continuously through a loudspeaker mounted in the ceiling of the chamber. Two pure tones (1 or 8 kHz; duration, 500 ms; 85 dB re 20 $\mu N/m^2$; rise time, 3 ms) were assigned as positive and negative conditional stimuli (CS^+ and CS^-) in a counterbalanced fashion. During training, the onset of the CS^+ was followed after 4 s by insertion of a drinking spout through a opening in the chamber wall. The rabbit was positioned such that a head extension of approximately 4 cm was required to reach the spout. Water reward (3 ml in 2 s) was delivered when oral contact was made with the spout. Spout contact responses were detected by a grounding circuit. CS^- presentation was also followed by spout insertion and spout contact responses were recorded, but no reward was delivered. Instead, spout contact responses were followed immediately by retraction of the spout.

Prior to training, rabbits were given daily sessions for acclimation to the conditioning chamber and spout presentations. Acclimation sessions consisting of 60 spout insertions at irregular intervals were given daily until the rabbits reached a criterion of at least 45 spout contact responses in a session. After acclimation, the rabbits received two preliminary training sessions during which baseline neuronal and behavioral data were recorded for comparison with later training sessions. In the first preliminary training session, the tones to be used as conditional stimuli were presented 60 times each without spout insertion or water reward. In the second session, the tone conditional stimuli were presented 60 times each along with explicitly unpaired water spout presentations. The rabbits could obtain water reward for spout contact responses. Following preliminary training, rabbits were given daily training sessions consisting of 120 trials (60 each with the CS^+ and CS^-, presented in an irregular order). The intertrial interval was 8, 13, 18, 23, or 28 s, with these values occurring in an irregular order. Training continued until the rabbits reached a criterion in which the percentage of spout contact responses on CS^+ trials exceeded the percentage of spout contact responses on CS^- trials by at least 50%. This discriminative performance had to be achieved in two consecutive training sessions. On the last day of training before they were sacrificed, all rabbits were given free access to water for 15 min in the training apparatus in order to assess possible motivational effects of lesions.

After the rabbits achieved the approach training criterion, discriminative avoidance training was initiated. Avoidance training sessions and continuing approach training sessions were given on alternating days. The tone conditional stimuli used during avoidance training were the same as those used during approach training, except that their predictive value was reversed relative to approach training. Thus, the tone that had been assigned as the CS^+ for approach training was used as the CS^- for avoidance training and the tone that had been assigned as the CS^- for approach training was used as the CS^+ for avoidance training. Discriminative avoidance training was administered while the rabbits occupied a rotating wheel apparatus designed for the instrumental aversive conditioning of small animals (Brogden & Culler, 1936). A 1.5-mA constant current shock delivered to the rabbits' footpads through the grid floor of the wheel served as an unconditional stimulus (US). The foot shock US had a maximum duration of 0.5 s and was terminated by wheel rotation. Prior to avoidance training, rabbits were given two preliminary training sessions similar to those employed in the approach task, the first consisting of 120 presentations of the tone conditional stimuli (60 with each CS) and the second consisting of 120 tone presentations with explicitly unpaired presentations of the foot-shock US. Regular discriminative avoidance training sessions consisted of of 120 trials (60 each with the CS^+ and CS^-, presented in an irregular order). The onset of the CS^+ was followed after 5 s by the US. Locomotion after the CS^+ prevented the scheduled US. The CS^- was not followed by shock. The intertrial interval was 8, 13, 18, 23, or 28 s, with these values occurring in an irregular order. The rabbits learned to step in the activity wheel in response to the CS^+ and to ignore the CS^-. Alternating sessions of approach and avoidance training were given daily until the rabbits attained the criterion, which required that the percentage of avoidance responses on CS^+ trials exceed the percentage of responses on CS^- trials by at least 60%. This discriminative performance had to be achieved in two consecutive training sessions.

Collection of neuronal data. The neuronal records were passed from the recording electrodes to a field-effect transistor (FET) that served as a high-impedance source follower located approximately 2.5 cm from the recording sites within the brain. The FET outputs were fed to a high-gain preamplifier (gain, 40000; $\frac{1}{2}$ amplitude cutoffs as 500 and 8000 Hz). The amplified records were fed through active band-pass filters ($\frac{1}{2}$ amplitude cutoffs at 600 and 8000 Hz; roll-off, 18 dB/octave). The records were then fed to Schmitt triggers with a threshold set to allow triggering at a mean rate of 110–190 spikes per second. With this setting, several of the larger spikes were sampled. In addition, the band-pass filter outputs were half-wave rectified and integrated. Schmitt trigger pulses were counted and the integrator output voltage was digitized on each trial (CS presentation) for 1.0 s, from 0.3 s before CS onset to 0.7 s after CS onset. A digital value was stored for each measure and electrode every 10 ms throughout the 1-s sampling interval. The Schmitt-trigger data provided an index of the firing frequency of the larger spikes, whereas integrated activity measured the fluctuations of the entire record, including activity below the triggering thresholds.

Histology. After the completion of training, euthanasia was administered via an overdose of sodium pentobarbital followed by transcardial perfusion with normal saline and 10% Formalin. The brains were frozen and sectioned at 40 μm and the sections were photographed while still wet (Fox & Eichman, 1959). After drying, the sections were

stained with a metachromatic Nissl and myelin stain using formol thionin (Donovick, 1974). Photographs and stained sections were used to verify recording electrode locations and lesion location. Lesion size was estimated using digitized images of the Beckman Institute Neuronal Pattern Analysis digital rabbit brain atlas (Payne, Hanlon, Cantey, Mungnirun, Duvel, Smith, Gimbel, Nelson, & Gabriel, 1999, http://soma.npa.uiuc.edu/isnpa/atlas/rabbit/). Ten coronal sections representing the rostral–caudal extent of the amygdala were selected and the nuclei within the amygdala were defined in each section using the atlases of Girgis and Shih-Chang (1981) and Urban and Richard (1972). The nuclei were digitally drawn onto a transparent layer over the sections of interest and color coded. The layer containing the color-coded nuclei was rendered invisible to the user so that the sections of interest could be seen. The region of damage was then digitally drawn in for each rabbit. The number of lesion pixels that overlapped pixels within each color-coded nucleus was automatically counted and a damage score was calculated [damage score = (number of lesion pixels/number of nuclear pixels) × 100]. This method provided a reusable template for quickly and objectively calculating accurate damage estimates. The mean damage score was 38.8% of the total amygdala (range, 16.6 to 55.2%). The mean damage scores for individual nuclei of the amygdala were as follows: CE, 81.4%; BL, 51.5%; basomedial, 32.0%, medial, 7.3%; and LA, 34.9%. The smallest and largest lesions are depicted in Fig. 1. Five rabbits with minimal damage to the CE nucleus were excluded from analysis. The damage scores for these rabbits ranged from 4.3 to 38.1% of the CE nucleus, with the larger lesions being bilaterally asymmetrical. Because the sizes of the lesions varied and some of the larger lesions included substantial damage to surrounding nuclei, extensive analyses were conducted to determine the effects of unintended damage to the BL or LA nuclei (see Results).

Data analysis. Because the rabbits required varying numbers of conditioning sessions to attain the criteria of approach and avoidance learning, the analyses were restricted to a set of training sessions common to all rabbits. The sessions were pretraining with unpaired CS and US presentations, the first conditioning session, the session of first significant behavioral discrimination (defined as the session half in which the percentage of trials with conditioned responses to the CS^+ first exceeded the percentage of trials with responses to the CS^- by at least 25%), and the criterial session. The percentage of trials in which a conditioned response (CR) occurred was submitted to a factorial, repeated measures analysis of variance (ANOVA) using the 2V program (BMDP Statistical Software). The factors used for the analysis were Group (two levels, control and lesion), CS (two levels, CS^+ and CS^-), and Training Session (four levels, as described above). Since the lesions could have impaired ongoing approach performance after the initiation of avoidance training, an additional analysis of approach behavior was conducted on data obtained after the introduction of avoidance training. This analysis used the same factors as other behavioral analyses, except that the session factor had four levels (the criterial session and the next three postcriterial approach training sessions).

Analysis of the neuronal data had the same form as the analysis of the CR percentage data, with an additional orthogonal factor, post-CS interval, denoting the successive epochs in which neuronal data were represented. All neuronal data (spike frequency

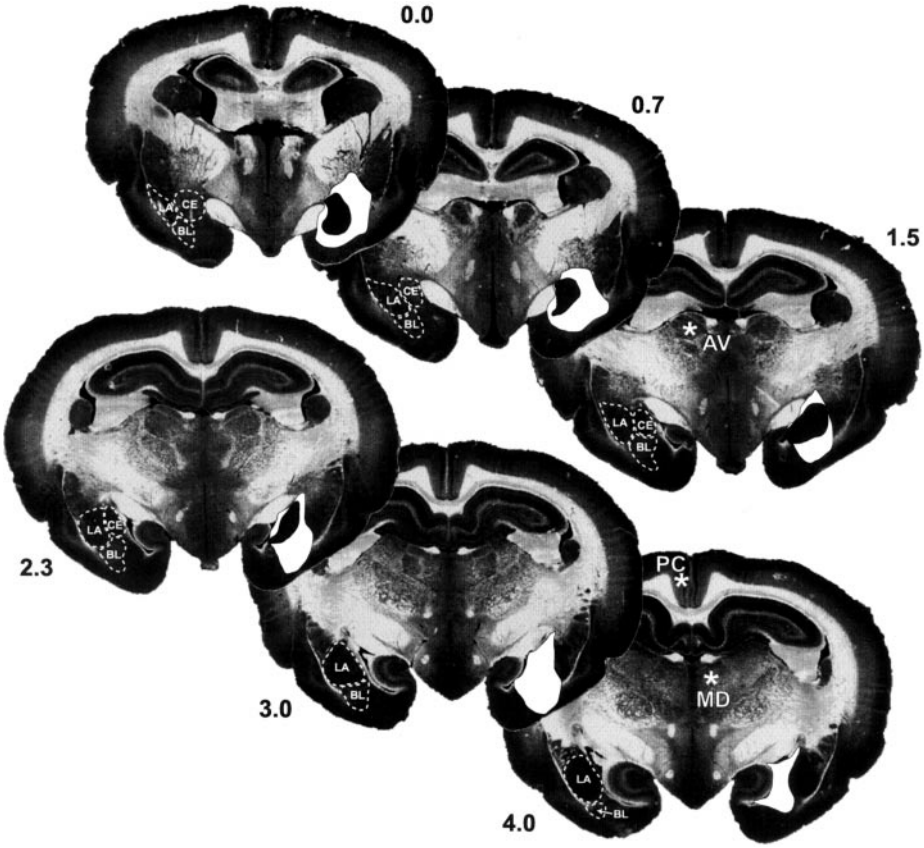

FIG. 1. Coronal sections used to quantify the lesions (see text for details) with stereotaxic coordinates (in mm posterior to bregma) shown. Each nucleus within the amygdala is identified and the largest (white) and smallest (black) lesions are shown on the right, although all lesions were bilateral. Recording sites, indicated by asterisks, were the posterior cingulate cortex (PC), the anterior ventral thalamic nucleus (AV), and the medial dorsal thalamic nucleus (MD).

and integrated activity) were analyzed using two different post-CS intervals, 40 consecutive 10-ms post-CS intervals and 4 consecutive 100-ms post-CS intervals. For the 10-ms post-CS intervals, the data were normalized to pre-CS baseline by converting them to *z* scores using the mean and standard deviation of data collected during the 300 ms immediately preceding CS onset. Similarly, data for the 100-ms intervals were converted to *T* scores (Student's *T* statistic), calculated for each of the four consecutive 100-ms post-CS intervals in comparison to 100 ms of pre-CS baseline data. These two measures were complimentary in that the 10-ms intervals allowed for fine-grained analysis of the neuronal response, while the 100-ms intervals provided a global measure that was less susceptible to short-term temporal variation. Corrections of the *F* tests due to violations of the sphericity assumption of repeated measures analysis were performed as needed following the procedure of Huynh and Feldt (1976). Factors yielding significant *F* ratios were further analyzed using simple effect tests following procedures described by Winer (1962). In order to assess general locomotor behavior, the number of wheel turn responses initiated during intertrial intervals was submitted to ANOVA using the same procedures as were used to assess conditioned responding. Analyses

were also conducted to assess the effects of lesions on motivation. These included analysis of water consumed during a free access period following training and latency to escape the foot shock US.

RESULTS

Discriminative Approach Behavior

No differences between control rabbits and rabbits with lesions were found in discriminative approach learning. Control rabbits required a mean of 16.6 sessions to attain the criterion, compared to 18.6 sessions in rabbits with lesions ($F[1, 20] = .58$, $p < .47$). No between-group differences in conditioned responding were found for any session of training (interaction of session, CS, and group factors; $F[2, 40] = .89$, $p < .43$, Fig. 2). The lesions had no effect on motivation. No between-group differences were found in the amount of water consumed during the free access period at the conclusion of training ($F[1, 9] = 0.56$, $p < .48$).

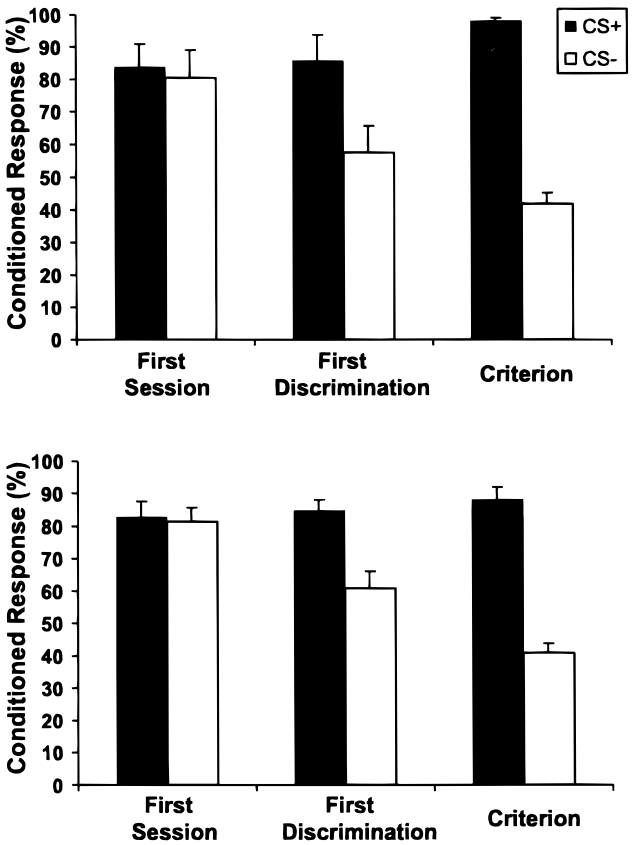

FIG. 2. Average percentage of conditioned responses to the CS^+ (black bars) and CS^- (white bars) in the discriminative approach task as a function of training session. Data are shown for control rabbits (top, $n = 10$) and rabbits with lesions (bottom, $n = 12$) for the following sessions: the first conditioning session, the session of first significant behavioral discrimination (First Discrimination), and the criterial session.

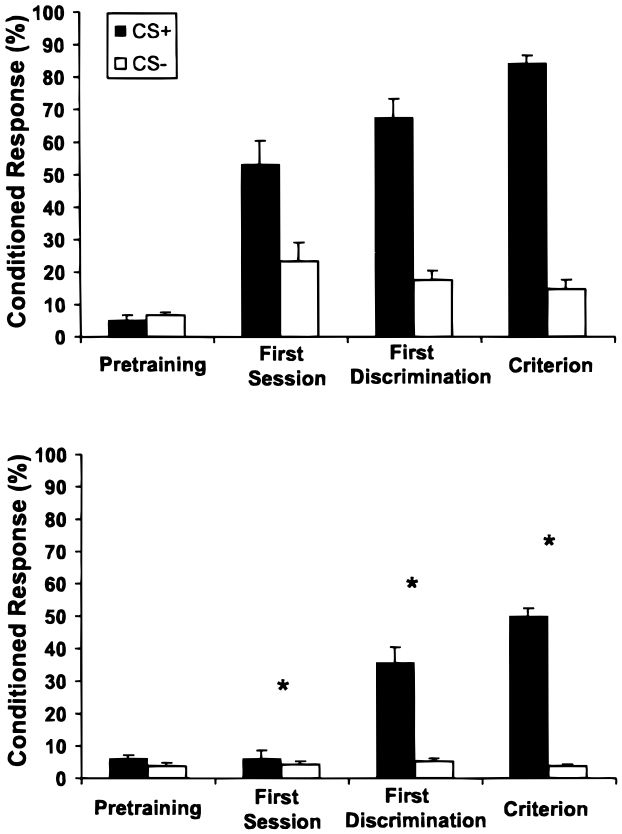

FIG. 3. Average percentage of conditioned responses to the CS^+ (black bars) and CS^- (white bars) in the discriminative avoidance task as a function of training session. Data are shown for control rabbits (top $n = 10$) and rabbits with lesions (bottom, $n = 12$) for the following sessions: pretraining with explicitly unpaired tone and foot shock presentations, the first conditioning session, the session of first significant behavioral discrimination (First Discrimination), and the criterial session.

Discriminative Avoidance Behavior

In contrast to approach learning, rabbits with lesions were severely impaired in discriminative avoidance learning. Six of the 12 rabbits with lesions failed to attain the avoidance criterion within 15 training sessions. The 6 rabbits with lesions that did attain the criterion were significantly delayed, requiring an average of 5.5 conditioning sessions compared to 3.0 sessions in control rabbits ($F[1, 14] = 12.87$, $p < .005$). Simple effect tests following a significant F ratio for the interaction of the Session, CS, and Group factors ($F[3, 60] = 4.58$, $p < .01$, Fig. 3) indicated that rabbits with lesions exhibited significantly less frequent responding to both the CS^+ and the CS^- during the first conditioning session and the session of first significant behavioral discrimination and they exhibited less responding to the CS^+ during the criterial session, relative to controls (all $p < .05$). Control rabbits showed significant discrimination during the first conditioning session ($p < .05$), whereas rabbits with lesions did not.

As in the case of approach learning, there were no indications that the lesions interfered with basic sensory, locomotor, or motivational processes. No group differences were found in the latency to respond to the foot shock US ($F[1, 18] = .10$, $p < .76$) or in the duration

of these unconditioned responses ($F[1, 18] = .14$, $p < .73$). No group differences were found in the number of times the rabbits initiated locomotor responses during intertrial intervals ($F[1, 18] = .85$, $p < .38$).

Effects of Lesion Size and Location on Avoidance Behavior

Because the size of the lesions varied and some of the larger lesions included substantial damage to the BL and LA nuclei, it was necessary to determine whether damage to these regions contributed to the avoidance learning impairment. To assess this possibility, the lesion group was divided into subgroups with differing amounts of damage to the neighboring nuclei (Table 1). In order to assess the effects of damage to the BL nucleus the lesion group was subdivided into two groups (BL Grouping), one containing rabbits with minimal BL damage (Minimal BL) and another containing all of the remaining rabbits with lesions (BL). A second grouping of the rabbits with lesions was carried out to assess the effects of damage to the LA nucleus. The lesion group was again subdivided into two groups (LA Grouping), one containing rabbits with minimal LA damage (Minimal LA) and another containing all of the remaining rabbits with lesions (LA). To assess the effects of BL and LA nuclear damage, the number of sessions required to attain the criterion and the percentage of trials on which a CR occurred were submitted to ANOVA. Each analysis used three groups, including control rabbits and the two resulting lesion groups (Minimal BL and BL or Minimal LA and LA).

Neither the BL Grouping nor the LA Grouping yielded lesion subgroups that differed in any behavioral measure. Furthermore, all of the lesion subgroups differed from control rabbits in the same manner as had the group composed of all rabbits with lesions. Individual comparisons following significant interactions of the session, CS, and group factors (comparison of Minimal BL, BL, and Control groups: $F[6, 57] = 2.54$, $p < .05$; comparison of Minimal LA, LA, and Control groups: $F[6, 57] = 2.4$, $p < .05$) indicated that the Minimal BL and BL groups did not differ in any respect nor did the Minimal LA and LA groups differ (all $p > .05$), but each of these lesion groups differed from control rabbits (all $p < .05$). Simple effect tests following a significant main effect of Group

TABLE 1
Lesion Location and Avoidance Behavior

			BL Grouping		LA Grouping	
	Control	All lesions	Minimal BL	BL	Minimal LA	LA
N	12	12	4	8	4	8
CE (%)	—	81	86	79	86	79
BL (%)	—	52	18	71	25	67
LA (%)	—	35	28	39	17	45
Sessions	3.0	9.8	13.0	8.0	12.0	8.6

Note. Data are shown for control rabbits and different groupings of rabbits with lesions: all rabbits with lesions (All lesions), rabbits with minimal damage to the BL nucleus (Minimal BL), rabbits with substantial damage to the BL nucleus (BL), rabbits with minimal damage to the LA nucleus (Minimal LA), and rabbits with substantial damage to the LA nucleus (LA). Shown in the columns are the number of subjects in each grouping (*N*), the percentage of damage to the CE, BL, and LA nuclei, and the mean number of sessions required to attain the criterion (Sessions) are given.

($F[2, 18] = 13.29$, $p < .001$) indicated that none of the lesion subgroupings differed in the number of sessions required to attain the criterion ($p > .05$), but that all of the lesion groups required more training sessions than controls (all $p < .05$, see Table 1 for means). In fact, the rabbits with more well-contained CE nuclear lesions (groups Minimal BL and Minimal LA) were numerically slower to attain the criterion than rabbits with lesions that included substantial BL damage (group BL) or rabbits with substantial LA damage (group LA), although these differences did not reach significance (comparison of Minimal BL and BL groups: $p < .10$; comparison of Minimal LA and LA: $p > .05$). Thus, these results showed equivalent behavioral deficits in rabbits with and without substantial BL or LA nuclear damage, suggesting that damage to these regions was unlikely to have been a factor in the learning deficit.

These results indicate that extensive damage to the BL or LA nuclei was not required to observe a learning impairment. Rabbits with well-confined CE nuclear lesions were as severely impaired as rabbits with larger lesions. All of the remaining analyses were therefore conducted using the full complement of rabbits with lesions.

Training-Induced Neuronal Activity: Brief Overview

Central nuclear lesions produced a marked impairment of avoidance learning, but had no effect on approach learning. Surprisingly, the lesions had minimal impact on cingulothalamic training-induced neuronal activity during avoidance learning. The lesions did attenuate training-induced activity in many of the sampled regions. However, this attenuation was most prevalent during discriminative approach learning, in which the rabbits with lesions were unimpaired. Despite the fact that the lesions were associated with an attenuation of neuronal activity, evidence of spared training-induced activity was found in all of the sampled regions during approach and avoidance learning.

Training-Induced Neuronal Activity: Posterior Cingulate Cortex

Central nuclear lesions attenuated, but did not block, the development of posterior cingulate cortical training-induced neuronal activity during approach learning (Fig. 4A). Individual comparisons following a significant interaction of the Session, CS, 100 ms Post-CS Interval, and Group factors ($F[9, 162] = 3.82$, $p < .005$, integrated activity, $F[9, 162] = 2.89$, $p < .05$, spike frequency) indicated significant discriminative training-induced activity (greater neuronal responses to the CS^+ than to the CS^-) in all four 100-ms post-CS intervals during the session of first significant behavioral discrimination and the criterial session in control rabbits ($n = 9$, $p < .05$) but no neuronal discrimination was observed in rabbits with lesions ($n = 11$, $p > .05$). Individual comparisons also indicated the development of excitatory training-induced neuronal activity in control rabbits (an increase in the magnitude of the CS-elicited responses during training, relative to the response during the pretraining session). The average neuronal response elicited by the CS^+ was significantly increased during the session of first significant behavioral discrimination and during the criterial session, relative to pretraining and the first conditioning session in control rabbits (all $p < .05$). No such increases in the neuronal response were observed in rabbits with lesions ($p > .05$).

The analysis of data from the control and lesion groups did not reveal training-induced

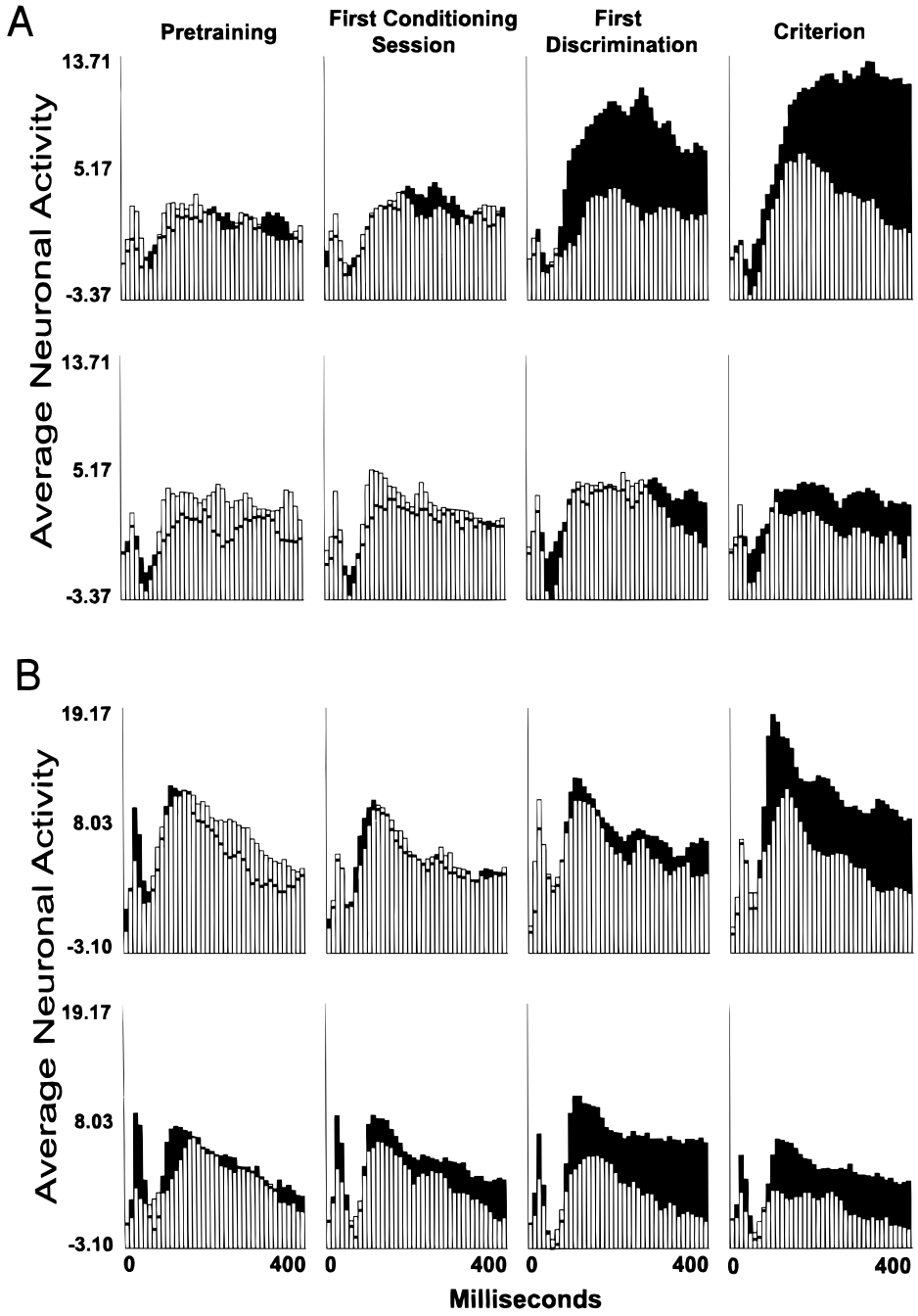

FIG. 4. Average integrated unit activity recorded in the posterior cingulate cortex during discriminative approach (A: top row, control; second row, lesion) and avoidance learning (B: top row, control; bottom row, lesion) for control rabbits ($n = 9$) and rabbits with lesions ($n = 11$). The data, in the form of z scores normalized with respect to a 300-ms pre-CS baseline, are shown in 40 consecutive 10-ms intervals after the onset of the CS^+ (black bars) and CS^- (white bars). Data are shown for the following sessions: pretraining with explicitly unpaired tone and foot shock presentations, the first conditioning session, the session of first significant behavioral discrimination (First Discrimination), and the criterial session.

neuronal activity in rabbits with lesions. However, separate analysis of the data from rabbits with lesions did indicate the development of excitatory and discriminative training-induced neuronal activity in the posterior cingulate cortex during discriminative approach learning. Individual comparisons following significant interactions of the Session and CS factors (10-ms post-CS intervals: $F[3, 54] = 5.78$, $p < .01$, integrated unit activity; $F[3, 54] = 3.56$, $p < .05$, spike frequency; 100-ms post-CS intervals: $F[3, 30] = 7.82$, $p < .001$, integrated activity; $F[3, 30] = 7.09$, $p < .005$, spike frequency) indicated that neurons in the posterior cingulate cortex of rabbits with lesions exhibited a preexisting sensory bias (greater responses to the tone assigned as the CS^- than to the tone assigned as the CS^+) during pretraining, but that significant discriminative activity (greater responses to the CS^+ than to the CS^-) was present during the criterial session ($p < .05$). Posterior cingulate cortical neurons also exhibited a significant increase in the response elicited by the CS^+ during the session of first significant behavioral discrimination and the criterial session, relative to pretraining (integrated activity, $p < .05$). Together, these results indicated that the lesions attenuated, but did not abolish, posterior cingulate cortical training-induced neuronal activity during discriminative approach learning.

Lesions of the CE nucleus had little effect on posterior cingulate cortical training-induced neuronal activity during discriminative avoidance learning (Fig. 4B). Individual comparisons following a significant interaction of the Session, CS, and Group factors (10-ms post-CS intervals: $F[3, 54] = 3.38$, $p < .05$, integrated unit activity; $F[3, 54] = 3.42$, $p < .05$, spike frequency; 100-ms post-CS intervals: $F[3, 54] = 3.97$, $p < .05$, integrated unit activity; $F[3, 54] = 3.39$, $p < .05$, spike frequency) indicated significant excitatory and discriminative training-induced neuronal activity in control rabbits during the criterial session ($p < .05$). Rabbits with lesions exhibited significant excitatory and discriminative training-induced neuronal activity during the session of first significant discrimination and the criterial session (all $p < .05$). Thus, rabbits with lesions exhibited training-induced activity earlier in training than control rabbits. However, these effects were likely due to group differences in the distribution of recording sites across cingulate cortical layers. Recording electrodes in 8 of the 11 of the rabbits with lesions were placed in cell layer V. Neurons in layer V have been shown to develop discriminative responses during the session of first significant behavioral discrimination (Gabriel, Vogt, Kubota, Poremba, & Kang, 1991b). In contrast, 6 of the 9 control rabbits had placements in layers IV ($n = 3$) and VI ($n = 3$), in which neurons typically do not develop discriminative responses until the criterial training session.

Training-Induced Neuronal Activity: AV Thalamic Nucleus

Central nuclear lesions attenuated excitatory training-induced neuronal activity, but did not disrupt the development of discriminative training-induced neuronal activity in the AV thalamic nucleus during discriminative approach learning (Fig. 5A). Individual comparisons following a significant interaction of the Session and CS factors (10-ms post-CS intervals: $F[3, 48] = 11.45$, $p < .0001$, integrated unit activity; $F[3, 48] = 8.40$, $p < .0005$, spike frequency; 100-ms post-CS intervals: $F[3, 48] = 10.62$, $p < .0005$, integrated unit activity; $F[3, 48] = 8.57$, $p < .001$, spike frequency) indicated significantly greater responses to the CS^+ than the CS^- during the session of first significant behavioral

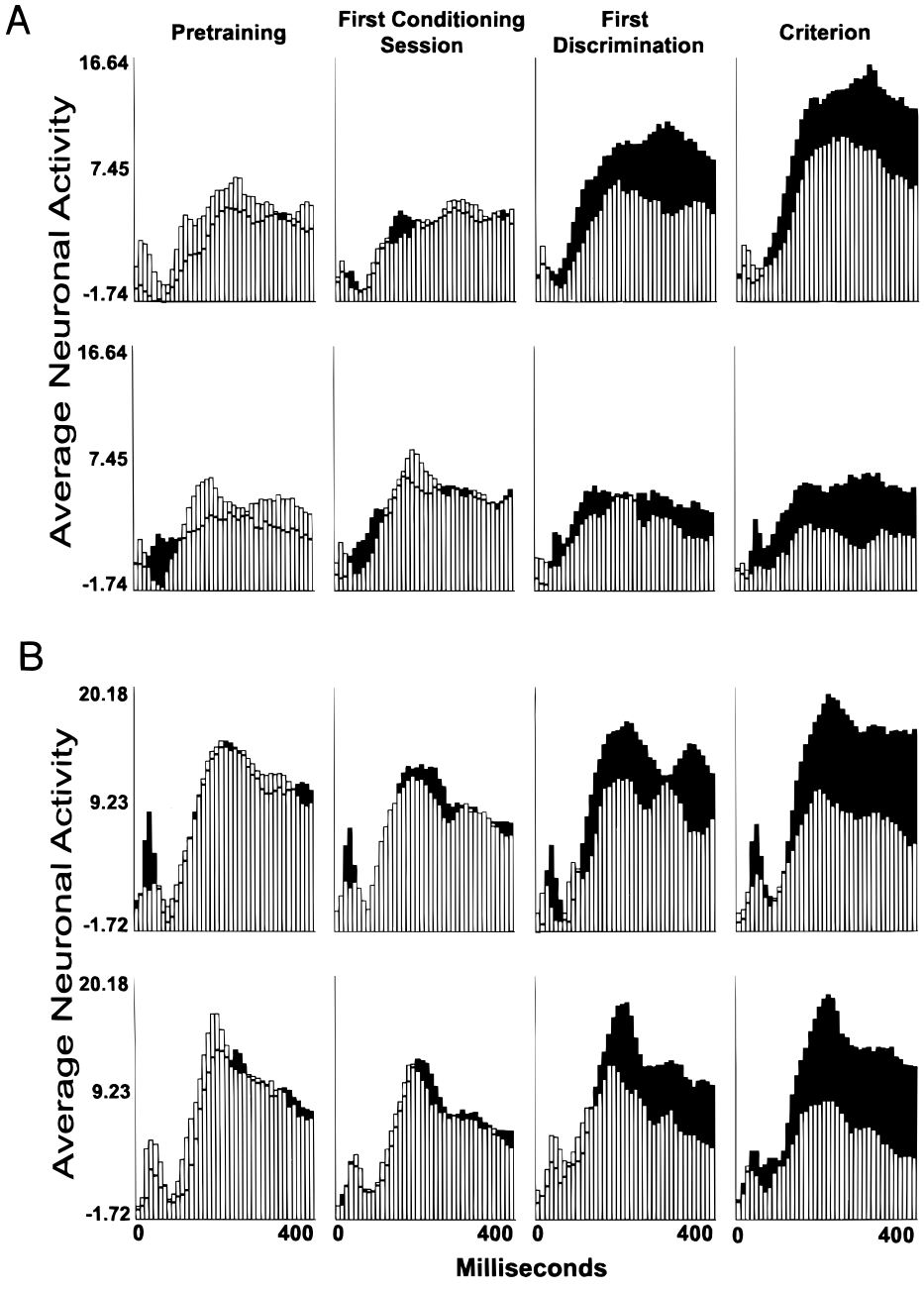

FIG. 5. Average integrated unit activity recorded in the anterior ventral thalamic nucleus during discriminative approach (A: top row, control; second row, lesion) and avoidance learning (B: top row, control; bottom row, lesion) for control rabbits ($n = 9$) and rabbits with lesions ($n = 9$). The data, in the form of z scores normalized with respect to a 300-ms pre-CS baseline, are shown in 40 consecutive 10-ms intervals after the onset of the CS^+ (black bars) and CS^- (white bars). Data are shown for the following sessions: pretraining with explicitly unpaired tone and foot shock presentations, the first conditioning session, the session of first significant behavioral discrimination (First Discrimination), and the criterial session.

discrimination and the criterial session (all $p < .05$). No group differences in the development of discriminative neuronal activity were found (e.g., interaction of the Session, CS, and Group factors, 10-ms intervals: $F[3, 48] = .94$, $p < .44$, integrated unit activity, $F[3, 48] = .33$, $p < .82$, spike frequency). Separate analyses of the data from rabbits with lesions confirmed that the lesions did not block the development of discriminative training-induced activity. Individual comparisons following a significant interaction of the Session and CS factors (10-ms intervals: $F[3, 24] = 7.19$, $p < .005$, integrated activity; $F[3, 24] = 4.61$, $p < .05$, spike frequency) indicated significant discriminative neuronal activity during the criterial session (all $p < .05$). However, the lesions did attenuate excitatory training-induced neuronal activity. Individual comparisons following a significant interaction of the Session and Group factors ($F[3, 48] = 5.58$, $p < .005$, integrated unit activity, $F[3, 48] = 4.33$, $p < .05$, spike frequency) indicated that the overall magnitude of the CS-elicited response increased during the session of first significant behavioral discrimination and the criterial session in control rabbits ($n = 9$), relative to pretraining and the first conditioning session (all $p < .05$). No such increases in the magnitude of the neuronal response were found in rabbits with lesions ($p > .05$, $n = 9$).

Central nuclear lesions did not disrupt AV thalamic training-induced neuronal activity during discriminative avoidance learning (Fig. 5B). No significant interactions involving the Group factor were found (e.g., interaction of the Session, CS, and Group factors, 10-ms post-CS intervals: $F[3, 42] = .01$, $p < .99$, integrated unit activity, $F[3, 42] = .38$, $p < .78$, spike frequency).

Training-Induced Neuronal Activity: MD Thalamic Nucleus

Central nuclear lesions attenuated excitatory training-induced activity, but did not disrupt the development of discriminative activity in the MD thalamic nucleus during discriminative approach learning (Fig. 6A). A significant interaction of the Session and Group factors was found in the spike frequency data (100-ms post-CS intervals: $F[3, 39] = 5.01$, $p < .001$; 10-ms post-CS intervals: $F[3, 39] = 3.93$, $p < .05$). Individual comparisons indicated the development of excitatory training-induced activity during the session of first significant discrimination and the criterial session in control rabbits but not in rabbits with lesions (all $p < .05$). These interactions did not attain significance in the integrated unit activity (100-ms post-CS intervals: $F[3, 39] = 2.64$, $p < .09$; 10-ms post-CS intervals: $F[3, 39] = 1.75$, $p < .20$). No group differences were found with respect to discriminative training-induced activity during discriminative approach learning (e.g., interaction of the Session, CS, and Group factors, 100-ms post-CS intervals: $F[3, 39] = .33$, $p < .80$, integrated unit activity, $F[3, 39] = .50$, $p < .67$, spike frequency).

Central nuclear lesions attenuated but did not block training-induced neuronal activity in the MD thalamic nucleus during discriminative avoidance learning (Fig. 6B). Individual comparisons following a significant interaction of the Session, CS, and Group factors for the integrated unit activity (100-ms post-CS intervals: $F[3, 42] = 3.58$, $p < .05$) indicated the development of significant discriminative and excitatory training-induced activity during the criterial session in control rabbits but not in rabbits with lesions (all $p < .05$). However, individual comparisons following an interaction of the Session, CS, and Group factors for the spike frequency data which approached significance (100-ms post-CS intervals: $F[3, 42] = 2.80$, $p < .06$) did indicate the development of excitatory and

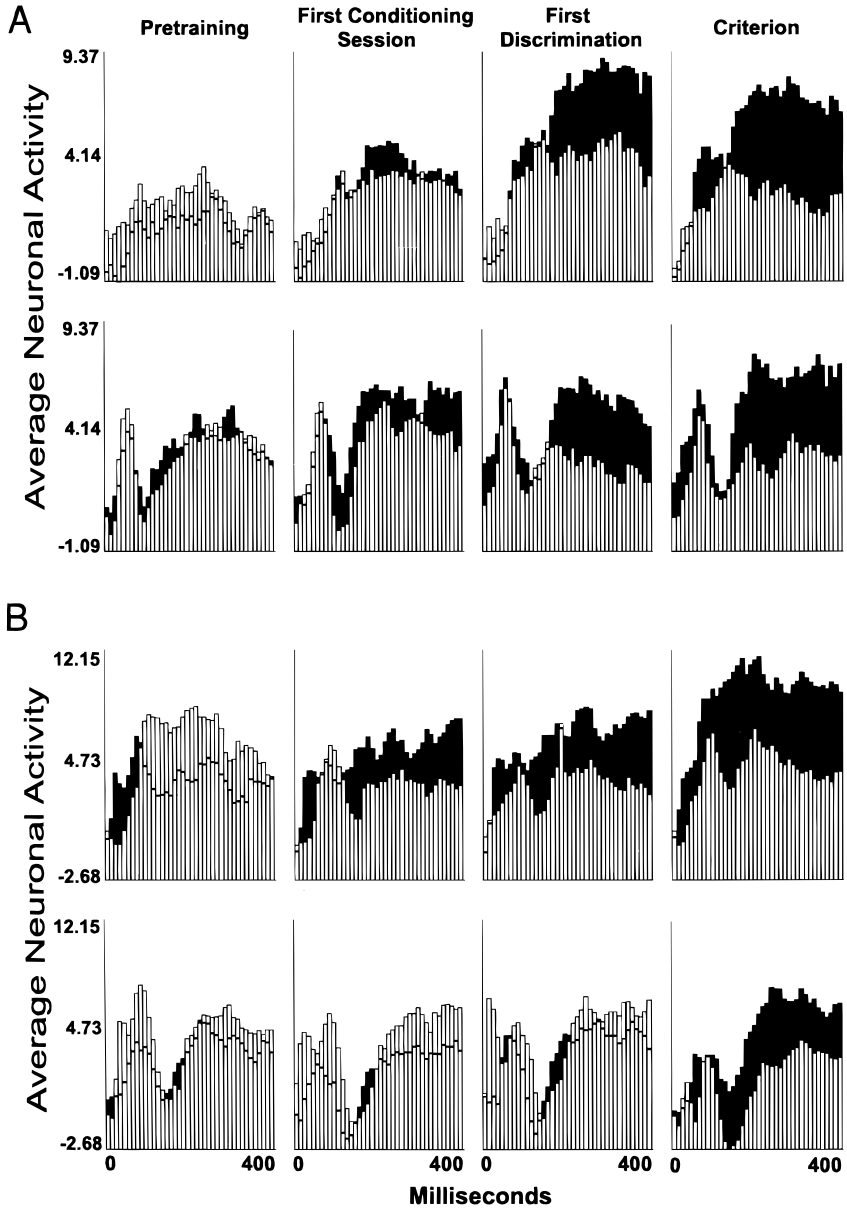

FIG. 6. Average integrated unit activity recorded in the medial dorsal thalamic nucleus during discriminative approach (A: top row, control; second row, lesion) and avoidance learning (B:top row, control; bottom row, lesion) for control rabbits ($n = 7$) and rabbits with lesions ($n = 8$). The data, in the form of z scores normalized with respect to a 300-ms pre-CS baseline, are shown in 40 consecutive 10-ms intervals after the onset of the CS^+ (blackbars) and CS^- (white bars). Data are shown for the following sessions: pretraining with explicitly unpaired tone and foot shock presentations, the first conditioning session, the session of first significant behavioral discrimination (First Discrimination), and the criterial session.

discriminative training-induced activity during the criterial session in rabbits with lesions (all $p < .05$). Furthermore, separate analyses of the data from rabbits with lesions confirmed the development of discriminative and excitatory training-induced activity. Individual comparisons following a significant interaction of the Session and CS factors (100-ms post-CS intervals: $F[3, 24] = 5.75$, $p < .01$, integrated unit activity, $F[3, 24] = 4.26$, $p < .05$, spike frequency) indicated significant discriminative and excitatory training-induced activity during the criterial session ($p < .05$).

Training-Induced Neuronal Activity during Avoidance Learning in Rabbits with Lesions: Comparison of Rabbits That Did and Did Not Attain the Avoidance Criterion

Although they were significantly delayed, half of the rabbits with lesions did attain the avoidance learning criterion. This raised the possibility that the intact training-induced neuronal activity during avoidance learning in the lesion group as a whole was due primarily to spared neuronal activity in the rabbits that learned. However, examination of the neuronal records revealed no evidence of greater disruption of neuronal activity in the rabbits that did not attain the criterion. Thus, training-induced neuronal activity was not more severely disrupted in rabbits that failed to attain the avoidance criterion and the intact neuronal activity in those subjects that did learn could not have accounted for the findings of intact training-induced activity in the lesion group as a whole.

Alternating Approach and Avoidance Task Performance

Initiation of avoidance training did not disrupt ongoing discriminative approach performance in control rabbits or rabbits with lesions. No significant interactions involving the Group or Session factors were found for conditioned responding in the approach task (e.g., interaction of Session, CS, and Group factors: $F[3, 57] = 1.96$, $p < .14$). Furthermore, there were no indications that initiation of avoidance training disrupted training-induced neuronal activity during continuing discriminative approach performance in any of the regions sampled. For example, the interaction of the Session, CS, and Group factors for the AV thalamic neuronal data was not significant ($F[3, 48] = 0.47$, $p < .71$, integrated unit activity). The same results were found for posterior cingulate cortical and MD thalamic neuronal activity. The predictive value of the conditional stimuli was reversed between the approach and avoidance tasks. Thus, the neurons could only exhibit the correct discriminative responses if they also reversed their responses on alternating days. Thus, cingulothalamic neurons exhibited task-appropriate discriminative responses which were unaffected by CE nuclear lesions.

DISCUSSION

Lesions centered in the CE nucleus of the amygdala impaired discriminative avoidance learning but had no effect on approach learning in the same subjects. Remarkably, rabbits with lesions failed to exhibit discriminative avoidance behavior even though they were able to use the same discriminative cues for approach behavior on alternating days. These results effectively ruled out interpretations of the deficit in avoidance learning as having

been due to any global inability to process the cues or to form associations between the cues and reinforcing events. The unimpaired responses to the foot shock US exhibited by rabbits with lesions further eliminated interpretations involving disruption of basic motoric, motivational, and pain processes.

The selective effects of CE nuclear lesions on avoidance learning may have been related to the aversive nature of the reinforcer. However, a growing body of data has indicated a CE nuclear role in various phenomena of appetitive learning (Hatfield et al., 1996; Holland & Gallgher, 1993; McIntyre, Ragozzino, & Gold, 1998; Gaffan & Murray, 1990).

A number of observations suggest that the critical determinant of CE nuclear involvement may be the level of emotional arousal induced by the learning situation. Note that arousal is engendered both by aversive and appetitive learning situations and is thus dissociable from hedonic value per se. Avoidance learning proceeds rapidly with little variation in subjects' performance. In contrast, approach learning proceeds slowly, performance is quite variable, and the behavioral discrimination is not as large at asypmtote. These observations suggest that discriminative avoidance learning engenders more arousal than discriminative approach learning. Thus, it may be that the CE nuclear lesions impaired the subjects' ability to use highly arousing reinforcers to accelerate learning. This interpretation is consistent with the notion that the amygdala facilitates memory storage in other brain regions in situations that are highly arousing or emotional (McGaugh, Cahill, & Roozendaal, 1996). Recent findings indicate that the amygdala is critically involved in learning when large rewards are employed, but not when small rewards are used (McIntyre et al., 1998). Indeed, it has been proposed that the CE nucleus is involved in regulating arousal during learning (Kapp, Whalen, Supple, & Pascoe, 1992).

Although avoidance learning was markedly retarded in subjects with lesions, significant learning did occur. Half of the subjects attained the criterion. Even among the subjects that did not attain the criterion, four of six did attain significant discrimination (25% more frequent responding to the CS^+ than to the CS^-) during the allotted 15-session training period. Comparison of approach and avoidance learning rates is instructive with regard to the role of the CE nucleus. Both control rabbits and rabbits with lesions exhibited slowly developing behavioral discrimination in the approach task. In the avoidance task, control rabbits exhibited very rapid learning. In contrast, rabbits with lesions learned slowly, as during approach learning. These observations suggest that the rabbits with lesions failed to modulate their learning rate in response to the urgency of the learning situation. This finding suggests that the CE nucleus of the amygdala functions to accelerate learning in emergency situations, which engender high arousal (Gabriel, 1992; Kapp et al., 1992). Consistent with this idea, disruption of training-induced neuronal activity during avoidance learning was observed in the MD nucleus, a region known to participate in the circuitry that promotes the rapid acquisition of avoidance learning (Gabriel, Sparenborg, & Kubota, 1989).

The lesions had no effect on approach learning, although they did attenuate AV thalamic and posterior cingulate cortical training-induced neuronal activity. Previous studies have shown that attenuation of training-induced neuronal activity in the AV thalamic nucleus does not impair avoidance learning as long as discriminative neuronal responses remain intact (Gabriel, Cuppernell, Shenker, Kubota, Henzi, & Swanson, 1995; Taylor, Freeman, Holt, & Gabriel, 1999). In the present study, clear evidence of discriminative training-induced activity was found in all of the monitored areas in rabbits with lesions. Thus, it

is perhaps not surprising that the attenuation of training-induced activity did not block approach learning. Nevertheless, the question still remains as to why the attenuation of training-induced neuronal activity was more prevalent during approach learning than during avoidance learning. During approach learning, a lesion-induced attenuation of training-induced neuronal activity was found in all three of the monitored regions. In contrast, a lesion-induced attenuation was found only in the MD thalamic nucleus during avoidance learning. In keeping with the above discussion of arousal processes, it is possible that the avoidance task was sufficiently arousing that any spared tissue in the amygdala was sufficient to transmit excitation to other brain regions, including the cingulothalamic circuitry. However, the less arousing approach task may have been insufficient to excite the damaged pathway, rendering the circuitry more susceptible to partial disruption by the lesions.

In previous studies, lesions involving extensive damage to the CE, BL, and LA nuclei were associated with a complete loss of both excitatory and discriminative training-induced neuronal activity in the cingulothalamic circuitry (Poremba & Gabriel, 1997). Discriminative neuronal activity effectively encodes stimulus significance by virtue of the greater responses to the CS^+ than to the CS^-. The importance of cingulothalamic significance coding is indicated by numerous studies showing that lesion-induced disruption of significance coding blocks learning (Gabriel et al., 1991a, 1983, 1989; Smith, Freeman, Boule, Kang, & Gabriel, 1997). In the present study, the damage to the BL and LA nuclei was much less extensive than that in previous studies and the disruption of discriminative training-induced activity was minimal. Thus, the current findings reinforce our view that cingulothalamic significance coding is one of multiple processes involved in the mediation of discriminative avoidance learning (Gabriel, 1993). In fact, the cingulothalamic neurons of rabbits with CE nuclear lesions correctly encoded stimulus significance, even under the seemingly demanding circumstances of alternating approach and avoidance training. Since the predictive value of the cues was reversed between the two tasks, cingulothalamic neurons could only correctly encode stimulus significance if their discriminative responses were also reversed on alternating days. The fact that CE nuclear lesions impaired avoidance learning while leaving cingulothalamic significance coding essentially intact suggests that the BL and LA amygdalar nuclei promote significance coding, as indexed by discriminative neuronal activity in the cingulothalamic circuitry, whereas CE nuclear projections to other brain regions are involved in mediating the effects of emotional arousal on learning.

Neither the route nor the mechanisms whereby the CE nucleus influences discriminative avoidance learning are known. The CE nucleus projects to several brain regions known to be involved in learning. The CE nucleus influences dopaminergic function via projections to the ventral tegmental area (VTA) and the substantia nigra (Amaral et al., 1992; Krettek & Price, 1978). Projections from the VTA have been implicated in an anterior cingulate cortical attentional response that is independent of discriminative neuronal activity (Taylor et al., 1999). Anterior cingulate cortical neurons exhibit robust excitatory and discriminative neuronal activity during the early stages of avoidance learning and lesions of the anterior cingulate cortex selectively disrupt the initial phase of avoidance learning (Gabriel et al., 1991a). Thus, the anterior cingulate cortex is part of an early learning system that promotes the rapid acquisition of avoidance behavior. The BL nucleus sends axonal projections to the anterior cingulate cortex (Krettek & Price, 1977a) and lesions

of the BL nucleus blocked the development of discriminative neuronal activity in the anterior cingulate cortex (Poremba & Gabriel, 1999). Thus, the rapid learning function of the anterior cingulate cortex may be dependent on both CE nuclear modulation of arousal, via the VTA, and BL nuclear modulation of anterior cingulate cortical significance coding. Response-related processing in the striatum may similarly depend on separate converging processes. The CE nucleus has been proposed to influence striatal function via its projections to the substantia nigra (Gallagher & Holland, 1994). Cingulothalamic neurons exhibit premotor discharges immediately preceding avoidance responses, suggesting that a "go" signal generated in the cingulothalamic circuitry is sent to the striatum to trigger the avoidance response (Kubota, Wolske, Poremba, Kang, & Gabriel, 1996). Thus, CE nuclear processes could modulate the excitability of striatal neurons under emergency learning conditions, thereby increasing the probability of avoidance responding upon receipt of a cingulothalamic "go" signal.

The CE nucleus also influences cholinergic functioning via its projections to the brainstem and basal forebrain cholinergic nuclei (Amaral et al., 1992; Hopkins & Holstege, 1978). Central nuclear projections to the basal forebrain have been implicated in various learning and attention functions (Gallagher & Holland, 1994; Muir, Dunnett, Robbins, & Everitt, 1992a; Muir, Robbins, & Everitt, 1992b). In a finding similar to those of the present study, lesions of the brainstem cholinergic nuclei impaired discriminative avoidance learning and attenuated excitatory neuronal activity but they did not block discriminative activity (Kubota, 1996). The present results suggest that disruption of CE nuclear inputs to the brainstem cholinergic system can disrupt the development of cingulothalamic excitatory activity. The magnitude of neuronal responses evoked by both the CS^+ and the CS^- increases significantly with the addition of a highly arousing stimulus, such as noncontingent foot-shock presentations (Gabriel & Saltwick, 1977). Thus, cingolothalamic excitatory neuronal activity may be related to the nonspecific arousal processes mediated by the CE nucleus of the amygdala. These observations are consistent with the idea that the CE nucleus modulates cortical attention functions through its projections to the basal forebrain and the brainstem cholinergic nuclei (Gallagher & Holland, 1994).

The results of the present study complement recent models of amygdalar function (Amorapanth et al., 2000; Everitt et al., 1991; Killcross et al., 1997) which suggest that the CE nucleus is involved in the Pavlovian conditioning of fear responses (e.g., conditioned freezing, fear-potentiated startle, autonomic responses). Two-process models of avoidance learning suggest that subjects first develop a Pavlovian fear response to a CS paired with an aversive reinforcer. The avoidance learning deficit observed here may have resulted from disruption of this Pavolovian learning process.

Another model suggests that the CE nucleus is involved in attentional processing of stimuli that predict reinforcement, whereas the BL nucleus is involved in assigning the motivational significance of a reinforcer to a predictive CS (Gallagher & Holland, 1994; Hatfield et al., 1996; Holland & Gallagher, 1993). Specifically, the CE nucleus is thought to be involved in incrementing attention to predictive stimuli. The present results are consistent with this model in that avoidance learning requires that subjects increment their attention to the CS^+. The cingulothalamic cuircuitry is involved in promoting attention to associatively significant cues. Cingolothalamic excitatory and discriminative neuronal activity represents an associative attentional response of the brain (Sparenborg & Gabriel,

1990; Taylor et al., 1999). Here, the lesions attenuated the attentional response of cingulothalamic neurons, but only in the low arousal conditions of approach learning. Thus, the role of the CE nucleus in attentional processing within the cingulothalamic circuitry may depend on the level of arousal induced by the learning situation.

REFERENCES

Amaral, D. G., Price, J. L., Pitkanen, A., & Carmichael, S. T. (1992). Anatomical organization of the primate amygdaloid complex. In J. LeDoux (Ed.), *The amgydala: Neurobiological aspects of emotion, memory, and mental dysfunction* (pp. 1–66). New York: Wiley-Liss.

Amorapanth, P., Ledoux, J. E., & Nader, K. (2000). Different lateral amygdala outputs mediate reactions and actions elicited by a fear-arousing stimulus. *Nature Neuroscience,* **3**(1), 74–79.

Brogden, W. J., & Culler, F. A. (1936). A device for motor conditioning of small animals. *Science,* **83,** 269.

Cador, M., Robbins, T. W., & Everitt, B. J. (1989). Involvement of the amygdala in stimulus-reward associations: Interaction with the ventral striatum. *Neuroscience,* **30,** 77–86.

Collins, D., & Pare, D. (1999). Reciprocal changes in the firing probability of lateral and central amygdala neurons. *Journal of Neuroscience,* **19**(2), 836–844.

Davis, M. (1990). Animal models of anxiety based on classical conditioning: The conditioned emotional response (CER) and the fear-potentiated startle effect. *Pharmacological Therapy,* **47,** 147–165.

Donovick, P. J. (1964). A metachromatic stain for neural tissue. *Stain Technology,* **49,** 49–51.

Everitt, B. J., Morris, K. A., O'Brian, A., & Robbins, T. W. (1991). The basolateral amygdala-ventral striatal system and conditioned place preference: Further evidence of limbic-striatal interactions underlying reward-related processes. *Neuroscience,* **42,** 1–18.

Fox, C. A., & Eichman, J. (1959). A rapid method for locating intracerebral electrode tracks. *Stain Technology,* **34,** 39–42.

Freeman, J. H., Jr., Cuppernell, C., Flannery, K., & Gabriel, M. (1996). Limbic thalamic, cingulate cortical and hippocampal neuronal correlates of discriminative approach learning in rabbits. *Behavioral Brain Research,* **80,** 123–136.

Gabriel, M. (1992). Neural substrates of avoidance learning: In L. R. Squire (Ed.), *Encyclopedia of learning and memory* (pp. 67–70). New York: Macmillan, Co.

Gabriel, M. (1993). Discriminative avoidance learning: A model system: In B. A. Vogt & M. Gabriel (Eds.), *Neurobiology of cingulate cortex and limbic thalamus: A comprehensive handbook* (pp. 478–523). Boston: Birkhäuser.

Gabriel, M., Cuppernell, C., Shenker, J., Kubota, Y., Henzi, V., & Swanson, D. (1995). Mamillothalamic tract transection blocks anterior thalamic training-induced neuronal plasticity and impairs discriminative avoidance behavior in rabbits. *Journal of Neuroscience,* **15**(2), 1437–1445.

Gabriel, M., Kubota, Y., Sparenborg, S. P., Straube, K., & Vogt, B. (1991a). Effects of cingulate cortical lesions of avoidance learning and training-induced unit activity in rabbits. *Experimental Brain Research,* **86,** 585–600.

Gabriel, M., Lampert, R. W., Foster, K., Orona, E., Sparenborg, S., & Maiorca, R. (1983). Anterior thalamic lesions and neuronal activity in cingulate cortices during discriminative avoidance behavior in rabbits. *Behavioral Neuroscience,* **97,** 675–696.

Gabriel, M., & Saltwick, S. (1977). Effects of unpaired footshock on rabbit limbic and auditory neuronal responses to tone stimuli. *Physiology and Behavior,* **19,** 29–34.

Gabriel, M., Sparenborg, S. P., & Kubota, Y. (1989). Anterior and medial thalamic lesions, discriminative avoidance learning and cingulate cortical neuronal activity in rabbits. *Experimental Brain Research,* **76,** 441–457.

Gabriel, M., Vogt, B. A., Kubota, Y., Poremba, A., & Kang, E. (1991b). Training-stage related neuronal plasticity in limbic thalamus and cingulate cortex during learning: A possible key to mnemonic retrieval. *Behavioral Brain Research,* **46,** 175–185.

Gaffan, E. A., Gaffan, D., & Harrison, S. (1988). Disconnection of the amgydala from visual association cortex impairs visual reward-association learning in monkeys. *Journal of Neuroscience,* **8**(9), 3144–3150.

Gaffan, D., & Murray, E. A. (1990) Amygdalar interaction with the mediodorsal nucleus of the thalamus and the ventromedial prefrontal cortex in stimulus-reward associative learning in the monkey. *Journal of Neuroscience,* **10**(11), 3479–93.

Gallagher, M., & Holland, P. (1994). The amygdala complex: Multiple roles in associative learning and attention. *Proceedings of the National Academy of Science USA,* **91,** 11771–11776.

Girgis, M., & Shih-Chang, W. (1981). *A new stereotaxic atlas of the rabbit brain.* St. Louis: Green.

Hatfield, T., Han, J., Conley, M., Gallagher, M., & Holland, P. (1996). Neurotoxic lesions of basolateral, but not central, amygdala interfere with pavlovian second-order conditioning and reinforcer devaluation effects. *Journal of Neuroscience,* **16**(16), 5256–5265.

Hitchcock, J., & Davis, M. (1986). Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the fear-potentiated startle paradigm. *Behavioral Neuroscience,* **100,** 11–22.

Holland, P. C., & Gallagher, M. (1993). Effects of amygdala central nucleus lesions on blocking and unblocking, *Behavioral Neuroscience,* **107**(2), 2235–2245.

Hopkins, D. A., & Holstege, G. (1978). Amygdalar projections to the mesencephalon, pons, and medulla oblongata in the cat. *Experimental Brain Research,* **32,** 529–547.

Huyhn, H., & Feldt, L. S. (1976). Estimation of the box correction for degrees of freedom from sample data in randomized block and split-plot factorial designs. *Journal of Educational Statistics,* 69–82.

Iwata, J., Chida, K., & LeDoux, J. (1987). Cardiovascular responses elicited by electrical stimulation of neurons in the central amygdaloid nucleus in awake, but not anesthetized rats resemble conditioned emotional responses. *Brain Research,* **418,** 183–187.

Kapp, B., Frysinger, R., Gallagher, M., & Haselton, J. (1979). Amygdala central nucleus lesions: Effect on heart rate conditioning in the rabbit. *Physiology and Behavior,* **23,** 1109–1117.

Kapp, B., Whalen, P., Supple, W., Jr., & Pascoe, J. (1992). In J. B. Aggleton (Ed.), *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction* (pp. 229–245). New York: Wiley-Liss.

Killcross, S., Robbins, T. W., & Everitt, B. J. (1997). Different types of fear-conditioned behaviour mediated by separate nuclei within the amygdala. *Nature,* **388,** 377–380.

Krettek, J., & Price, J. (1977a). Projections from the amygdaloid complex to the cerebral cortex and thalamus in the rat and cat. *Journal of Comparative Neurology,* **172**(4), 687–722.

Krettek, J., & Price, J. (1977b). Projections from the amygdaloid complex & adjacent olfactory structures to the entorhinal cortex and to the subiculum in the rat and cat. *Journal of Comparative Neurology,* **172,** 723–752.

Krettek, J., & Price, J. (1978). Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. *Journal of Comparative Neurology,* **178,** 225–254.

Kubota, Y. (1996). *Brainstem cholinergic modulation of the limbic learning circuit.* Unpublished doctoral dissertation, University of Illinois, Urbana.

Kubota, Y., Wolske, M., Poremba, A., Kang, E., & Gabriel, M. (1996). Stimulus-related and movement-related single-unit in rabbit cingulate cortex and limbic thalamus during performance of discriminative avoidance behavior. *Brain Research,* **721,** 22–38.

LeDoux, J. E. (1993). Emotional memory: In search of systems and synapses. In F. M. Crinella and J. Yu (Eds.), *Brain mechanisms: Papers in memory of Robert Thompson* (pp. 149–157). New York: New York Academy of Sciences.

LeDoux, J. E., Iwata, J., Cicchetti, P., & Reis, D. (1988). Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *Journal of Neuroscience,* **8**(7), 2517–2529.

Liang, K., McGauch, J., Martinez, J., Jensen, R., Vasquez, B., & Messing, R. (1982). Post-training amygdaloid lesions impair retention of an inhibitory avoidance response. *Behavioral Brain Research,* **4,** 237–249.

Maren, S., & Fanselow, M. S. (1996). The amygdala and fear conditioning: Has the nut been cracked? *Neuron,* **16,** 237–240.

Maren, S., Poremba, A., & Gabriel, M. (1991). Basolateral amygdaloid multi-unit neuronal correlates of discriminative avoidance learning in rabbits. *Brain Research,* **549,** 311–316.

McGaugh, J. L., Cahill, L., & Roozendaal, B. (1996). Involvement of the amygdala in memory storage: Interaction with other brain systems. *Proceedings of the National Academy of Science, USA,* **24,** 13508–13514.

McIntyre, C. K., Ragozzino, M. E., & Gold, P. E. (1998). Intra-amygdala infusions of scopolamine impair performance on a conditioned place preference task but not a spatial radial maze task. *Behavioral Brain Research,* **95**(2), 219–226.

Muir, J. L., Dunnett, S. B., Robbins, T. W., & Everitt, B. J. (1992a). Attentional functions of the forebrain cholinergic systems: Effects of intraventricular hemicholinium, physostigmine, basal forebrain lesions and intracortical grafts on a multiple-choice serial reaction time task. *Experimental Brain Research,* **89**(3), 611–622.

Muir, J. L., Robbins, T. W., & Everitt, B. J. (1992b). Disruptive effects of muscimol infused into the basal forebrain on conditional discrimination and visual attention: Differential interactions with cholinergic mechanisms. *Psychopharmacology,* **107**(4), 541–550.

Nagel, J., & Kemble, E. (1976). Effects of amygdaloid lesions on the performance of rats in four passive avoidance tasks. *Physiology and Behavior,* **17,** 245–250.

Parent, M., & McGaugh, J. (1994). Posttraining infusion of lidocaine into the amygdala basolateral complex impairs retention of inhibitory avoidance training. *Brain Research,* **661,** 97–103.

Payne, J., Hanlon, J., Cantey, J., Mungnirun, K., Duvel, A., Smith, D., Gimbel, K., Nelson, M., & Gabriel, M. (1999). High-resolution digital brain atlases for behavioral neuroscience. *Society for Neuroscience Abstracts,* **25,** 877.

Pitkänen, A., Savander, V., & LeDoux, J. (1997). Organization of intra-amygdaloid circuitries in the rat: An emerging framework for understanding the functions of the amygdala. *Trends in Neuroscience,* **20**(11), 517–523.

Poremba, A., & Gabriel, M. (1997). Amygdalar lesions block discriminative avoidance learning and cingulothalamic training-induced plasticity in rabbits. *Journal of Neuroscience,* **17**(13), 5237–5244.

Poremba, A., & Gabriel, M. (1999). Amygdala neurons mediate acquisition but not maintenance of instrumental avoidance behavior in rabbits. *Journal of Neuroscience,* **19**(21), 9635–9641.

Porrino, L. J., Crane, A. M., & Goldman-Rakic, P. S. (1981). Direct and indiarect pathways from the amygdala to the frontal lobe in rhesus monkey. *Journal of Comparative Neurology,* **198,** 121–136.

Roozendaal, B., Koolhaas, J. M., & Bohus, B. (1993). The central amygdala is involved in conditioning but not in retention of active and passive shock avoidance in male rats. *Behavioral and Neural Biology,* **2,** 143–149.

Smith, D. M., Freeman, J. H., Jr., Boule, M., Kang, E., & Gabriel, M. (1997). Thalamic lesions, appetitively motivated discrimination learning and training-induced neuronal activity in rabbits. *Society for Neuroscience Abstracts,* **23,** 1617.

Smith, Y., & Pare, D. (1994). Intra-amygdaloid projections of the lateral nucleus in the cat: PH-L anterograde labeling combined with post-embedding GABA and glutamate immunocytochemistry. *Journal of Comparative Neurology,* **342,** 232–248.

Sparenborg, S., & Gabriel, M. (1990). Neuronal encoding of conditional stimulus duration in the cingulate cortex and the limbic thalamus of rabbits. *Behavioral Neuroscience* **104,** 919–933.

Swanson, L. W., & Petrovich, G. D. (1998). What is the amygdala? *Trends in Neurosciences* **21,** 323–331.

Taylor, C. L., Freeman, J. H., Jr., Holt, W., & Gabriel, M. (1999). Impairment of cingulothalamic learning-related neuronal coding in rabbits exposed to cocaine in utero: General and sex-specific effects, Behavioral Neuroscience, **113**(1), 62–77.

Urban, I., & Richard, P. (1972). *A stereotaxic atlas of the new zealand white rabbit brain.* Springfield: Thomas.

Winer, B. J. (1962). *Statistical principles in experimental design.* New York: McGraw–Hill.