Behavioral Neuroscience
1996, Vol. 110, No. 4, 718–726

0735-7044/96/$3.00

Retrograde Abolition of Conditional Fear After Excitotoxic Lesions in the Basolateral Amygdala of Rats: Absence of a Temporal Gradient

Stephen Maren, Gal Aharonov, and Michael S. Fanselow
University of California, Los Angeles

The role of the basolateral amygdala (BLA) in the acquisition and expression of Pavlovian fear conditioning was examined in 80 rats. Excitotoxic lesions were made in the BLA using *N*-methyl-D-aspartate 7 days before or 1, 14, or 28 days after Pavlovian fear conditioning. Conditioning consisted of three pairings of a tone with an aversive footshock in a novel chamber, and freezing behavior served as an index of conditional fear. BLA lesions abolished conditional freezing to both the contextual and acoustic conditional stimuli at all training-to-lesion intervals, and the magnitude of the impairment did not vary as a function of the training-to-lesion interval. Reacquisition training elevated levels of freezing in rats with BLA lesions but did not reduce the magnitude of their deficit in relation to that of controls. These results reveal that neurons in the BLA have an enduring role in the expression of conditional fear.

In the last decade, a great deal of progress has been made in identifying the neural substrates of simple forms of aversive learning in mammals (Davis, 1992; Fanselow, 1994; Gabriel, 1990; Kapp, Whalen, Supple, & Pascoe, 1992; LeDoux, 1995; McGaugh, 1989). One form of aversive learning that has been extensively investigated in this regard is Pavlovian fear conditioning in rats. In the fear-conditioning task, rats are placed in a novel observation chamber and presented with a discrete conditional stimulus (CS), such as a tone, that signals the occurrence of an aversive footshock unconditional stimulus (US). After a few such pairings, rats come to exhibit a conditional fear response to the CS that is characterized by increases in arterial blood pressure, potentiated acoustic startle, and somatomotor immobility (i.e., freezing).

Concerning the neural substrates of Pavlovian fear conditioning, it is now established that neurons in the amygdala play an essential role. For example, excitotoxic lesions placed in either the central nucleus of the amygdala (CEA) or the basolateral amygdaloid complex (BLA, comprising the lateral, basolateral, and basomedial nuclei) produce severe impairments in both the acquisition (Campeau & Davis, 1995; Sananes & Davis, 1992) and expression (Campeau & Davis, 1995; Helmstetter, 1992; Sananes & Davis, 1992) of conditional fear. In addition, reversible inactivation of the BLA disrupts the acquisition and expression of conditional fear (Helmstetter & Bellgowan, 1994), and associative neuronal discharges develop rapidly in the BLA during aversive conditioning (Maren, Poremba, & Gabriel, 1991). Because the BLA is a site for the convergence of CSs and USs (Romanski, Clugnet, Bordi, & LeDoux, 1993), it may be a substrate for CS–US association during Pavlovian fear conditioning (e.g., LeDoux, 1995). In contrast, the CEA, which is a major recipient of axons from the BLA and source of projections to brain structures involved in generating fear responses, may be involved in executing both conditional and unconditional fear responses (CRs and URs; e.g., Davis, 1992).

Although it is recognized that the amygdala is required for both the acquisition and expression of conditional fear, it is not clear whether it has an enduring or temporary role in expressing conditional fear after training. For instance, certain neural systems (e.g., the hippocampus) have been reported to have only a temporary role in the expression of conditional fear to contextual CSs after training (J. J. Kim & Fanselow, 1992). To determine the role of the amygdala in the expression of conditional fear after training, Davis and his colleagues examined the impact of posttraining CEA lesions on the expression of fear-potentiated startle in rats. In this behavioral paradigm, conditional fear is manifest as an enhancement of the acoustic startle reflex in the presence of a discrete CS (usually a light) that has been paired with footshock. Previous work from Davis's laboratory has demonstrated that either electrolytic or excitotoxic CEA lesions prevent the acquisition of fear-potentiated startle (Campeau & Davis, 1995; Hitchcock & Davis, 1986). Recently, these investigators have reported that electrolytic lesions of the CEA given either 6 or 30 days after training produced a similar and massive impairment in fear-potentiated startle (M. Kim & Davis, 1993b). This suggests that the CEA (or axons that traverse it) has an essential and enduring role in the expression of conditional fear following conditioning.

The enduring role of the CEA in expressing fear-potentiated startle is perhaps not surprising given that the CEA may be the final common output pathway for the performance of conditional fear responses (e.g., LeDoux, Iwata, Cicchetti, & Reis, 1988). An important and yet unanswered question is the role of the BLA in the expression of conditional fear after training. Work by McGaugh and his associates (e.g., Liang, McGaugh, Martinez, Jensen, Vasquez, & Messing, 1982) suggests

Stephen Maren, Gal Aharonov, and Michael S. Fanselow, Department of Psychology, University of California, Los Angeles.

This work was supported by National Institute of Mental Health Grant MH39786 to Michael S. Fanselow. Stephen Maren was supported by National Institute of Mental Health Training Grant MH15795.

Correspondence concerning this article should be addressed to Stephen Maren, who is now at the Department of Psychology, University of Michigan, 525 East University Avenue, Ann Arbor, Michigan 48109-1109. Electronic mail may be sent via Internet to maren@umich.edu.

that the BLA has a temporary role in the expression of inhibitory avoidance conditioning, an instrumental task in which rats are trained to avoid a compartment associated with footshock. However, there are currently no data concerning the involvement of the BLA in the expression of Pavlovian fear conditioning over time following training. To address this issue, we have examined the impact of selective *N*-methyl-D-aspartate (NMDA) lesions of the BLA, which reportedly spare neurons in the CEA and fibers of passage in both structures (Hatfield, Graham, & Gallagher, 1992; Sananes & Davis, 1992), on Pavlovian fear conditioning in rats. BLA lesions were made either 7 days before or 1, 14, or 28 days after fear conditioning, in which tones were paired with aversive footshocks. Following training, recovery from surgery, or both, conditional freezing to the tone CS and the context of the conditioning chamber was assessed in separate extinction tests. Fear to both classes of stimuli was assessed to examine the possibility that the BLA is differentially involved in the expression of cued and contextual fear.

Two predictions can be made concerning the impact of selective BLA lesions on conditional fear. If the BLA has a temporary role in the expression of conditional fear (e.g., McGaugh, 1989), then excitotoxic BLA lesions should disrupt the expression of conditional fear when made either before or shortly after conditioning but not long after conditioning. Alternatively, if the BLA has an enduring role in the expression of conditional fear (e.g., Davis, 1992) or is required for the performance of conditional fear, then BLA lesions should attenuate conditional fear when made either before or anytime after conditioning. The results of the present study are consistent with the latter possibility and suggest that neurons in the BLA are required for both the acquisition and long-term expression of conditional fear in rats.

Method

Subjects

The subjects were 80 adult male Long-Evans rats (300–500 g) born and reared in the Department of Psychology vivarium at the University of California, Los Angeles. After weaning, the rats were group-housed in same-sex cohorts. At the beginning of the experiment, the rats were individually housed in standard stainless steel cages on a 14:10-hr light–dark cycle (lights on at 7:00 am) with free access to food and tap water. After individual housing, the rats were handled daily (10–20 s per rat) for 5 days to acclimate them to the experimenter.

Behavioral Apparatus

Four identical observation chambers (28 × 21 × 22 cm; Lafayette Instrument Co., North Lafayette, IN) were used for both conditioning and contextual fear testing. The chambers were constructed from aluminum (sidewalls) and Plexiglas (rear wall, ceiling, and hinged front door). The chambers were situated in chests located in a brightly lit and isolated room. A videocamera placed in front of the observation chambers allowed each subject's behavior to be observed and recorded by an experimenter in an adjacent room. The floor of each chamber consisted of 18 stainless steel rods (4 mm diameter) spaced 1.5 cm apart (center to center). The rods were wired to a shock generator and scrambler (Lafayette Instrument Co., North Lafayette, IN) for the delivery of footshock USs. A speaker located on one wall of the chamber permitted the delivery of acoustic CSs. The chambers were cleaned with a 5% ammonium hydroxide solution, and stainless steel pans containing a thin film of the same solution were placed underneath the grid floors before rats were placed inside. Background noise (70 dB, A scale) was supplied by ventilation fans in each chest and adjacent shock scramblers.

An additional set of four observation chambers (28 × 21 × 22 cm; Lafayette Instrument Co., North Lafayette, IN) located in an adjacent room was used for testing conditional fear to the tone. These chambers were constructed as described above and were situated in chests located in a quiet, dimly lit, and isolated room. A number of modifications were made to this testing environment to minimize its similarity to the training context. Ambient light in the room was provided by a single lamp equipped with a 15-W red light bulb. The floor of each chamber consisted of 17 vertically staggered stainless steel rods (4 mm diameter) spaced 1.5 cm apart (center to center), and the outside of the rear wall of the chambers was covered with white construction paper. In addition, a white, opaque Plexiglas tent was inserted into each chamber so that the apex of the tent contacted the roof of the chamber and the open base of the tent fit into the bottom corners of the chamber. A speaker located on one wall of the chamber permitted the delivery of acoustic CSs. Background noise (70 dB, A scale) was supplied by a white noise generator. The chambers were cleaned with a 1% acetic acid solution, and stainless steel pans containing a thin film of the same solution were placed underneath the grid floors before rats were placed inside. A videocamera placed in front of the observation chambers allowed each subject's behavior to be observed and recorded by an experimenter in an adjacent room.

Procedure

The rats were randomly assigned to a 2 × 4 (Lesion × Interval; $n = 10$ per group) design. For this design, rats received either *N*-methyl-D-aspartate (NMDA) lesions in the BLA (procedure adapted from Sananes & Davis, 1992) or sham surgery 7 days before (anterograde) or 1, 14, or 28 days after (retrograde) Pavlovian fear conditioning.

On the conditioning day, the rats were transported to the laboratory and placed in the conditioning chambers in squads of 4 rats; the chamber position was counterbalanced for each squad and group. The rats received three tone (10 s, 90 dB, 2 kHz) and footshock (2 s, 1.0 mA) pairings (74-s intertrial interval [ITI]) 3 min after being placed in the chambers. Sixty-four seconds following the final shock, the rats were returned to their home cages.

For rats that received surgery prior to training (anterograde groups), fear conditioning on the training day was assessed by measuring freezing during the 64-s period before the first tone–shock trial and the 64-s periods following each of the three tone–shock trials (i.e., immediate postshock freezing). Briefly, an observer who was unaware of the experimental conditions scored each rat for freezing (behavioral immobility except for movement necessitated by respiration) every 8 s for a total of eight observations per animal. Twenty-four hours after training, fear conditioning to the context was assessed by returning the rats to the conditioning chambers and scoring freezing during an 8-min extinction test, yielding a total of 64 observations per rat. On the following day, fear conditioning to the tone was assessed in an "off-baseline" extinction test; that is, the tone test was conducted in a context different from the training context. The rats were placed in observation chambers that were distinct from those used during conditioning and, after 2 min, presented with an 8-min tone. For rats receiving surgery after training, the context and tone tests were performed 1 week following surgery to allow for recovery.

One week after the tone test, rats in the 1-day retrograde groups were given reacquisition training. The rats were returned to the original training context and given 10 unsignaled footshocks (2 s, 1.0 mA) with a 64-s ITI. Freezing was scored as described above during an

immediate-context extinction test (the 10-min period following the final footshock) and a delayed-context extinction test (10 min) the following day. Unsignaled training was used for reacquisition because we were specifically interested in examining fear during an immediate extinction test, which would not be possible with an off-baseline tone test.

Surgery

Rats were anesthetized with an intraperitoneal injection of Nembutal (sodium pentobarbital, 65 mg per kilogram of body weight) and mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The scalp was incised and retracted, and head position was adjusted to place bregma and lambda in the same horizontal plane. Small burr holes (2 mm diameter) were drilled bilaterally in the skull for the placement of 28-gauge cannulae in the BLA (3.3 mm posterior to bregma, 5.0 mm lateral to the midline). A 10-μl Hamilton syringe was mounted in an infusion pump (Harvard Apparatus, South Natick, MA) and connected to the injection cannula with polyethylene tubing. NMDA (20 μg/μl; Sigma Chemical Co., St. Louis, MO) in 100-mM phosphate-buffered saline (PBS, pH = 7.6) was infused (0.1 μl/min) 8.0 mm ventral to brain surface (0.2 μl) and 7.5 mm ventral to brain surface (0.1 μl) for each penetration. Five minutes were allowed after each infusion for diffusion of the drug. Rats receiving sham surgery before training (anterograde groups) were split into two groups. Both groups were treated as above except that one group received PBS infusions, and the other group received neither an infusion nor cannula implantation. The two sham groups did not differ from one another on either histological or behavioral measures and were collapsed; the remainder of the retrograde sham groups did not receive either cannula implants or PBS infusions. After surgery, the incision was closed with stainless steel wound clips, and the rats were allowed to recover on a heating pad before returning to their home cage.

Histology

Histological verification of lesion location was performed after behavioral testing. Rats were perfused across the heart with 0.9% saline followed by 10% formaldehyde solution. After extraction from the skull, the brains were postfixed in 10% formaldehyde solution for 2 days and 10% formaldehyde, 30% sucrose solution until sectioning. Coronal sections (50 μm thick, taken every 200 μm) were cut on a cryostat (−16 °C) and wet-mounted on glass microscope slides with 70% ethanol. After drying, the sections were stained with 0.25% thionin to visualize neuronal cell bodies.

Data Analysis

For each test period, the freezing data were transformed to a percentage of total observations, a probability estimate that is amenable to analysis with parametric statistics. These probability estimates of freezing were analyzed using an analysis of variance (ANOVA). Planned comparisons in the form of Fisher least significant difference (LSD) tests were performed following a significant omnibus *F* ratio. All data are represented as means plus or minus the standard errors of the means (*SEM*s).

Results

Lesion Placement

Figure 1 shows thionin-stained coronal sections, and Figure 2 shows maximum and minimum cell loss from representative rats receiving either BLA lesions or sham surgery. Examination of tissue sections from the rats receiving intra-amygdala NMDA infusion revealed a consistent pattern of brain damage. In 32 of the 40 rats, near-total bilateral lesions of the BLA were observed. In these rats, NMDA destroyed cell bodies throughout the rostral-caudal extent of the BLA, yielding extensive bilateral damage in the posterior divisions of the basolateral, basomedial, and lateral nuclei; damage in anterior divisions of the basolateral and lateral nuclei was more variable. Eight rats (2 rats in each of the four training-to-lesion groups) had incomplete (unilateral or partial bilateral) BLA

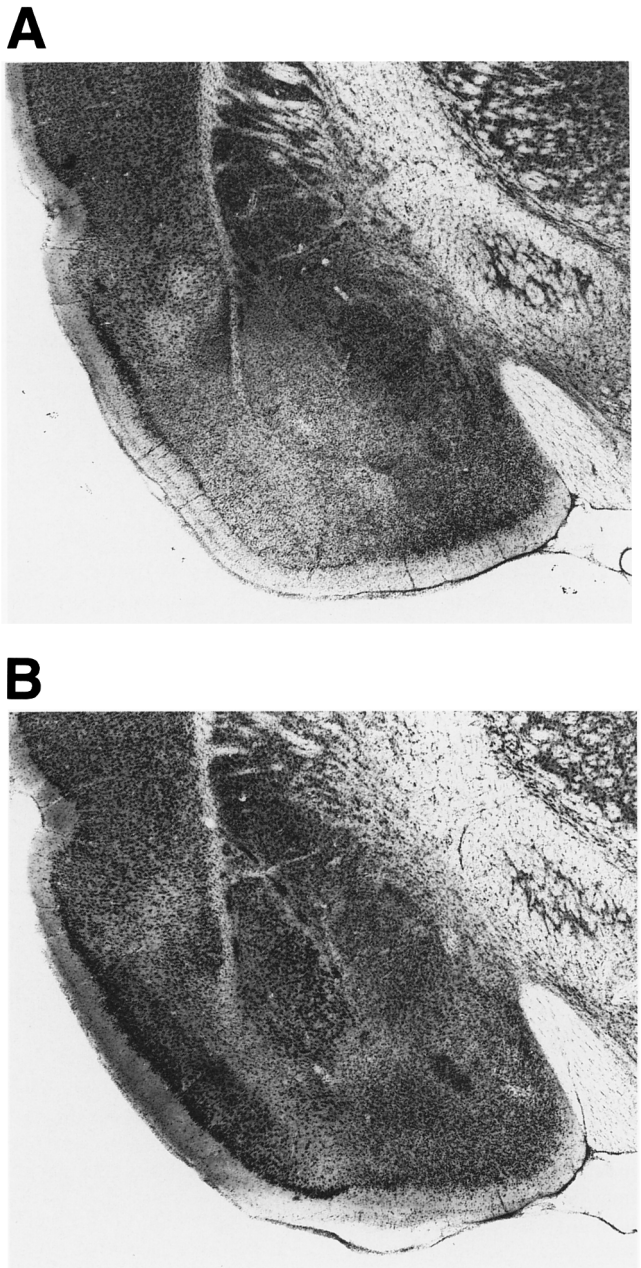

Figure 1. Photomicrographs showing coronal sections through the left amygdala in representative rats receiving either (A) *N*-methyl-D-aspartate lesions in the basolateral amygdaloid complex or (B) sham surgery.

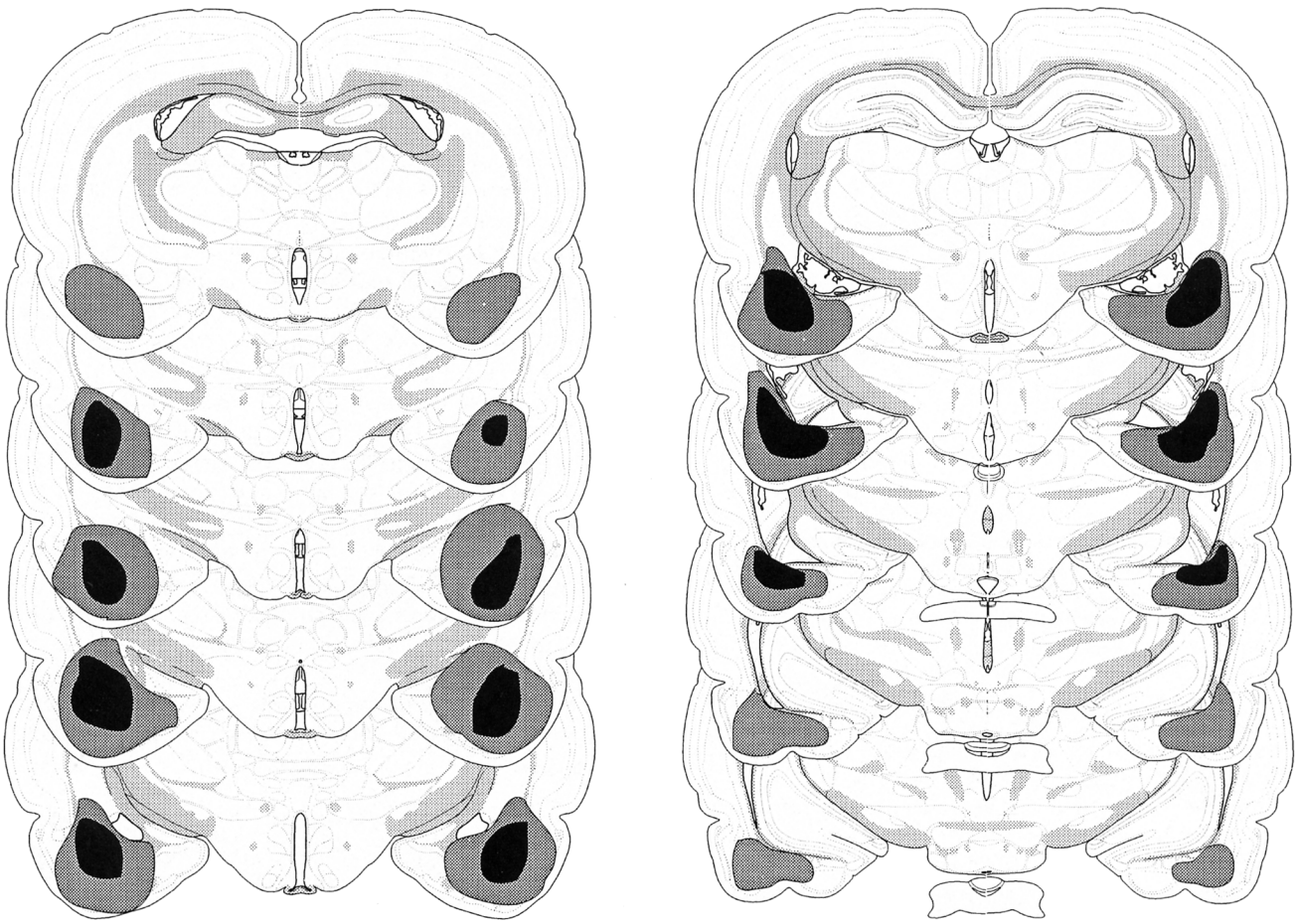

Figure 2. Schematic representation of maximum (gray) and minimum (black) cell loss in rats with excitotoxic basolateral amygdaloid lesions.

lesions and were excluded from the analysis. This left 8 rats in each of the BLA lesion groups and 10 rats in each of the sham surgery groups.

In addition to the BLA, cell loss in the cortical and medial amygdaloid nuclei was observed in the majority of rats. However, only minor CEA damage was observed in lesioned subjects. Specifically, cell loss in the CEA was most often associated with ventricular hypertrophy near its caudal pole. For the most part, extra-amygdala damage was limited. When present, it was observed in the amygdalohippocampal transition zone and ventrolateral entorhinal cortex; damage to the perirhinal cortex adjacent to the amygdala was not observed. Thus, the lesions were generally selective for the amygdala and the BLA in particular.

Conditional Freezing

As a measure of acquisition of conditional fear, immediate postshock freezing on the conditioning day was scored in the rats that had received surgery before training (anterograde groups). As is shown in Figure 3, rats with BLA lesions exhibited substantially less freezing on the training day than did sham rats. This was confirmed in an ANOVA that revealed significant effects of lesion, $F(1, 16) = 37.6, p < .0001$, and trial, $F(3, 48) = 28.5, p < .0001$, and a significant Lesion × Trial interaction, $F(3, 48) < 16.2, p < .0001$. Planned comparisons indicated that postshock freezing after the third tone–shock trial was higher than preshock freezing in the sham rats ($p < .0001$) but not in the BLA rats ($p = .07$).

Conditional freezing on the posttraining extinction tests is shown in Figure 4. In comparison with the nearly asymptotic level of fear in rats receiving sham surgery, conditional fear to both the context and tone CS in rats with BLA lesions was massively attenuated. Separate ANOVAs for context and tone freezing revealed a significant effect of lesion (context, $F(1, 3) = 498.1, p < .0001$; tone, $F(1, 3) = 857.9, p < .0001$) and a nonsignificant Lesion × Interval interaction (context, $F(3, 64) < 1, p = .53$; tone, $F(3, 64) < 1, p = .62$). Thus, the severe deficit in conditional freezing did not vary as a function of training-to-lesion interval. A significant main effect of training-to-lesion interval was obtained for context, $F(3, 64) = 3.6, p < .05$, but not tone, $F(3, 64) < 1, p = .62$, reflecting the overall increase in context freezing across the training-to-lesion intervals. However, planned comparisons indicated that freezing in rats with BLA lesions did not reliably increment across the

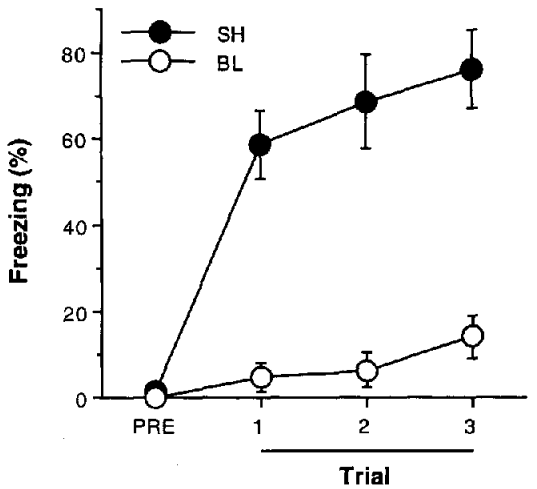

Figure 3. Mean percentage of freezing (±*SEM*) during training in sham rats (SH, solid circles) and rats with excitotoxic basolateral amygdaloid (BLA) lesions (BL, open circles). Freezing was scored during the 64-s period preceding the first tone–shock trial (PRE) and during the 64-s period following footshock on each of the three tone–shock trials. Sham rats showed reliably greater levels of freezing than did rats with excitotoxic lesions of the BLA.

training-to-lesion interval for either context or tone freezing ($Fs < 2.5, ps > .08$).

It has been suggested that reacquisition training can reinstate conditional fear that has been abolished by a posttraining amygdala lesion (M. Kim & Davis, 1993a; Parent, Avila, & McGaugh, 1995). To test this, a subset of the rats reported above (1-day rats) received extensive reacquisition training (10 unsignaled shocks) following the tone extinction test. For comparison, Figure 5 shows immediate postshock freezing during the first conditioning session prior to surgery (TRAIN), freezing during the delayed context test following surgery (TEST 1, same data as in Figure 4), freezing during an immediate test following the 10 unsignaled reacquisition trials (TEST 2), and freezing during the delayed context test 24 hr following retraining (TEST 3). As is shown, reacquisition training incremented the levels of freezing in both sham rats and rats with BLA lesions; however, in comparison with sham rats, the magnitude of the deficit in rats with BLA lesions was not altered by reacquisition training. These observations were confirmed in an ANOVA that revealed a significant effect of lesion, $F(1, 16) = 83.2, p < .0001$, and of phase, $F(3, 48) = 10.9, p < .0001$, and a significant Lesion × Phase interaction, $F(3, 48) = 10.5, p < .0001$. Planned comparisons ($ps < .01$) confirmed that rats with BLA lesions exhibited greater levels of freezing after reacquisition training in comparison with the levels of freezing on the postsurgery context test, although these higher levels of freezing were still reliably lower than those observed during training prior to surgery. In contrast, reacquisition training augmented freezing in sham rats to levels that were reliably greater than those on the pre-reacquisition tests. These results suggest that rats with BLA lesions are capable of some degree of conditional freezing, although this level of freezing is severely retarded in relation to sham controls despite the extensive reacquisition training.

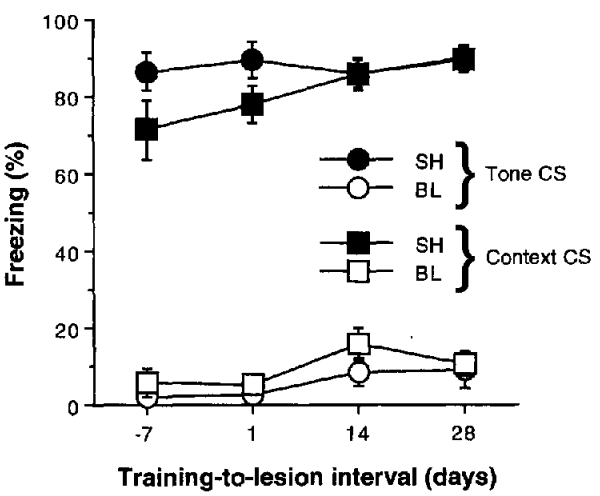

Figure 4. Mean percentage of freezing (±*SEM*) averaged across the 8-min tone (circles) and context (squares) extinction tests in sham rats (SH, solid circles) and rats with excitotoxic basolateral amygdaloid (BLA) lesions (BL, open circles). Rats received surgery either 7 days before (−7) or 1, 14, or 28 days after fear conditioning. Excitotoxic BLA lesions abolished conditional freezing to both the context and tone conditional stimuli (CSs) at all training-to-lesion intervals.

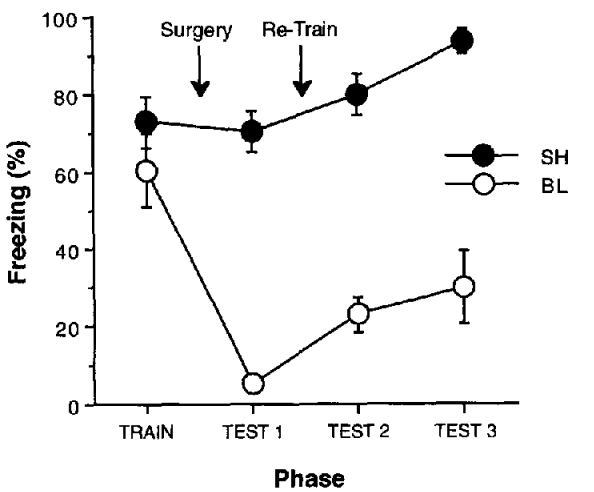

Figure 5. Mean percentage of freezing (±*SEM*) in sham rats (SH, solid circles) and rats with excitotoxic basolateral amygdaloid (BLA) lesions (BL, open circles) from the 1-day group during four phases of training. Shown is freezing during training (TRAIN; average of three 64-s postshock blocks), freezing during the postsurgery context test (TEST 1; 8-min average; same data as shown for 1-day rats in Figure 4), freezing during an immediate context test following 10 context-shock reacquisition trials (TEST 2; 10-min average), and freezing during a delayed context test 24 hr following reacquisition training (TEST 3; 10-min average). Excitotoxic BLA lesions produced severe impairments in conditional freezing to the context CS despite extensive reacquisition training, although levels of freezing in rats with BLA lesions did reliably increase in relation to pre-reacquisition levels.

Locomotor Activity

The absence of conditional freezing in rats with BLA lesions may have been due to a lesion-induced increase in locomotor activity that interfered with freezing. Indeed, excitotoxic BLA lesions have been reported to increase locomotor activity in an open field (Parent et al., 1995). To examine the effects of BLA lesions on locomotor activity in the conditioning chambers used in the present study, we scored rats in the anterograde (7-day) group for "crossovers," defined as locomotion of the rat from one side of the conditioning chamber to the other, during the 3-min preshock period on the first conditioning session. The mean (plus or minus the standard error of the mean) number of crossovers was 12.3 ± 1.2 for sham rats and 15.5 ± 1.8 for rats with BLA lesions; this difference was not statistically reliable, $F(1, 16) = 2.4, p = .14$.

Discussion

The present results indicate that selective excitotoxic lesions of the BLA produce severe deficits in the acquisition and expression of Pavlovian fear conditioning in rats. These deficits in conditional freezing were observed to both contextual and discrete CSs and were evident for both immediate and delayed freezing. The effects of selective BLA lesions on the acquisition of conditional fear are consistent with BLA lesion-induced deficits in the acquisition of fear-potentiated startle (Campeau & Davis, 1995; Sananes & Davis, 1992) and inhibitory avoidance conditioning (Parent et al., 1995). In the present study, the nearly asymptotic levels of conditioning in the sham rats indicates that BLA lesions produced a severe impairment in a robust aversive memory. Thus, it is unlikely that deficits in rats with BLA lesions were due to limited original training. Consistent with this, extensive reacquisition training did not alleviate the deficit in BLA rats. Nonetheless, rats with BLA lesions did increment their level of freezing following reacquisition, although this modest increase in freezing may have been mediated by spared tissue in the BLA. Additional footshocks during reacquisition did not serve to reinstate performance, suggesting that the deficit in rats with BLA lesions was not the result of a retrieval failure due to the omission of shock in the extinction tests. Moreover, deficient conditional freezing in rats with BLA lesions was not due to a lesion-induced increase in locomotor activity. Collectively, these results reveal that neurons in the BLA are required for both the acquisition and long-term expression of Pavlovian fear conditioning in rats.

As in other fear-motivated tasks (Campeau & Davis, 1995; Parent et al., 1995; Sananes & Davis, 1992), BLA lesions produced a robust deficit in the expression of conditional fear following training. Importantly, the retrograde attenuation of conditional fear produced by BLA lesions did not vary as a function of training-to-lesion interval; that is, it did not exhibit a temporal gradient. This suggests that the BLA has a relatively stable role in the expression of conditional fear following training. The severe and enduring disruption of conditional freezing following BLA lesions accords with a recent report of a similarly stable impairment in fear-potentiated startle following electrolytic CEA lesions (M. Kim & Davis, 1993b). In this case, CEA lesions abolished the expression of fear-potentiated startle when made either 6 or 30 days following training. Together, these reports provide strong support for the view that both the BLA and CEA are necessary for the expression of learned fear for at least 1 month following conditioning.

This view contrasts with that of McGaugh and colleagues who assert that the amygdala has a temporary role in the expression of aversive memories (Liang et al., 1982; McGaugh, 1989). This group has found, for instance, that posttraining electrolytic lesions of the amygdala disrupt retention of an inhibitory avoidance response when made 2 days, but not 10 days, after training (Liang et al., 1982). However, examination of these results reveals that the similar retention latencies in the 10-day rats was due primarily to a decrease in the retention latency of control rats rather than to an increase in the latency in rats with lesions. Thus, retention latencies did not reliably differ in rats lesioned 2, 5, or 10 days after training, although latencies in the control subjects showed a significant decrease across these intervals. More recent work using excitotoxic lesions reveals that combined BLA and CEA lesions produce a massive deficit in the expression of inhibitory avoidance conditioning up to 1 month following training (Parent, West, & McGaugh, 1994). Together with the present results, these data suggest that the long-term expression of both inhibitory avoidance conditioning and Pavlovian fear conditioning requires neurons in the amygdala.

Nonetheless, there are data that suggest that the role of the amygdala in instrumental avoidance conditioning is different from its role in Pavlovian fear conditioning. For instance, extensive training blunts the affects of amygdala lesions on the expression of avoidance conditioning (Parent, Tomaz, & McGaugh, 1992; Parent et al., 1994) but not fear-potentiated startle (Kim & Davis, 1993a). In addition, learning-related unit firing in the BLA is maximal early in avoidance training and diminishes thereafter (Maren et al., 1991). These data suggest that the BLA is required for the early stages of avoidance learning, whereas it has a more enduring role in the expression of conditional fear. This pattern of results may be related to the fact that avoidance learning involves the acquisition of both conditional fear and a conditional avoidance response. In this regard, substantial data indicate that conditional fear is acquired early in avoidance conditioning and diminishes over the course of training as the avoidance response is learned (e.g., Mineka & Gino, 1980).

The similar effect of anterograde and retrograde BLA lesions on conditional freezing is consistent with a general role for the BLA in both the learning and performance of conditional fear. However, a number of factors other than learning or performance deficits might account for the effects of BLA lesions on fear conditioning. It is possible, for instance, that BLA lesions produced a general sensory deficit that disrupted the rats' ability to perceive the contextual or acoustic CSs or shock USs. Several points argue against this, however. For example, it is unlikely that BLA lesions produced a CS processing deficit, because rats with BLA lesions have been shown to exhibit normal learning in appetitively motivated learning tasks that require the processing of discrete conditional cues (e.g., Cahill & McGaugh, 1990). Furthermore,

anterograde deficits in aversive conditioning are not likely to be due to a US processing deficit, because excitotoxic BLA lesions do not alter footshock sensitivity (Cahill & McGaugh, 1990; Sananes & Davis, 1992). That is, the footshock UR does not appear to be altered by BLA lesions. Moreover, retrograde deficits in fear conditioning cannot be explained by a US processing deficit, because the lesions in the present study produced deficits in freezing during shock-free extinction tests. Alternatively, deficits in the expression of conditional freezing following BLA lesions may have been due to a motor hyperactivity that interfered with freezing. However, the present results indicate that BLA lesions do not increase motor activity prior to conditioning. These findings do not support the view that deficits in conditional fear are due to sensory or motor deficits produced by BLA lesions.

Recognizing that the effects of BLA lesions are not simply due to sensory or motor impairments, the question at hand is whether the lesions affected the learning or performance of conditional fear. From a performance point of view, it might be argued that BLA lesions disrupted the ability of shock-associated CSs to elicit a "central fear state" and consequent freezing, without affecting the memory for fear conditioning. Therefore, by this view, the amygdala serves only to coordinate behavioral output (e.g., freezing) upon retrieval of an aversive memory. Of course, this performance account requires that BLA lesions attenuate fear URs. In accordance with this, electrolytic lesions of either CEA or BLA eliminate the unconditional analgesia produced by exposure to a cat (Fox & Sorenson, 1994; Sorenson, Greenfield, Shetty, & Younas, 1994). Similarly, excitotoxic BLA lesions increase open field activity (Parent et al., 1995), which by some accounts (e.g., R. J. Blanchard, Kelley, & Blanchard, 1974) would indicate a reduced level of unconditional fear in BLA rats. There are currently no data on the effects of selective, excitotoxic BLA lesions on unconditional freezing, but it has been reported that large, electrolytic lesions of the amygdala including both the BLA and CEA reduce cat-elicited freezing in rats (D. C. Blanchard & Blanchard, 1972). Thus, the BLA, like the CEA, may have a critical role in the performance of both conditional and unconditional fear. A general role for the BLA in the performance of fear responses is consistent with the severe BLA lesion-induced attenuation of conditional freezing observed at all training-to-lesion intervals in the present study.

Of course, the putative involvement of the BLA in the performance of fear does not prohibit the formation and storage of CS–US associations in the amygdala. Indeed, a number of pieces of evidence suggest that the BLA may be a site for CS–US association during fear conditioning. First, anatomical and electrophysiological studies indicate that multimodal auditory and somatosensory information converge in the BLA (LeDoux, Farb, & Ruggiero, 1990; Mello, Tan, & Finch, 1992; Romanski et al., 1993; Turner & Herkenham, 1991), a necessary condition for involvement of the BLA in acquisition of tone–shock associations. Additionally, the BLA is a unique target in the amygdala for projections from the hippocampal formation (Canteras & Swanson, 1992; Ottersen, 1982; Swanson & Kohler, 1986; Wyss, 1981), and convergence of somatosensory and multimodal hippocampal inputs in the BLA may underlie context–shock associations. Second, we have recently discovered that BLA synapses exhibit an NMDA receptor-dependent long-term potentiation (LTP) following high-frequency stimulation of the hippocampal formation in vivo (Maren & Fanselow, 1995). In addition, high-frequency stimulation of the thalamic medial geniculate nucleus (MGN, the source of auditory projections to the BLA) also produces LTP in the BLA (Clugnet & LeDoux, 1990). The identification of amygdaloid LTP in vivo is important, because current neurobiological theories of memory formation posit a role for activity-dependent synaptic plasticity, such as LTP, in learning and memory (e.g., Maren & Baudry, 1995). Third, neurons in the BLA exhibit associative discharges during the acquisition of conditional avoidance responses motivated by footshock (Maren et al., 1991). Fourth, intra-amygdala infusions of NMDA receptor antagonists, which block BLA LTP (e.g., Maren & Fanselow, 1995), produce impairments in the acquisition of conditional fear (Campeau, Miserendino, & Davis, 1992; Fanselow & Kim, 1994; Miserendino, Sananes, Melia, & Davis, 1990). Collectively, these studies provide support for the view that the BLA is involved in the acquisition of CS–US associations during fear conditioning. Insofar as the BLA is involved in association formation, the present data suggest that the BLA is involved in the long-term expression of these associations following training.

The foregoing discussion implicates the BLA in both the learning and the performance of conditional fear in rats. That the BLA is involved in both of these functions is consistent with amygdaloid anatomy and physiology. As has been suggested, the learning functions of the BLA may be mediated by associative synaptic plasticity at sites of CS–US convergence in the BLA (Clugnet & LeDoux, 1990; Maren & Fanselow, 1995; Romanski et al., 1993), whereas the performance functions of the BLA may be mediated by projections to neurons in the CEA involved in the generation of fear responses (LeDoux et al., 1988). Thus, the BLA and CEA appear to serve as sensory and motor interfaces, respectively, for the amygdala in both acquisition and expression of conditional fear. Of course, the BLA is not the only locus for sensory convergence and learning-related plasticity in Pavlovian fear-conditioning circuits. For example, there is evidence for associative plasticity in the MGN (Edeline & Weinberger, 1992; Supple & Kapp, 1989) and the CEA (Applegate, Frysinger, Kapp, & Gallagher, 1982; Pascoe & Kapp, 1985), and CS–US convergence in both of these areas has been implicated in the acquisition of conditional fear (Cruikshank, Edeline, & Weinberger, 1992; Kapp et al., 1992). Further studies are required to understand the contributions of these structures to the expression of conditional fear following training.

References

Applegate, C. D., Frysinger, R. C., Kapp, B. S., & Gallagher, M. (1982). Multiple unit activity recorded from amygdala central nucleus during Pavlovian heart rate conditioning in rabbit. *Brain Research, 238,* 457–462.

Blanchard, D. C., & Blanchard, R. J. (1972). Innate and conditioned reactions to threat in rats with amygdaloid lesions. *Journal of Comparative and Physiological Psychology, 81,* 281–290.

Blanchard, R. J., Kelley, M. J., & Blanchard, D. C. (1974). Defensive

reactions and exploratory behavior in rats. *Journal of Comparative and Physiological Psychology, 87,* 1129–1133.

Cahill, L., & McGaugh, J. L. (1990). Amygdaloid complex lesions differentially affect retention of tasks using appetitive and aversive reinforcement. *Behavioral Neuroscience, 104,* 532–543.

Campeau, S., & Davis, M. (1995). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *Journal of Neuroscience, 15,* 2301–2311.

Campeau, S., Miserendino, M. J., & Davis, M. (1992). Intra-amygdala infusion of the *N*-methyl-D-aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. *Behavioral Neuroscience, 106,* 569–574.

Canteras, N. S., & Swanson, L. W. (1992). Projections of the ventral subiculum to the amygdala, septum, and hypothalamus: A PHAL anterograde tract-tracing study in the rat. *Journal of Comparative Neurology, 324,* 180–194.

Clugnet, M. C., & LeDoux, J. E. (1990). Synaptic plasticity in fear conditioning circuits: Induction of LTP in the lateral nucleus of the amygdala by stimulation of the medial geniculate body. *Journal of Neuroscience, 10,* 2818–2824.

Cruikshank, S. J., Edeline, J. M., & Weinberger, N. M. (1992). Stimulation at a site of auditory-somatosensory convergence in the medial geniculate nucleus is an effective unconditioned stimulus for fear conditioning. *Behavioral Neuroscience, 106,* 471–483.

Davis, M. (1992). The role of the amygdala in fear and anxiety. *Annual Review of Neuroscience, 15,* 353–375.

Edeline, J. M., & Weinberger, N. M. (1992). Associative retuning in the thalamic source of input to the amygdala and auditory cortex: Receptive field plasticity in the medial division of the medial geniculate body. *Behavioral Neuroscience, 106,* 81–105.

Fanselow, M. S. (1994). Neural organization of the defensive behavior system responsible for fear. *Psychonomic Bulletin & Review, 1,* 429–438.

Fanselow, M. S., & Kim, J. J. (1994). Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L -2-amino-5-phosphonovaleric acid to the basolateral amygdala. *Behavioral Neuroscience, 108,* 210–212.

Fox, R. J., & Sorenson, C. A. (1994). Bilateral lesions of the amygdala attenuate analgesia induced by diverse environmental challenges. *Brain Research, 648,* 215–221.

Gabriel, M. (1990). Functions of anterior and posterior cingulate cortex during avoidance learning in rabbits. *Progress in Brain Research, 85,* 467–482.

Hatfield, T., Graham, P. W., & Gallagher, M. (1992). Taste-potentiated odor aversion learning: Role of the amygdaloid basolateral complex and central nucleus. *Behavioral Neuroscience, 106,* 286–293.

Helmstetter, F. J. (1992). The amygdala is essential for the expression of conditional hypoalgesia. *Behavioral Neuroscience, 106,* 518–528.

Helmstetter, F. J., & Bellgowan, P. S. (1994). Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. *Behavioral Neuroscience, 108,* 1005–1009.

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

Kapp, B. S., Whalen, P. J., Supple, W. F., & Pascoe, J. P. (1992). Amygdaloid contributions to conditioned arousal and sensory information processing. In J. P. Aggleton (Ed.), *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction* (pp. 229–254). New York: Wiley-Liss.

Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256,* 675–677.

Kim, M., & Davis, M. (1993a). Electrolytic lesions of the amygdala block acquisition and expression of fear-potentiated startle even with extensive training but do not prevent reacquisition. *Behavioral Neuroscience, 107,* 580–595.

Kim, M., & Davis, M. (1993b). Lack of a temporal gradient of retrograde amnesia in rats with amygdala lesions assessed with the fear-potentiated startle paradigm. *Behavioral Neuroscience, 107,* 1088–1092.

LeDoux, J. E. (1995). Emotion: Clues from the brain. *Annual Review of Psychology, 46,* 209–235.

LeDoux, J. E., Farb, C., & Ruggiero, D. A. (1990). Topographic organization of neurons in the acoustic thalamus that project to the amygdala. *Journal of Neuroscience, 10,* 1043–1054.

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

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

Maren, S., & Baudry, M. (1995). Properties and mechanisms of long-term synaptic plasticity in the mammalian brain: Relationships to learning and memory. *Neurobiology of Learning and Memory, 63,* 1–18.

Maren, S., & Fanselow, M. S. (1995). Synaptic plasticity in the basolateral amygdala induced by hippocampal formation stimulation *in vivo*. *Journal of Neuroscience, 15,* 7548–7564.

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. (1989). Involvement of hormonal and neuromodulatory systems in the regulation of memory storage. *Annual Review of Neuroscience, 12,* 255–287.

Mello, L. E., Tan, A. M., & Finch, D. M. (1992). Convergence of projections from the rat hippocampal formation, medial geniculate and basal forebrain onto single amygdaloid neurons: An in vivo extra- and intracellular electrophysiological study. *Brain Research, 587,* 24–40.

Mineka, S., & Gino, A. (1980). Dissociation between conditioned emotional response and extended avoidance performance. *Learning & Motivation, 11,* 476–502.

Miserendino, M. J., Sananes, C. B., Melia, K. R., & Davis, M. (1990). Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. *Nature (London), 345,* 716–718.

Ottersen, O. P. (1982). Connections of the amygdala of the rat: 4. Corticoamygdaloid and intraamygdaloid connections as studied with axonal transport of horseradish peroxidase. *Journal of Comparative Neurology, 205,* 30–48.

Parent, M. B., Avila, E., & McGaugh, J. L. (1995). Footshock facilitates the expression of aversively motivated memory in rats given post-training amygdala basolateral complex lesions. *Brain Research, 676,* 235–244.

Parent, M. B., Tomaz, C., & McGaugh, J. L. (1992). Increased training in an aversively motivated task attenuates the memory-impairing effects of post-training *N*-methyl-D-aspartate-induced amygdala lesions. *Behavioral Neuroscience, 106,* 789–797.

Parent, M. B., West, M., & McGaugh, J. L. (1994). Memory of rats with amygdala lesions induced 30 days after footshock-motivated escape training reflects degree of original training. *Behavioral Neuroscience, 108,* 1080–1087.

Pascoe, J. P., & Kapp, B. S. (1985). Electrophysiological characteristics

of amygdaloid central nucleus neurons during Pavlovian fear conditioning in the rabbit. *Behavioural Brain Research, 16,* 117–133.

Romanski, L. M., Clugnet, M. C., Bordi, F., & LeDoux, J. E. (1993). Somatosensory and auditory convergence in the lateral nucleus of the amygdala. *Behavioral Neuroscience, 107,* 444–450.

Sananes, C. B., & Davis, M. (1992). *N*-methyl-D-aspartate lesions of the lateral and basolateral nuclei of the amygdala block fear-potentiated startle and shock sensitization of startle. *Behavioral Neuroscience, 106,* 72–80.

Sorenson, C. A., Greenfield, J. P., Shetty, R. S., & Younas, B. (1994). A proposed neural circuit for cat-induced analgesia. *Society for Neuroscience Abstracts, 20,* 366.

Supple, W., Jr., & Kapp, B. S. (1989). Response characteristics of neurons in the medial component of the medial geniculate nucleus during Pavlovian differential fear conditioning in rabbits. *Behavioral Neuroscience, 103,* 1276–1286.

Swanson, L. W., & Kohler, C. (1986). Anatomical evidence for direct projections from the entorhinal area to the entire cortical mantle in the rat. *Journal of Neuroscience, 6,* 3010–3023.

Turner, B. H., & Herkenham, M. (1991). Thalamoamygdaloid projections in the rat: A test of the amygdala's role in sensory processing. *Journal of Comparative Neurology, 313,* 295–325.

Wyss, J. M. (1981). An autoradiographic study of the efferent connections of the entorhinal cortex in the rat. *Journal of Comparative Neurology, 199,* 495–512.

Received August 8, 1995
Revision received October 27, 1995
Accepted December 4, 1995 ■