

## THE LATERAL AMYGDALA PROCESSES THE VALUE OF CONDITIONED AND UNCONDITIONED AVERSIVE STIMULI

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**Abstract**—The amygdala is critical for acquiring and expressing conditioned fear responses elicited by sensory stimuli that predict future punishment, but there is conflicting evidence about whether the amygdala is necessary for perceiving the aversive qualities of painful or noxious stimuli that inflict primary punishment. To investigate this question, rats were fear conditioned by pairing a sequence of auditory pips (the conditioned stimulus, or CS) with a brief train of shocks to one eyelid (the unconditioned stimulus, or US). Conditioned responding to the CS was assessed by measuring freezing responses during a test session conducted 24 h after training, and unconditioned responding to the US was assessed by measuring head movements evoked by the eyelid shocks during training. We found that pre-training electrolytic lesions of the amygdala's lateral (LA) nucleus blocked acquisition of conditioned freezing to the CS, and also significantly attenuated unconditioned head movements evoked by the US. Similarly, bilateral inactivation of the amygdala with the GABA-A agonist muscimol impaired acquisition of CS-evoked freezing, and also attenuated US-evoked responses during training. However, when amygdala synaptic plasticity was blocked by infusion of the NR2B receptor antagonist ifenprodil, acquisition of conditioned freezing was impaired but shock reactivity was unaffected. These findings indicate that neural activity within the amygdala is important for both predicting and perceiving the aversive qualities of noxious stimuli, and that synaptic plasticity within LA is the mechanism by which the CS becomes associated with the US during fear conditioning. © 2005 Published by Elsevier Ltd on behalf of IBRO.

**Key words:** fear conditioning, freezing, ifenprodil, muscimol, shock.

Pavlovian fear conditioning is an associative learning task in which subjects are presented with a neutral conditioned stimulus (CS) paired with an innately aversive unconditioned stimulus (US). As a result of such pairing, the CS comes to elicit behavioral, autonomic, and endocrine re-

sponses that are characteristically expressed in the presence of threatening or dangerous stimuli. Pavlovian fear conditioning is severely impaired by disruption of the amygdala, indicating that the amygdala plays an important role in learning to respond defensively to stimuli that predict punishment (Blanchard and Blanchard, 1972; LeDoux et al., 1990; Davis, 1992). However, it is unclear whether the amygdala is necessary for perceiving the aversiveness of noxious stimuli (physical pain, loud noises, foul odors, etc.) that are responsible for inflicting primary punishment.

Some rodent fear conditioning studies have reported that amygdala lesions selectively impair acquisition and expression of conditioned fear responses to the CS, without altering unconditioned reflex responses to the innately aversive US (Cahill and McGaugh, 1990; Sananes and Davis, 1992; Maren, 1998; Wallace and Rosen, 2001). These findings have been interpreted by some as evidence that the amygdala is critical for learning to anticipate future punishments based on predictive cues, but not for the animal's innate experience of punishment by a primary aversive reinforcer. In other words, it has been suggested from these findings that the amygdala plays a critical role in the *prediction* of punishment (which may be identified with the emotion of fear), but does not contribute to the *perception* of punishment (which may be identified with pain, disgust, or other noxious sensations).

However, this interpretation is problematic for two reasons. First, several studies have reported that lesions of the amygdala in rats attenuate unconditioned responses to innately aversive stimuli, contradicting the evidence cited above that amygdala lesions do not affect unconditioned responses to an aversive US (Blanchard and Blanchard, 1972; Hitchcock et al., 1989; Kim and Davis, 1993; Vazdarjanova et al., 2001; Borszcz and Leaton, 2003). Second, it is difficult to determine whether an animal perceives a stimulus as "aversive" based upon limited observations of the animal's behavioral responses to the stimulus. Demonstrating this point, Borszcz and Leaton (2003) have shown that electrolytic lesions of the amygdala's central nucleus (CeA) spare unconditioned motor reflex responses to electric shock while simultaneously attenuating unconditioned vocalization afterdischarges (VADs) evoked following the shock. Importantly, the same CeA lesions that selectively abolished VADs (but not motor reflexes) also abolished auditory fear conditioning (Borszcz and Leaton, 2003), and shock levels that were below threshold for evoking VADs were incapable of supporting fear conditioning in unlesioned rats (Borszcz, 1995). These findings suggest that shock-evoked motor reflexes are mediated by spinal and brainstem circuits that

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**Abbreviations:** CeA, central nucleus; CS, conditioned stimulus; CX, context; HAB, habituation; LA, lateral nucleus; LED, light-emitting diode; MUS-HI, high dose of muscimol; MUS-LO, low dose of muscimol; RETEST, retested; TEST, testing; US, unconditioned stimulus; VAD, vocalization afterdischarge; VEH, vehicle.

do not participate in signaling the emotive aversiveness of the shock, whereas shock-evoked VADs are mediated by higher structures (including CeA) that are involved in signaling the aversiveness of the shock. Based on these observations, Borszcz and Leaton (2003) argued that the amygdala contains a neural substrate for perceiving the emotive aversiveness of the shock, and that the shock must activate this substrate in order to serve as an effective US during fear conditioning.

This interpretation is consistent with classical models of fear learning, which postulate that CS–US pairing causes the CS to become associated with an internal emotional representation of the US, which is distinct from sensory representations of the US and from motor circuits controlling simple reflex responses to the US (Konorski, 1967; Wagner and Brandon, 1989). According to such models, CS–US pairing causes the CS to become an “emotional substitute” for the US, so that the CS subsequently elicits emotional responses (but not necessarily motor reflex responses) similar to those elicited by the US. Supporting this model, there is strong evidence that the amygdala’s lateral (LA) nucleus is a critical site where the CS becomes associated with the US during fear conditioning through synaptic plasticity (Fendt and Fanselow, 1999; Fanselow and LeDoux, 1999; LeDoux, 2000; Maren, 2001; Blair et al., 2001; Lee and Kim, 2004; but for an alternative view see Cahill et al., 1999). Such plasticity is exactly what would be required for the CS to gain access to an emotional representation of the aversive qualities of the US if such a representation were indeed stored in the amygdala. If this proposal is correct, then it should be expected that the amygdala participates not only in the prediction of future punishment when the CS occurs, but also in the perception of present punishment when the US occurs.

To test this hypothesis, the present study investigated the role of the amygdala in predicting and perceiving aversive stimuli. Rats were fear conditioned by pairing a sequence of auditory pips (the CS) with a brief train of shock pulses to the eyelid (the US). We found that electrolytic lesions or temporary inactivation of LA with muscimol impaired acquisition and expression of conditioned freezing responses to the auditory CS, and concurrently impaired unconditioned responses to the eyelid shock US. By contrast, blocking synaptic plasticity in the amygdala with infusions of ifenprodil only impaired acquisition but not expression of freezing to the CS, without affecting unconditioned reflex responses to the shock US. Based on these and other findings, we conclude that neural activity within LA is important for both predicting and perceiving the aversive qualities of noxious stimuli, and that synaptic plasticity within LA is the mechanism by which the CS becomes an emotional substitute for the US during fear conditioning.

## EXPERIMENTAL PROCEDURES

### Subjects and surgery

Male Sprague–Dawley rats weighing 350–400 g were reduced to 85% of their *ad libitum* weight through limited daily feeding, so that

they would be motivated to perform a food-pellet chasing task during the experiment (see below). Amygdala lesion and cannula implantation surgeries were performed under sterile conditions while rats were deeply anesthetized with Nembutal (40 mg/kg). In all rats, silver wires (75  $\mu$ m diameter, stripped of insulation 2 mm from the tip) were threaded through the skin of the left eyelid for delivery of the periorbital shock US. Rats in experiment 1 received electrolytic lesions of the amygdala, and the lesion control group consisted of six rats from another experiment (which used identical conditioning protocols) that were chronically implanted with recording electrodes in the amygdala (using methods described in Repa et al., 2001). These rats were chosen as controls because like the lesion group, they had electrode tips inserted into the amygdala and sustained similar damage to overlying tissue, but no current was passed through the recording electrodes so the amygdala remained fully intact. Rats in experiments 2 and 3 were bilaterally implanted with intracranial infusion cannulae in LA. Postsurgical analgesics (2 mg/kg ketoprofen) were given daily for 3 days after all surgeries.

**Electrolytic amygdala lesions.** A stainless steel, monopolar electrode insulated with epoxy to within 200  $\mu$ m of the tip was lowered through an incision in the dura to the targeted position within LA. Lesions were made at two different locations in each hemisphere (2.3 mm posterior, 5.1 mm lateral, 8 mm ventral to bregma; 3.2 mm posterior, 5.3 mm lateral, 8.1 mm ventral to bregma) by passing positive current (1.0 mA) through the electrode at each lesion site for 10 s. The stimulating electrode was then removed and a headstage connector for the periorbital shock electrodes was fixed to the skull with bone cement.

**Intracranial infusion cannula.** Stainless steel guide cannulae (22-gauge) were bilaterally implanted into the dorsal tip of the LA amygdala (3.0 mm posterior,  $\pm$ 5.3 mm lateral, and 8.0 mm dorsal to bregma) and secured to the skull (along with a headstage connector for the periorbital shock cable) using surgical screws and bone cement.

All experimental procedures were thoroughly reviewed and approved in advance by the UCLA Animal Research Committee in accordance with U.S. government and international guidelines concerning the use of animals in research.

### Fear conditioning

Rats were fear conditioned using a protocol similar to a previous study by Moita et al. (2003). All training and test sessions were conducted while unrestrained rats navigate freely in a small experimental chamber (36 $\times$ 24 $\times$ 44 cm). A thin cable from the top of the chamber was attached to the rat’s head for delivery of the shock to the eyelid electrodes, but this cable had sufficient slack that it did not restrict the animal’s movements in any way. The cable’s headstage was mounted with two infrared light-emitting diodes (LEDs) which were monitored by an overhead video tracking system for automatic scoring of conditioned and unconditioned responses. Throughout all sessions of the experiment, rats foraged for 20 mg food pellets dropped from an overhead dispenser, providing a baseline of motor activity against which freezing behavior could easily be detected by the automated scoring system (Moita et al., 2003). Rats underwent fear conditioning which consisted of 16 pairings of an auditory CS with an electric shock US. The CS was a sequence of 20 75 dB white noise pips (each 250 ms duration) presented at a rate of 1 Hz. The US was a 1.4 s train of eight very brief shock pulses (1.5 mA for 2 ms) delivered at a rate of 5 Hz. The US began 300 ms after the offset of the final white noise pip of the CS.

### Conditioned and unconditioned responses

Conditioned responses to the CS (freezing during the white noise pips) and unconditioned responses to the US (head movement

during eyelid shocks) were both measured by the overhead video tracking system which sampled the LEDs attached to the rat's head at a rate of 30 Hz. Freezing to the CS was assessed by an automated scoring algorithm as described in Moita et al. (2003). Head movement responses elicited by the eyelid shock US were quantified by calculating the absolute distance (in cm) traveled by the headstage LEDs during successive position samples, and then summing over all successive position samples during the 1.5 s shock train to obtain the total distance traveled by the head during the shock train. This distance was then divided by the 1.5 s duration of the shock period to obtain the mean head speed (in cm/s) during the shock.

### Drug infusions and histology

Fifteen minutes prior to training (experiment 2) or testing (experiment 3), rats received bilateral intra-amygdala infusions of vehicle (VEH) solution (0.5  $\mu$ l/side) containing either the GABA-A agonist muscimol (high dose (MUS-HI) 1.0 nmol/side, or low dose (MUS-LO) 0.2 nmol/side) to block neural activity in the amygdala, the NR2B antagonist ifenprodil (4.0  $\mu$ mol/side) to block synaptic plasticity in the amygdala, or VEH only in the control group. The VEH for all infusions was 0.1 M PBS containing 0.1% tartaric acid, because tartaric acid was used for dissolving the ifenprodil (Rodrigues et al., 2001). All solutions were infused at a rate of 0.25  $\mu$ l/min through 28-gauge injector cannulae attached to a 1.0  $\mu$ l Hamilton syringe via polyurethane tubing (as in Wilensky et al., 1999). After drug infusion, the cannulae were left in place for an additional 1 min to allow diffusion of the drug away from the cannulae tip, after which the dummy cannulae were replaced. At the end of the experiment, cannula placements were verified by fixing the brains in formalin and cutting 50  $\mu$ m sections through the amygdala to visually identify the tips of the injectors in each rat. Two rats from the ifenprodil group and one from the MUS-HI group were eliminated from the study and replaced because the injector tips were not within 0.25 mm of the LA amygdala in either hemisphere.

## RESULTS

### Experiment 1: effects of amygdala lesions on fear acquisition and shock reactivity

**Histology.** Bilateral electrolytic amygdala lesions were targeted at the dorsal tip of the LA. Lesions were made in eight rats, but two of the rats were eliminated from the study

because LA was not bilaterally damaged. Fig. 1 shows the extent of each lesion in the remaining six rats, all of which exhibited at least partial damage to LA in both hemispheres. In some rats (T49 and T50) the lesions were small and well restricted to the LA, while in other rats (M42 and M43) the lesions were more widespread, encompassing portions of the caudate and CeA. The basal nucleus of the amygdala was largely spared in all cases. Rats in the unlesioned control group were bilaterally implanted with chronic recording electrodes in LA, so they sustained similar damage to overlying tissue from the electrode tracks as in the lesion group.

**Conditioned freezing.** Rats were fear conditioned using a protocol similar to a previous study (Moita et al., 2003). During each experimental session, they were placed in an experimental chamber where they foraged for small food pellets dropped from an overhead dispenser. While foraging, rats underwent fear conditioning in which the auditory CS was paired with an electric shock US (see Experimental Procedures). Fear learning was assessed by automated scoring of freezing behavior, a standard index of conditioned fear (Blanchard and Blanchard, 1969).

Fig. 2a illustrates the design of the experiment. Rats were first given six unpaired habituation (HAB) trials, in which the CS was presented alone. This was followed by 16 training (TRAIN) trials in which the CS was paired with the US. After a 24 h delay, rats were given a testing (TEST) session in which the CS was again presented alone. Fig. 2b shows that rats in both groups (lesion and control) froze very little to the context (CX) or auditory pips (CS) during the HAB session prior to CS–US pairings. During the TEST session (24 h after CS–US pairing), rats in the control group showed greatly enhanced freezing to the CS but not the CX ( $2 \times 2$  ANOVA  $F_{(1)}=35.69$ ,  $P<0.0001$  for stimulus;  $F_{(5)}=18.75$ ,  $P<0.0001$  for session;  $F_{(5)}=6.97$ ,  $P=0.003$  for stimulus $\times$ session). Rats in the lesion group did not show enhanced freezing to the CS or CX ( $2 \times 2$  ANOVA  $F_{(1)}=0.04$ ,  $P=0.85$  for stimulus;

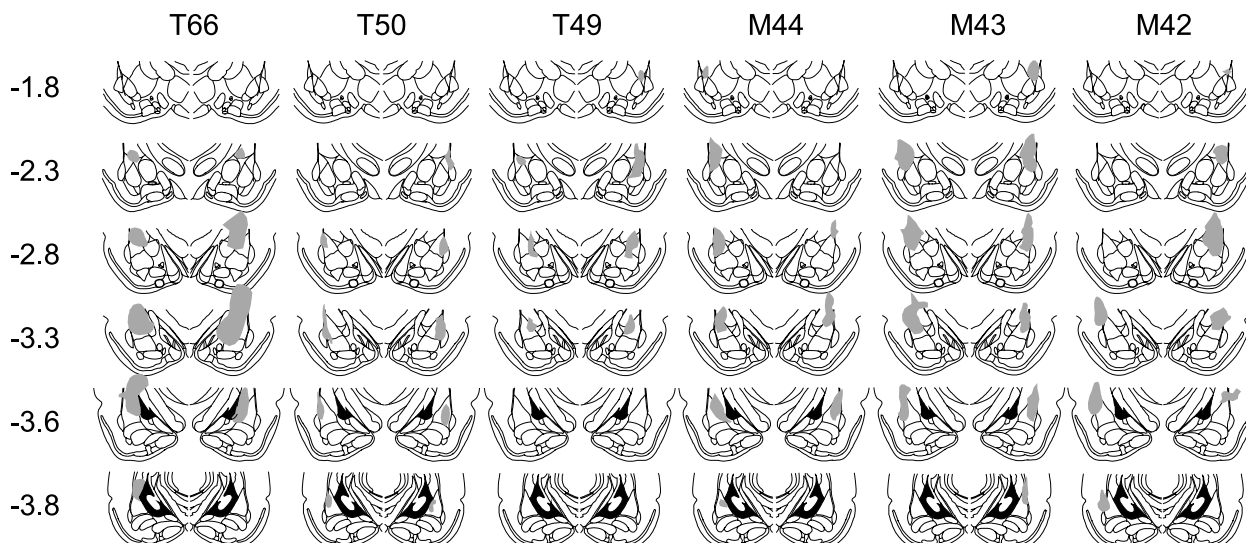
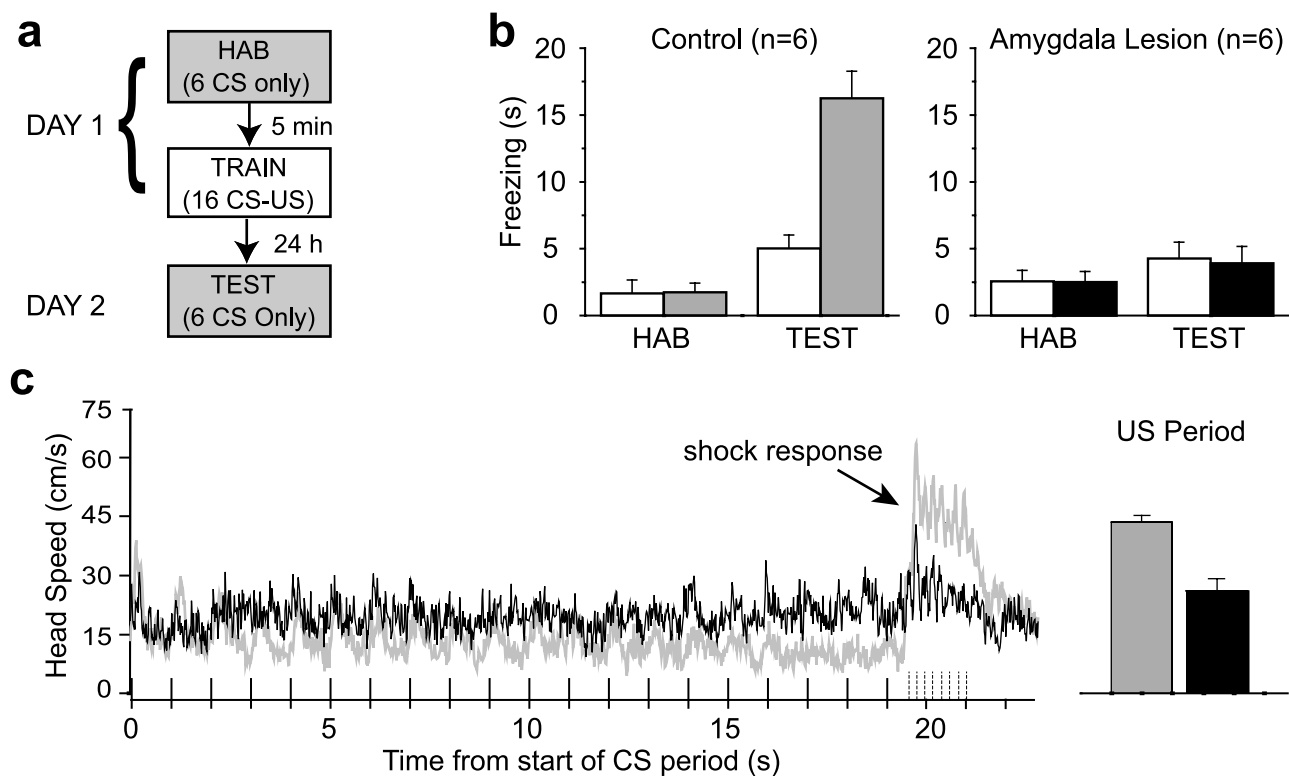


Fig. 1. Histological reconstruction of amygdala lesions. Gray shading shows the area destroyed by electrolytic lesions in each of the six lesioned rats.



**Fig. 2.** Impairment of conditioned freezing and shock reactivity following amygdala lesions. (a) Design of the auditory fear conditioning experiment. (b) Bar graphs show average time spent freezing (y axis) during the CS period (shaded bars) and pre-CS period (white bars) of the HAB and TEST sessions for the control (left panel) and amygdala lesion (right panel) groups. (c) Line graph shows the mean head speed (y axis) of rats in the control (gray trace) and amygdala lesion (black trace) groups at each time point during the training trial (x axis) averaged over the 16 CS–US pairing trials during training. Along the x axis, the onset of auditory pips denoted by solid hash marks and the onset of shock pulses denoted by dotted hash marks. Bar graph at right shows the mean head speed during the 1.5 s shock delivery period, plotted on the same scale as the y axis of the line graph.

$F_{(5)}=1.08$ ,  $P=0.42$  for session;  $F_{(5)}=0.26$ ,  $P=0.92$  for stimulus $\times$ session). Thus, LA lesions abolished conditioned freezing responses to the auditory CS, in agreement with many previous findings (see Davis, 1992; Fendt and Fanselow, 1999; LeDoux, 2000; Maren, 2001).

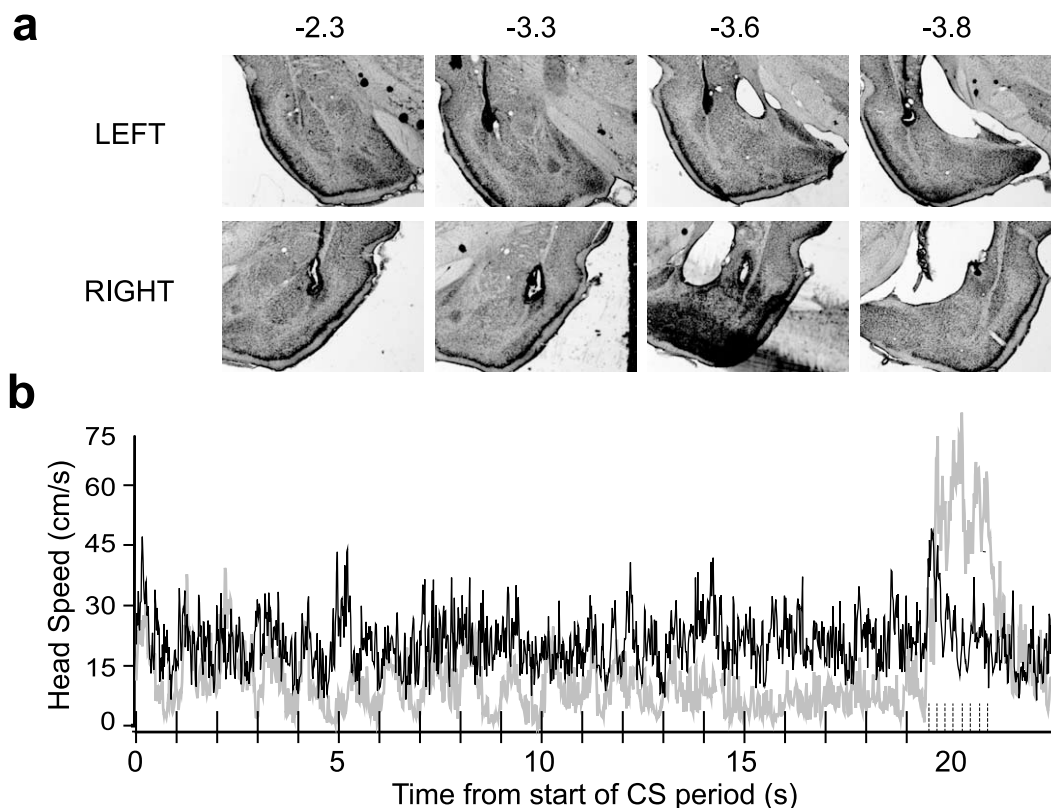
**Responses to shock.** Rats typically exhibit a burst of motor activity in response to electric shock (Fanselow, 1982). We measured shock-evoked motor activity by using data from the video tracker to analyze the rats' head movements during pip-shock pairing trials. When the shock US was delivered to the eyelid, rats in the control group exhibited robust head movements during the shocks (Fig. 2c). These head movements were greatly attenuated in the lesion group (black trace is below the gray trace during the US period in Fig. 2c). Statistical analysis confirmed that head movements during the shock train were significantly reduced in the lesioned group relative to the control group (independent  $t_{(10)}=5.24$ ,  $P=0.0004$ ). This reduction was observed equally in rats that received large lesions of the amygdala and surrounding tissues and in rats that received small focal lesions confined to the LA nucleus. Fig. 3a shows histology sections from a single rat (T50; see Fig. 1) which sustained small lesions that selectively destroyed the dorsal tip of LA while sparing most of the other amygdala nuclei. Fig. 3b (black trace) shows head movement data from this same rat, along with data from a single

rat in the control group (gray trace). The graph demonstrates that shock reactivity was attenuated in this rat even though lesions were mainly confined to the dorsal tip of LA.

#### Experiment 2: effects of drug infusions on fear acquisition and shock reactivity

To further examine the amygdala's role in shock reactivity during fear conditioning, rats were implanted with intracranial infusion cannulae in the amygdala (see placements in Fig. 4a). To be included in the study, placements were required to be within 0.25 mm of LA in both hemispheres. Several rats were eliminated from the study and then replaced (one from the high dose of MUS-HI group, two from the IFEN group, and one from the VEH group) because cannula tips were not correctly implanted in both hemispheres. Although cannula tips were targeted at LA, it cannot be ruled out that some of the drug solution may have diffused into adjacent structures such as CeA and the basal nucleus of the amygdala.

After recovery from surgery, the rats were trained in a fear conditioning protocol similar to that for experiment 1. The design of the experiment is summarized in Fig. 4b. Prior to the training session, rats received bilateral intra-LA infusions of VEH solution (0.5  $\mu$ L/side) containing either a MUS-HI (1.0 nmol/side), a MUS-LO (0.2 nmol/side), or ifenprodil (IFEN; 4.0  $\mu$ mol/side). A control group received



**Fig. 3.** Example of shock reactivity impairment following restricted lesions of LA. (a) Photomicrographs show serial sections of electrolytic amygdala lesions in rat T50 (number above each pair of sections denotes coordinates relative to bregma, in mm). (b) Line graph shows the mean head speed of rat T50 (black trace) compared with a typical rat from the unlesioned control group (gray trace) at each time point during training trials (see Fig. 2c caption).

pre-training infusions of the VEH alone. Immediately after the test session on the second day, rats that had been trained with muscimol or ifenprodil were infused with VEH alone, and then retrained with 16 additional CS–US pairings. Retrained rats were then retested (RETEST) on the following day.

**Conditioned freezing.** Fig. 4c shows that in all groups, rats froze very little to the CX or CS before conditioning (HAB session). When tested 24 h after pip-shock pairings (TEST session), rats in the VEH group showed greatly enhanced freezing to the CS but not the CX ( $2 \times 2$  ANOVA  $F_{(1)}=42.54$ ,  $P<0.0001$  for stimulus;  $F_{(5)}=16.85$ ,  $P<0.0001$  for session;  $F_{(5)}=7.6$ ,  $P=0.002$  for stimulus  $\times$  session). By contrast, rats in the MUS-HI group did not show enhanced freezing to the CS or CX during the test session ( $2 \times 2$  ANOVA  $F_{(1)}=0.3$ ,  $P=0.59$  for stimulus;  $F_{(5)}=0.52$ ,  $P=0.76$  for session;  $F_{(5)}=0.31$ ,  $P=0.89$  for stimulus  $\times$  session). When rats in the MUS-HI group were retrained with injections of VEH alone, they showed normal fear conditioning to the CS when RETEST on the following day ( $2 \times 2$  ANOVA  $F_{(1)}=22.43$ ,  $P=0.0005$  for stimulus;  $F_{(5)}=12.21$ ,  $P=0.0002$  for session;  $F_{(5)}=6.19$ ,  $P=0.005$  for stimulus  $\times$  session), indicating that the MUS-HI did not cause a permanent impairment in fear conditioning. These findings are consistent with previous studies showing that inactivation of the amygdala with muscimol temporarily impairs acquisition of auditory fear conditioning (Helmstetter and Bellgowan, 1994; Muller et al., 1997).

Rats in the MUS-LO group showed slightly enhanced freezing to the CS during the test session, and also showed slightly enhanced CX freezing ( $2 \times 2$  ANOVA  $F_{(1)}=2.68$ ,  $P=0.13$  for stimulus;  $F_{(5)}=9.84$ ,  $P=0.0006$  for session;  $F_{(5)}=0.26$ ,  $P=0.93$  for stimulus  $\times$  session). When rats in the MUS-LO group were retrained with injections of VEH alone, they showed normal fear conditioning to the CS when RETEST on the following day ( $2 \times 2$  ANOVA  $F_{(1)}=32.83$ ,  $P<0.0001$  for stimulus;  $F_{(5)}=9.53$ ,  $P=0.0007$  for session;  $F_{(5)}=5.84$ ,  $P=0.006$  for stimulus  $\times$  session), indicating that the MUS-LO did not cause a permanent impairment in fear conditioning. These findings are consistent with previous reports that muscimol infusions into LA have a dose-dependent effect on acquisition of auditory fear conditioning (Wilensky et al., 1999).

Rats in the IFEN group failed to show conditioned freezing to the CS or CX during the first test session ( $2 \times 2$  ANOVA  $F_{(1)}=0.9$ ,  $P=0.36$  for stimulus;  $F_{(5)}=0.75$ ,  $P=0.60$  for session;  $F_{(5)}=0.78$ ,  $P=0.58$  for stimulus  $\times$  session), but showed normal conditioning when retrained with injections of the VEH alone ( $2 \times 2$  ANOVA  $F_{(1)}=8.56$ ,  $P=0.0127$  for stimulus;  $F_{(5)}=5.61$ ,  $P=0.007$  for session;  $F_{(5)}=2.36$ ,  $P=0.1$  for stimulus  $\times$  session). These results are consistent with previous reports that blocking LA plasticity with ifenprodil impairs acquisition of auditory fear conditioning (Rodrigues et al., 2001).

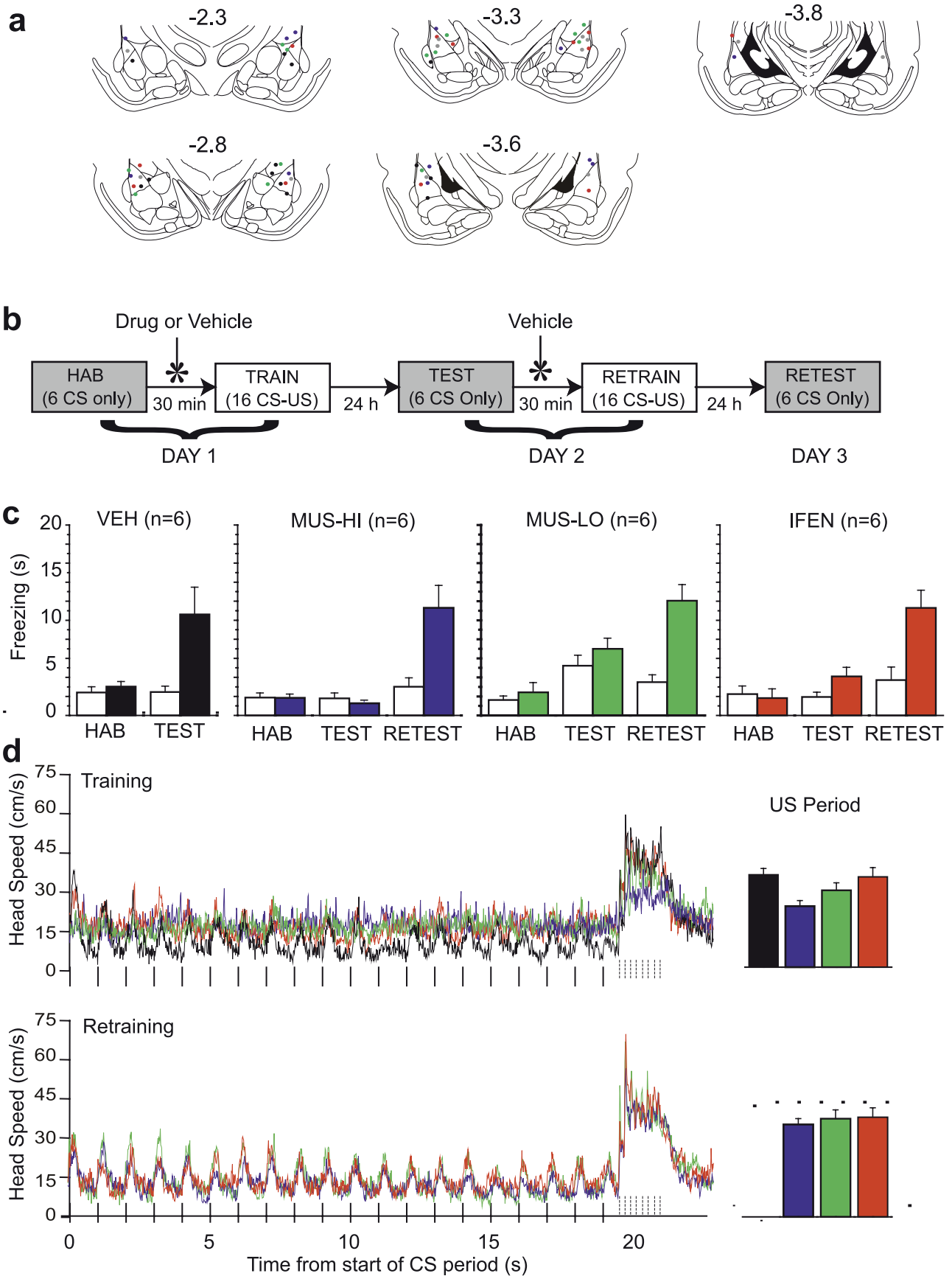


Fig. 4

Taken together, the results of Fig. 4 support the view that neural activity (which is blocked by muscimol) and synaptic plasticity (which is blocked by ifenprodil) in LA are both necessary for acquisition of conditioned freezing responses to an auditory CS, in agreement with previous studies (Helmstetter and Bellgowan, 1994; Muller et al., 1997; Wilensky et al., 1999; Rodrigues et al., 2001).

**Responses to shock.** Fig. 4d shows evoked head movements during training (top graph) and retraining (bottom graph) sessions for the VEH, MUS-HI, MUS-LO, and IFEN groups. During training, the VEH and IFEN groups show robust head movement responses evoked by the US, indicating normal shock reactivity for rats in these groups (US response for VEH vs. IFEN:  $t_{(10)}=0.19$ ;  $P=0.85$ ). By contrast, the MUS-HI group shows significantly attenuated responses to the eyelid shock US (US response for VEH vs. MUS-HI:  $t_{(10)}=3.8$ ;  $P=0.003$ ), much like the amygdala lesion group in experiment 1. The MUS-LO group shows partially attenuated US responses, but this attenuation does not reach statistical significance (US response for VEH vs. MUS-LO:  $t_{(10)}=1.62$ ;  $P=0.14$ ). During retraining with injections of VEH only, rats in the IFEN, MUS-HI, and MUS-LO groups showed normal head movement responses to the US (independent  $t$ -test VEH group in training vs. IFEN group in retraining:  $t_{(10)}=0.92$ ;  $P=0.37$ ; VEH group in training vs. MUS-HI group in retraining:  $t_{(10)}=0.36$ ;  $P=0.73$ ; VEH group in training vs. MUS-LO group in retraining:  $t_{(10)}=0.83$ ;  $P=0.43$ ). Thus, the attenuation of shock reactivity in muscimol groups on day 1 was not permanent.

In summary, the results shown in Fig. 4d indicate that neural activity in the amygdala contributes to the head-jerking response elicited by the US, since these responses were attenuated by infusions of muscimol into the amygdala. However, synaptic plasticity in the amygdala does not appear to contribute to the US-evoked head movement response, since the response was unaffected by infusions of ifenprodil into the amygdala.

### Experiment 3: effects of drug infusions on fear expression

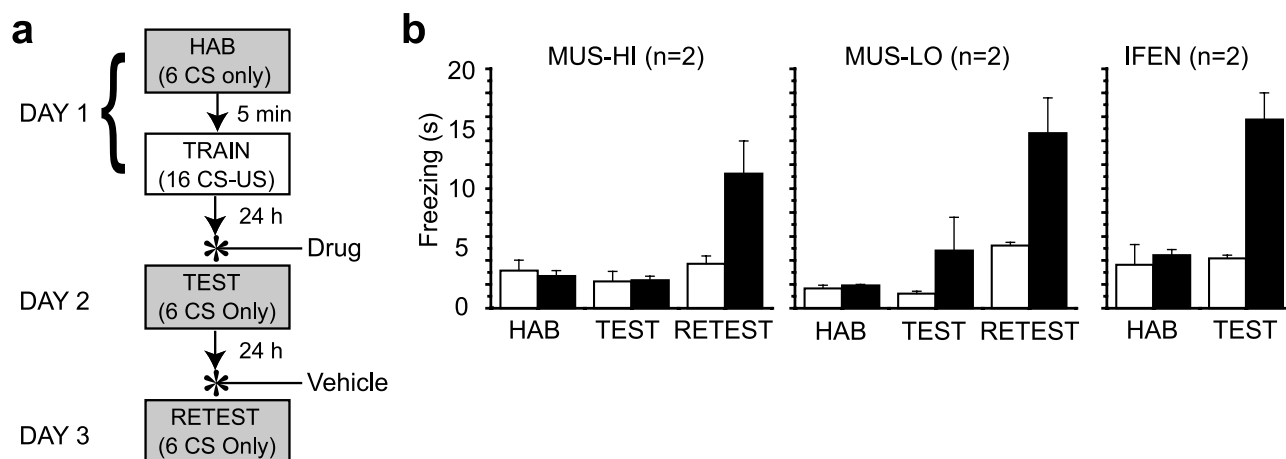
A group of six rats was trained drug-free using a fear-conditioning protocol that was similar to that used for experiment 1 and 2 above. Rats were given six HAB trials followed by 16 drug-free pairings of the CS and US on day 1 (Fig. 5). Prior to the test session on day 2, rats received a pre-TEST infusion of 1.0 nmol/side MUS-HI ( $n=2$  rats), 0.2 nmol/side MUS-LO ( $n=2$  rats), or 4.0  $\mu$ mol/side ifenprodil (IFEN,  $n=2$  rats). Histological analysis confirmed that infusion sites were within 0.25 mm of the border of the basolateral amygdala (see Fig. 4). Rats showed no conditioned freezing to the CS or CX when tested immediately after injections of a MUS-HI

or MUS-LO ( $2 \times 2$  ANOVA for MUS-HI and MUS-LO groups combined:  $F_{(1)}=3.53$ ,  $P=0.09$  for stimulus;  $F_{(3)}=0.73$ ,  $P=0.59$  for session;  $F_{(3)}=1.0$ ,  $P=0.45$  for stimulus  $\times$  session). However, when RETEST on the following day immediately after injections of VEH alone, these same rats showed freezing to the CS but not the CX ( $2 \times 2$  ANOVA for MUS-HI and MUS-LO groups combined:  $F_{(1)}=9.26$ ,  $P=0.01$  for stimulus;  $F_{(3)}=3.55$ ,  $P=0.047$  for session;  $F_{(3)}=1.71$ ,  $P=0.22$  for stimulus  $\times$  session). This result indicates that neural activity in the amygdala is essential for the expression of conditioned fear, in accordance with previous findings (Helmstetter and Bellgowan, 1994; Muller et al., 1997). Rats showed normal conditioned freezing to the CS (and normal absence of freezing to the CX) when they were tested immediately after injections of ifenprodil into the amygdala ( $2 \times 2$  ANOVA  $F_{(1)}=19.22$ ,  $P=0.012$  for stimulus;  $F_{(1)}=17.52$ ,  $P=0.014$  for session;  $F_{(1)}=14.53$ ,  $P=0.019$  for stimulus  $\times$  session). Thus, it was not necessary to retest these rats on the following day. This finding replicates a previous demonstration that infusion of ifenprodil into LA does not block fear expression (Rodrigues et al., 2001), and implies that synaptic plasticity in the amygdala is not required for the expression of conditioned fear, despite the fact that it is required for fear acquisition.

## DISCUSSION

In this study, we found that bilateral lesions or inactivation of the amygdala's LA nucleus impaired acquisition and expression of auditory fear conditioning, as reported in many previous studies (for review see Fendt and Fanselow, 1999; LeDoux, 2000; Maren, 2001; Blair et al., 2001). However, we also found that unconditioned responses evoked by the shock US were impaired by lesions or inactivation of the amygdala. This observation indicates that amygdala disruption may have attenuated the aversive reinforcement value of the US, and this could be one reason why acquisition of fear conditioning was impaired by amygdala damage. However, reduction of the US reinforcement value cannot fully account for the fear conditioning deficits we observed, because amygdala inactivation also impaired expression of CS-evoked freezing in rats that were previously trained drug-free (as in Helmstetter and Bellgowan, 1994; Muller et al., 1997; Wilensky et al., 1999). This result indicates that neural activity in the amygdala was required not only for unconditioned responses to the US, but also for conditioned responses to the CS. In addition, we found that blocking synaptic plasticity in the amygdala with ifenprodil impaired acquisition (but not expression) of auditory fear conditioning (as in Rodrigues et al., 2001), without affecting motor responses to the shock US. Thus, neural activity in the amygdala appears to be required for expression of both conditioned and unconditioned responses to aversive stimuli, whereas synaptic plasticity ap-

**Fig. 4.** Effects of intra-amygdala infusions of muscimol and ifenprodil upon shock reactivity and acquisition of freezing. (a) Histological reconstruction of cannula placements: black=VEH group, blue=MUS-HI group, green=MUS-LO group, red=IFEN group, gray=expression group. (b) Design of the infusion experiment. (c) Bar graphs show average time spent freezing ( $y$  axis) during the CS period (colored bars) and pre-CS period (white bars) of the HAB, TEST, and RETEST sessions for the VEH (black), MUS-HI (blue), MUS-LO (green), and IFEN (red) groups. (d) Line graph shows the mean head speed of rats in each group (indicated by colors as in 'b') at each time point during the training trial (see Fig. 2c caption). Bar graphs at right shows the mean head speed during the 1.5 s shock delivery period, plotted on the same scale as the  $y$  axis of the line graph.



**Fig. 5.** Effects of intra-amygdala infusions of muscimol and ifenprodil upon expression of conditioned freezing. (a) Design of the infusion experiment. (b) Bar graphs show average time spent freezing during the CS period (black bars) and pre-CS period (white bars) of the HAB, TEST, and RETEST sessions following MUS-HI (left graph), MUS-LO (middle graph), and ifenprodil (right graph).

appears to be required only for acquisition of conditioned responses, and not for expression of conditioned or unconditioned responses.

Our findings are consistent with prior data showing that amygdala damage impairs unconditioned responses to aversive stimuli in rats (Blanchard and Blanchard, 1972; Hitchcock et al., 1989; Kim and Davis, 1993; Borszcz and Leaton, 2003). However, contrary to our present findings, several previous studies have reported that amygdala lesions impair fear acquisition without affecting motor reflex responses to a footshock US (Cahill and McGaugh, 1990; Sananes and Davis, 1992; Maren, 1998). But in agreement with our results, other studies have reported that amygdala lesions can attenuate motor responses to footshock during fear conditioning (Hitchcock et al., 1989; Kim and Davis, 1993). In attempting to interpret these discrepant findings, it is important to recognize that shock-evoked motor responses are mediated by multiple brain circuits, including low-level circuits in the spinal cord and brainstem. Therefore, even if amygdala lesions attenuate the reinforcement value of shock and thereby render it less aversive, rats might continue to exhibit normal spinal and brainstem reflex responses when a shock is delivered (Borszcz and Leaton, 2003). Thus, unconditioned responses to shock may consist of at least two dissociable components, which we shall refer to here as “reflexive” versus “emotive” components. Since the amygdala is regarded as an emotional processing structure, it might be expected that amygdala lesions should attenuate emotive shock responses (which depend upon the amygdala) without affecting reflexive shock responses (which depend upon low-level reflex circuits). Different methods for delivering shock and measuring motor activity may favor detection of one of these response components over the other, and this might explain why some studies have observed attenuation of shock reactivity following amygdala lesions, while other studies have not (see Borszcz and Leaton, 2003). In addition, differing effects of amygdala lesions on shock reactivity might also be due to differences in the extent and

nature of the tissue damage caused by different lesion methods used in discrepant studies (see Koo et al., 2004).

In the present study, shock reactivity was quantified using a video tracker to measure the speed of head movements evoked by a train of shock pulses to the eyelid in freely behaving rats. This method has not previously been used to investigate the amygdala’s role in shock reactivity. Several different motor behaviors contributed to the head movements detected by the tracker, including flexion of the neck muscles and locomotion across the floor of the chamber during the shock. These various motor components were probably driven by several different motor circuits, possibly including trigeminal and brainstem reflex circuits as well as higher level emotive circuits. Our finding that amygdala disruption attenuated shock-evoked head movements suggests that the head-movement response depended at least partly upon emotive circuits involving the amygdala. However, lesions and inactivation of the amygdala did not completely abolish shock-evoked head movements in our experiments, and the residual response may have been governed by lower-level reflexive circuits that were left intact following amygdala disruption.

A large body of evidence from anatomical, physiological, and behavioral studies indicates that the LA nucleus of the amygdala is a critical site of neural plasticity where memories of the CS–US association are stored during auditory fear conditioning (for review see Fendt and Fanselow, 1999; Fanselow and LeDoux, 1999; LeDoux, 2000; Maren, 2001; Blair et al., 2001; for an alternative view see Cahill et al., 1999). Our present findings are in accordance with this view, since auditory fear conditioning was impaired by blocking neural activity or synaptic plasticity in LA. However, we also observed that localized electrolytic lesions of LA impaired unconditioned responses to the shock US, as did muscimol infusion targeted at the LA nucleus. These results suggest that in addition to being a site of synaptic plasticity that supports fear learning, neural activity in the LA nucleus may also contribute to representing the aversive reinforcement value of the US.

In summary, our findings support the conclusion that neural activity in LA is important not only for predicting the occurrence of punishment in the future, but also for perceiving the emotive aversiveness of punishment occurring in the present. This conclusion is consistent with the hypothesis that during fear conditioning, animals store an association between the CS and an internal representation of the emotional properties of the US (Konorski, 1967; Wagner and Brandon, 1989), and that the amygdala provides the neural substrate for such emotional representations of sensory stimuli (Klüver and Bucy, 1937; Weiskrantz, 1956). Our findings also support the view that synaptic plasticity in the amygdala provides the mechanism by which a neutral CS acquires aversive valence when it is paired with an aversive US, since blocking amygdala plasticity impaired acquisition of fear conditioning (as in Rodrigues et al., 2001). However, amygdala plasticity does not appear to be necessary for perceiving the aversiveness of a shock US (since blocking amygdala plasticity did not affect US-evoked head movements) or for conditioned freezing to the CS (since blocking amygdala plasticity does not impair expression of previously acquired freezing CRs).

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