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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 responses 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.

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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 μm 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 μm 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, ±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 (36×24×44 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