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Differential Contribution of Amygdala and Hippocampus to Cued and Contextual Fear Conditioning

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The contribution of the amygdala and hippocampus to the acquisition of conditioned fear responses to a cue (a tone paired with footshock) and to context (background stimuli continuously present in the apparatus in which tone–shock pairings occurred) was examined in rats. In unoperated controls, responses to the cue conditioned faster and were more resistant to extinction than were responses to contextual stimuli. Lesions of the amygdala interfered with the conditioning of fear responses to both the cue and the context, whereas lesions of the hippocampus interfered with conditioning to the context but not to the cue. The amygdala is thus involved in the conditioning of fear responses to simple, modality-specific conditioned stimuli as well as to complex, polymodal stimuli, whereas the hippocampus is only involved in fear conditioning situations involving complex, polymodal events. These findings suggest an associative role for the amygdala and a sensory relay role for the hippocampus in fear conditioning.

In classical fear conditioning, an emotionally neutral conditioned stimulus (CS), such as a light or tone, is paired with an aversive unconditioned stimulus (US), usually footshock. The CS, by virtue of its relationship with the US, acquires aversive properties and comes to elicit responses characteristically elicited by threatening stimuli. Thus, a tone that has previously been paired with footshock elicits "freezing," defecation, piloerection, stereotyped increases in arterial pressure and heart rate, and the release of adrenal hormones into the circulation (e.g., R. J. Blanchard & D. C. Blanchard, 1969; Bolles & Fanselow, 1980; LeDoux, 1987; Smith & DeVito, 1984). Because these "fear" or defense responses are not elicited by the CS before the temporal pairing of the CS with the US, they can be referred to as learned or conditioned emotional responses.

Conditioned emotional responses are also elicited by placing an animal in a chamber in which an aversive US has previously been experienced (D. C. Blanchard & R. J. Blanchard, 1972; Bolles & Fanselow, 1980; McCarty, Kvetnansky, Lake, Thoa, & Kopin, 1978). In this situation, the conditioned emotional responses are elicited not by a stimulus that was explicitly paired with the US in a temporally specific manner but instead by some combination of the various background or contextual stimuli that were present in the chamber when the US occurred and remain present when the animal is returned to the chamber.

Although the emotional responses elicited by contextual and cued CSs are identical, the information processing demands underlying the two forms of fear conditioning are very different. First, in contextual conditioning the CS is not restricted to a single sensory modality. Second, unlike an explicit CS, contextual CSs are continuously present and are thus not delivered to the animal in a precise, time-dependent manner in relation to the US. Third, contextual CSs are predictive of the general situation in which the US is likely to occur but are not predictive of the onset of any particular US. These observations suggest that different neural pathways may mediate the analysis of the stimulus properties of explicit and contextual stimuli but that common pathways may be involved in the expression of the conditioned emotional responses elicited by either kind of CS.

Considerable evidence now points to the amygdala as an essential link in the neural system underlying fear conditioning (e.g., Davis, Hitchcock, & Rosen, 1987; Kapp, Pascoe, & Bixler, 1984; Kapp, Wilson, Pascoe, Supple, & Whalen, 1990; LeDoux, 1987, 1990). In fact, lesions of the amygdala interfere with the acquisition and expression of emotional responses conditioned to cued (Gentile, Jarrel, Teich, McCabe, & Schneiderman, 1986; Hitchcock & Davis, 1986; Iwata, LeDoux, & Reis, 1986; Kapp, Frysinger, Gallagher, & Haselton, 1979) and contextual (D. C. Blanchard & R. J. Blanchard, 1972) CSs. The amygdala, particularly the central nucleus of the amygdala, has connections with brain stem and spinal areas controlling the motor expression of emotional responses (Hopkins & Holstege, 1978; Krettek & Price, 1978a; LeDoux, Iwata, Cicchetti, & Reis, 1988; Price & Amaral, 1981; Schwaber, Kapp, & Higgins, 1980) and may be a common output channel through which conditioned emotional responses are expressed in the presence of both an explicit CS and contextual stimuli.

Recent studies have also made some progress in understanding how the brain transmits auditory CS information to the amygdala. For very simple auditory stimuli (undiscriminated tones), the CS is transmitted through the auditory system to the medial geniculate body (MGB) and from there directly to the lateral nucleus of the amygdala (AL; for review see LeDoux, 1990). In contrast, if an auditory discrimination is

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required, then the CS is transmitted from the MGB to the auditory cortex and then to the amygdala (Gentile et al., 1986; Jarrell, Gentile, Romanski, McCabe, & Schneiderman, 1987), most likely to the AL (LeDoux, Ruggiero, Forest, Stornetta, & Reis, 1987). The AL, in turn, projects to the central nucleus of the amygdala both directly (Krettek & Price, 1978b) and by way of the basolateral nucleus (Farb, Go, & LeDoux, 1991; Pitkanen & Amaral, 1991). These latter projections thus complete the auditory CS pathway to the central amygdala, which controls the expression of the emotional responses. This general scheme for auditory fear conditioning may also apply to other sensory systems, especially the visual system (LeDoux, 1990; LeDoux, Romanski, & Xagoraris, 1989).

Much less is known about how contextual stimuli are evaluated for emotional significance and come to control emotional responses. However, it is generally believed that the hippocampus, as part of its general role in spatial processing functions (O'Keefe & Nadel, 1978; Olton, Becker, & Handlemann, 1979), plays an important role in contextual processing (Nadel, Willner, & Kurtz, 1985; Sutherland & McDonald, 1990; Sutherland & Rudy, 1989; Winocur & Olds, 1978). The hippocampus receives inputs from cortical areas that integrate information across sensory modalities (Amaral, 1987; Herzog & van Hoesen, 1975; Jones & Powell, 1970; Mesulam, van Hoesen, Pandya, & Geschwind, 1977), and this kind of functional architecture may underlie the modality-independent aspects of contextual processing. Interestingly, the subiculum, a major output of the hippocampal formation, projects directly to the AL (Ottersen, 1982). Although the contribution of the hippocampus to contextual processing in fear conditioning has never been examined, the connection between the subiculum and the AL suggests a plausible route through which the contextual information processing in the hippocampus might interact with the emotional response control mechanisms of the amygdala.

Unfortunately, this relatively straightforward scenario, whereby direct links between sensory processing systems and the amygdala are responsible for explicitly cued fear conditioning and more circuitous routes involving several sensory processing systems, the hippocampus, and the amygdala are responsible for contextual fear conditioning, is complicated by a recent study in which Selden, Everitt, and Robbins (1989) found that although excitotoxic lesions of the amygdala interfered with the acquisition of conditioned responses to acoustic clicks paired with shock, the same lesions had no effect on the passive avoidance of the place in which shock occurred. The response to the clicks was used to measure conditioning to an explicit cue and the passive avoidance response was used as a measure of contextual conditioning. Why Selden et al. failed to find an effect of amygdala damage on passive avoidance conditioning is not clear because a host of other studies have observed deleterious effects of amygdala damage (see McGaugh, 1990; Panksepp, Sacks, & Crepeau, 1991; Sarter & Markowitsch, 1985). However, our main concern is with neural mechanisms underlying Pavlovian contextual conditioning rather than with neural basis of passive avoidance. Passive avoidance is an instrumental response and is thus an indirect measure of Pavlovian contextual conditioning. Contextual conditioning is more directly assessed by measuring conditioned responses, such as freezing, that are elicited directly by the context in which the US occurs (R. J. Blanchard & D. C. Blanchard, 1969; Fanselow, 1980).

The purpose of the present study was therefore to reexamine the role of the amygdala and to assess the possible contribution of the hippocampus to contextual fear conditioning. Modification of procedure described by Helmstetter and Fanselow (1989) allowed us to monitor in a single chamber and test situation the development of conditioned freezing responses in the presence of a cued CS (tone) paired with a US (shock) and in the presence of contextual stimuli (apparatus cues) present during CS–US pairing. Once acceptable conditioning parameters were determined, we examined the effects of lesions of the amygdala and dorsal hippocampus on the acquisition of conditioned freezing to the cued CS and to the context in which explicit CS–US pairing occurred. We hypothesized that lesions of the amygdala would interfere with freezing responses elicited both by the cue CS and by contextual stimuli but that lesions of the hippocampus would only interfere with freezing responses elicited by contextual stimuli.

Materials and Method

Animals

Male Sprague-Dawley rats, which weighed 275–300 g upon arrival, were housed in groups of 2 for 1 week after arrival to become acclimatized to laboratory conditions. They were provided with free access to lab chow and water and were maintained on a 12:12-hr light–dark cycle (lights on at 6:00 a.m.). After 1 week, some rats underwent surgery and were then housed individually for the remainder of the experiment.

Animals were randomly assigned to groups in two experiments. The first experiment examined the effects of parametic variations in the intensity of the US (0.3 mA, $n = 4$; 0.5 mA, $n = 12$; 1.0 mA, $n = 8$; 2.0 mA, $n = 4$) on the acquisition of freezing to explicit and contextual CSs. The second experiment examined the effects of brain lesions on the acquisition of freezing responses to explicit and contextual CSs (amygdaloid lesions, $n = 8$; hippocampal lesions, $n = 25$; neocortical lesions, $n = 11$).

Behavioral Method

Apparatus and stimuli. For aversive classical conditioning, the rats were placed individually in a rodent conditioning chamber (Coulbourn Instrs. Inc., Lehigh Valley, PA, Model E10-10) enclosed by a sound-attenuating cubicle (Coulbourn Instrs. Inc., Model E10-20). Stimulus presentation was controlled by a microprocessor and a digital I/O board (Opto 22). The CS was an 800-Hz tone produced by a frequency generator (Coulbourn Instrs. Inc., Model S81-06), amplified to 80 dB (Archer Mini Amplifier), and presented for 20 s through a speaker located in the front panel of the chamber. The US was a brief (500 ms) distributed delivery of direct current produced by a grid floor shocker (Coulbourn Instrs. Inc., Model E13-08). The intensity of the US was varied (0.3, 0.5, 1.0, and 2.0 mA) in the first experiment, which was designed to determine optimal conditioning parameters. Based on the results of this experiment, the 0.5 mA US was selected for use in the lesion study.

Procedure. On Day 0, the animals were allowed 20 min to acclimate to the conditioning box before the start of training trials. They remained in the conditioning chamber for an additional 20 min without stimulus presentation and were then returned to their home cages. On Days 1 and 2, conditioning trials (which consisted of two

trials per day during which the US was presented during the last 500 ms of the 20-s CS) were given. The intertrial interval varied randomly between 60 and 120 s. Extinction trials (two presentations of the CS alone) began on Day 3 and continued for 3 additional days.

Freezing, which was used as the index of conditioned fear, was assessed by viewing the animals through a peephole in the sound-attenuating chamber and using stopwatches to measure freezing time. Freezing was defined as the absence of all movement except for respiratory-related movements. Scoring of freezing was performed by one of two observers. One of these was naive as to the purpose of the experiment and the expected effects of the manipulations. Comparison of results from the two observers for animals within a given group showed no differences.

Freezing during the pre-CS period (the 20-s period immediately preceding the onset of the CS) was used as a measure of contextual fear conditioning, and freezing during the 20-s delivery of the CS was used as a measure of cued fear conditioning. Particular weight was given to the amount of time spent freezing during the first pre-CS and the first CS period on each day because freezing during these periods reflects effects of US presentations on the previous day. In contrast, freezing during the pre-CS and CS periods of Trial 2 is potentially confounded by the lingering effects of the US presented moments earlier during Trial 1.

Stereotaxic Placement of Brain Lesions

Brain lesions were placed in the amygdala (n = 12), dorsal hippocampus (n = 25), or neocortex overlying the dorsal hippocampus (n = 11). Animals were anesthetized with pentobarbital (40 mg/kg) and placed in a stereotaxic frame. The cranium was exposed, and a small hole was made over the lesion site using a dental drill. Monopolar stainless steel electrodes insulated with epoxy to within 200 μm of the tip were lowered through an incision in the dura into the target brain area. The cathode was connected to the open skin wound. Lesions were made by passing anodal constant current (1 mA, 15–20 s) through the electrode. All lesions were bilateral, with placement guided by coordinates modified from an atlas of the rat brain (Paxinos & Watson, 1986). The anterior–posterior (AP), medial–lateral (ML), and dorsal–ventral (DV) coordinates were computed in relation to the interaural line. Bilateral lesion sites included the amygdala (AP = 6.2, ML = ±4.7, DV = 1.8), hippocampus (two lesions: AP = 4.2, ML = ±2.2, DV = 6.5; AP = 5.7, ML = ±1.8, DV = 6.6), and neocortex above the hippocampus (two lesions: AP = 4.2, ML = ±2.2, DV = 8.5; AP = 5.7, ML = ±2.2, DV = 8.5). After surgery the wound was closed, and the animal was placed under a heat lamp until fully recovered from anesthesia and was then returned to its home cage in the animal housing area. Ten to 14 days were allowed for recovery from surgery.

Histology

After completion of behavioral studies, animals were given an overdose of sodium pentobarbital (120 mg/kg) and perfused with saline, which was followed by 10% buffered formalin. Brains were postfixed in buffered formalin, frozen, and cut on a microtome into 40-μm sections. Every fourth section was taken, mounted on a gelatin-coated slide, and then stained with thionin.

Results

Experiments were first conducted on unoperated rats to determine appropriate US parameters to establish cued and contextual fear conditioning. Comparisons were made between groups given conditioning trials with 0.3, 0.5, 1.0, and 2.0 mA shocks as the US.

Acquisition of conditioned responses was examined by measuring freezing on Days 1, 2, and 3 (Figure 1). On Day 1, before the first CS–US pairing occurred, animals in all groups exhibited exploratorylike movement for a majority of the time during the pre-CS period and during the CS. Little or no freezing was observed. On Day 2 (after two CS–US pairings on Day 1), animals in the 0.3- and 0.5-mA intensity groups exhibited freezing during the CS but not during the pre-CS period, with the 0.3-mA group freezing less than the 0.5-mA group during the CS. Animals conditioned with 1.0 or 2.0 mA exhibited freezing during both the pre-CS and CS periods. On Day 3 (after two pairings on Day 1 and two more pairings on Day 2), the rats in the 0.3-mA group still showed very little freezing during the pre-CS period but did show some freezing during the CS. Animals in the 0.5-, 1.0-, and 2.0-mA groups showed freezing during the pre-CS and during the CS on Day 3. Extinction was tested on Days 4–7. As shown in Figure 1, extinction was more rapid with less intense shocks for both CS and contextual freezing, and within each US intensity group, extinction to the context was more rapid than extinction to the cued CS.

A three-way analysis of variance (ANOVA) with two grouping variables (stimulus type and US intensity) and one repeated measure (Test Days 1–7) was performed on these data. The main effects of stimulus type, $F(1, 52) = 23.12$, $p < .001$, US intensity, $F(3, 52) = 28.08$, $p < .001$, and test day, $F(6, 312) = 56.26$, $p < .001$, were all significant, as was the three-way interaction: Stimulus Type × Intensity × Day, $F(18, 312) = 1.69$, $p < .05$. Post hoc analysis with the Tukey test indicated that on Day 3 there was more freezing during the pre-CS ($p < .001$) and CS ($p < .05$) in the 0.5-mA group than in the 0.3-mA group. No other comparisons between adjacent US intensity groups were significant. Within US intensity groups, freezing during the pre-CS ($p < .001$) and CS ($p < .001$) were significantly different on Day 2 for the 0.5-mA group but not for any other day for this group and not for any day for the other groups. These analyses indicated that for the 0.5-mA group a separate assessment of the rate of acquisition of freezing responses to a specific cue and context could be made. This intensity was therefore used in the brain lesion study.

Lesions were placed bilaterally in the amygdala or dorsal hippocampus. Controls were unoperated. An additional control group received lesions of the sensorimotor cortex dorsal to the hippocampus because this area was damaged in the hippocampus-lesioned animals.

The effects of the lesions are shown in Figure 2. Amygdala-lesioned animals showed little or no freezing throughout the course of the experiment. In contrast, although hippocampus-lesioned animals showed very little freezing during the pre-CS (context test) period, they exhibited the normal pattern of freezing in the presence of the CS. Cortex-lesioned animals exhibited the same pattern of freezing as did the unoperated controls. Thus, lesions of the amygdala appeared to interfere with the acquisition of conditioned freezing to both the context and the cue, whereas lesions of the hippocampus appeared to

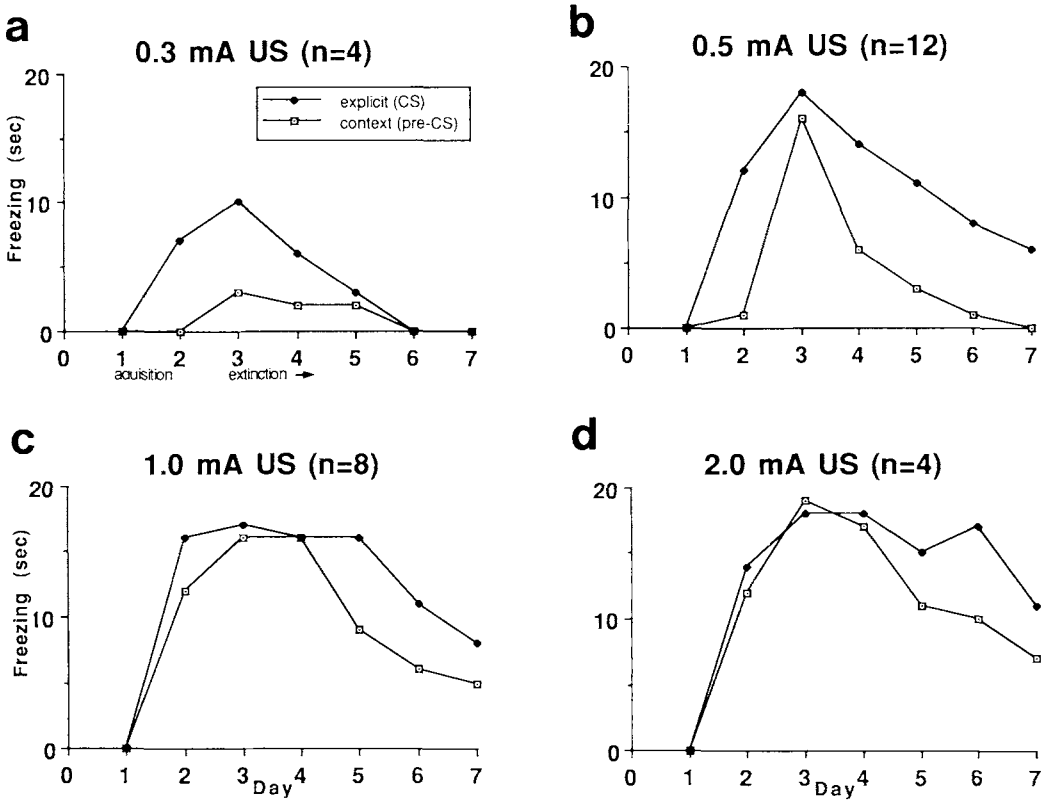

Figure 1. Effects of variation of unconditioned stimulus (US) magnitude (footshock intensity) on the acquisition of conditioned freezing responses to a cued conditioned stimulus (CS; tone paired with footshock) and to the context in which tone–shock pairings occurred. (Each group received two CS–US pairings on Days 1 and 2. Freezing was measured during the 20-s period before the CS and during the CS on the first trial of each day. The pre-CS period was used as a measure of contextual conditioning and freezing during the CS as a measure of explicit conditioning. Responses on a given day reflect the effects of the conditioning session on the previous day. Thus, on Day 1, freezing during the pre-CS and CS periods of trial 1 is measured in naive animals. On Day 2, freezing reflects the two conditioning trials on Day 1 and freezing on Day 3 reflects the conditioning trials on Day 2. Freezing on Days 4–7 reflect the extinction trials [no US presentation] of Days 3–6.)

interfere with the acquisition of contextual but not cue-elicited freezing.

An ANOVA with two grouping variables (lesion group and stimulus type) and one repeated measure (Test Days 1–7) was performed on freezing response data. The main effect of lesion group, $F(3, 98) = 47.58, p < .001$, stimulus type, $F(1, 98) = 172.29, p < .001$, and test day, $F(6, 588) = 90.46, p < .001$, were all significant, as was the Lesion Group × Stimulus Type × Test Day interaction, $F(18, 588) = 3.569, p < .001$. Post hoc analysis with the Tukey test showed a significant difference between the amygdala-lesioned animals and controls during the pre-CS ($p < .001$) and CS ($p < .001$) on Days 2–7. Animals with lesions of the hippocampus also showed significantly reduced freezing during the pre-CS, compared with unoperated controls, on Days 2–7 ($p < .001$), but there was no significant change in the amount of time spent freezing during the CS on any day. Lesions of the neocortex above the hippocampus had no significant effect on freezing to the CS or context during either test, compared with unoperated controls.

The average amount of freezing during the pre-CS and CS periods on Day 3 is shown in Figure 3. The standard errors illustrated for this day are representative across the other days of the experiment.

Lesions of the amygdala (Figure 4) typically destroyed the lateral, basolateral, and central nuclei. There was also variable damage to the overlying posterior caudate-putamen, especially ventrally. Lesions of the dorsal hippocampus (Figure 5) included areas CA1, CA2, and CA3, as well as the dentate gyrus and dorsal subiculum. In some hippocampus-lesioned animals, thalamic areas (lateral dorsal or lateral posterior nuclei or both) underlying the dorsal hippocampus were damaged. No differences were found in the behavioral effects of the lesions from animals with ($n = 8$) and without ($n = 16$) thalamic damage. Also, part of the overlying neocortex was damaged to some extent in all hippocampus-lesioned animals. Control lesions of the overlying sensorimotor cortex (Figure 6) did not invade the hippocampus and, as indicated, had no effect.

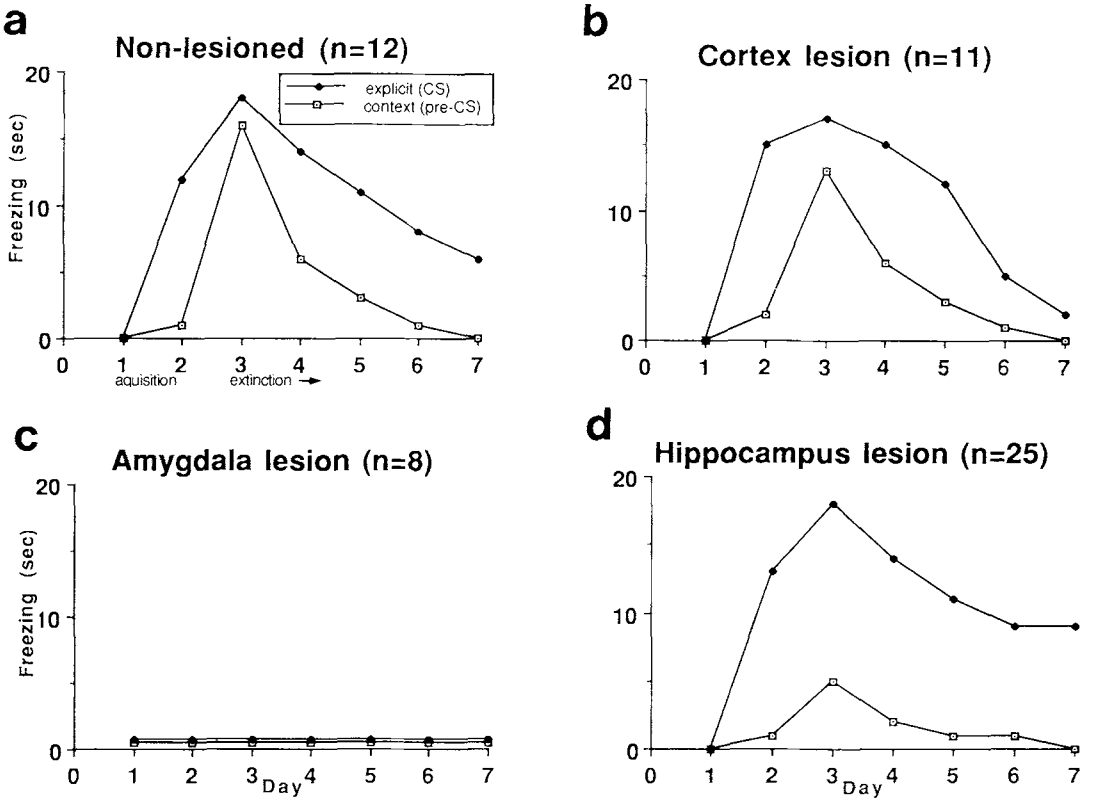

Figure 2. Effects of lesions of the amygdala and hippocampus on the acquisition of conditioned freezing responses to a cued conditioned stimulus (CS) and to contextual stimuli. (Lesions of the amygdala [c] interfere with conditioning to the cued CS and to the context, whereas lesions of the hippocampus [d] only interfere with contextual conditioning, compared with controls [a]. Lesions of the cortex above the hippocampus [b] have no effect on either form of conditioning. Conclusions are based on analysis of variance and post hoc tests.)

Discussion

In the present study, electrolytic lesions of the amygdala disrupted the acquisition of freezing responses to an explicit cue (a tone paired with footshock) and to the context in which tone–shock pairing took place. In contrast, lesions of the dorsal hippocampus interfered with the acquisition of freezing responses to contextual stimuli but not to the cued CS. These findings suggest that the amygdala is an essential component in the neural system of fear conditioning, regardless of the type of stimulus input serving as the CS, and that the hippocampus, although not necessary for conditioning with an explicit CS, is necessary for the conditioning of fear responses to contextual stimuli. Thus, divergent but overlapping brain mechanisms mediate conditioning to specific cues and contextual stimuli.

Our findings concerning the amygdala are largely consistent with past studies showing that lesions of the amygdala interfere with fear conditioning in situations involving both explicit (Davis et al., 1987; Gentile et al., 1986; Iwata et al., 1986; Kapp et al., 1979) and contextual (D. C. Blanchard & R. J. Blanchard, 1972) CSs. They are, however, inconsistent with the study by Selden et al. (1989), which suggested that the amygdala is not involved in contextual fear conditioning. In that study, the extent to which rats avoided entering the compartment in which clicks had previously been paired with shocks was examined. Contextual classical conditioning was thus assessed indirectly through the measurement of a fear-motivated instrumental avoidance response. In contrast, freezing is a classically conditioned fear response and is thus a more direct measure of contextual classical fear conditioning. Both the present study and the study by D. C. Blanchard and R. J. Blanchard (1972) indicate that amygdala lesions interfere with contextually induced freezing. But this difference in the way contextual conditioning was measured in our study and in the Selden et al. study does not readily explain the different results obtained. Many other studies have reported that lesions of the amygdala interfere with passive avoidance conditioning (see Sarter & Markowitsch, 1985). Another difference between the Selden et al. study and the present study is that they made neurotoxic lesions whereas we made electrolytic lesions. Our results could therefore be due to the interruption of fibers of passage rather than to amygdaloid damage per se, whereas their results could be due solely to amygdaloid damage. However, we have conducted a pilot study using animals with neurotoxic lesions of the amygdala and have replicated our

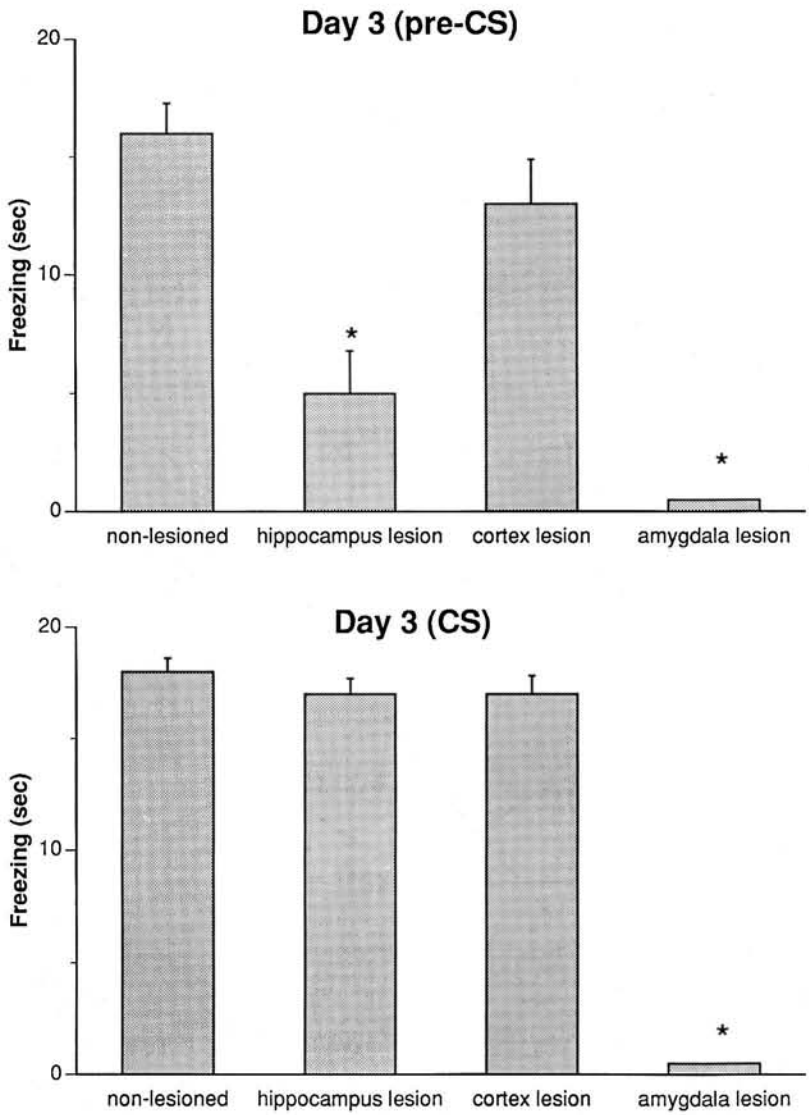

Figure 3. Freezing during the preconditioned stimulus (pre-CS; context test) and CS (cue test) periods on Day 3. (Values shown are $M \pm SE$. The SE for the amygdala group was too small to illustrate. The SE values shown for each group for Day 3 are representative of other days of the experiment. $^*p < .001$, in relation to the control group.)

effects of amygdaloid damage on contextual conditioning. Why Selden et al. failed to find an effect of lesions of the amygdala on their contextual procedure (passive avoidance conditioning) remains unclear.

The failure of hippocampal lesions to affect conditioned fear reactions to the tone CS used in this study is consistent with the results of several past studies (e.g., Rickert, Bennett, Lane, & French, 1978; Thomas, 1988). Further, the involvement of the hippocampus in contextual conditioning is consistent with the effects of hippocampal lesions on contextual processing, as studied in other tasks (Nadel et al., 1985; Winocur & Olds, 1978), and with several theories of hippocampal function that emphasize the role of this structure in spatial, contextual, and configural processing (O'Keefe & Nadel, 1978; Olton et al., 1979; Nadel, 1991; Sutherland & Rudy, 1989). None of this previous research on the hippocampus has involved fear conditioning. However, a recent study by Kim and Fanselow (1991) also found that hippocampal lesions interfere with contextual fear conditioning. Our results, together with those of Kim and Fanselow, suggest a new behavioral model for examining the contribution of the hippocampus to contextual processing.

We suggest that in fear conditioning the amygdala is involved in the formation of associations between an aversive US and of any of a variety of types of CSs, ranging from the simplest to the most complex. The exact CS pathway used by the amygdala in a given situation depends on the processing demands of the situation. The amygdala receives inputs from sensory processing areas of the thalamus, modality-specific sensory processing areas of the neocortex, higher order corti-

Figure 4. Amygdala lesions typically included portions of the lateral, basolateral, and central nuclei. (The striatum was variably damaged from case to case. Lesioned area is indicated by stippling. ABl = basolateral nucleus, amygdala; ABm = basomedial nucleus, amygdala; ACo = cortical nucleus, amygdala; AH = anterior hypothalamus; AL = lateral nucleus, amygdala; AMe = medial nucleus, amygdala; AST = amygdalostriatal transition area; CA1–CA3 = fields of Ammon's horn; CPu = caudate putamen; DEn = dorsal endopiriform nucleus; DG = dentate gyrus; f = fornix; GP = globus pallidus; ic = internal capsule; LH = lateral hypothalamus; LV = lateral ventricle; ot = optic tract; RT = reticular thalamic nucleus; Thal = thalamus; VM = ventromedial thalamic nucleus; 3V = third ventricle.)

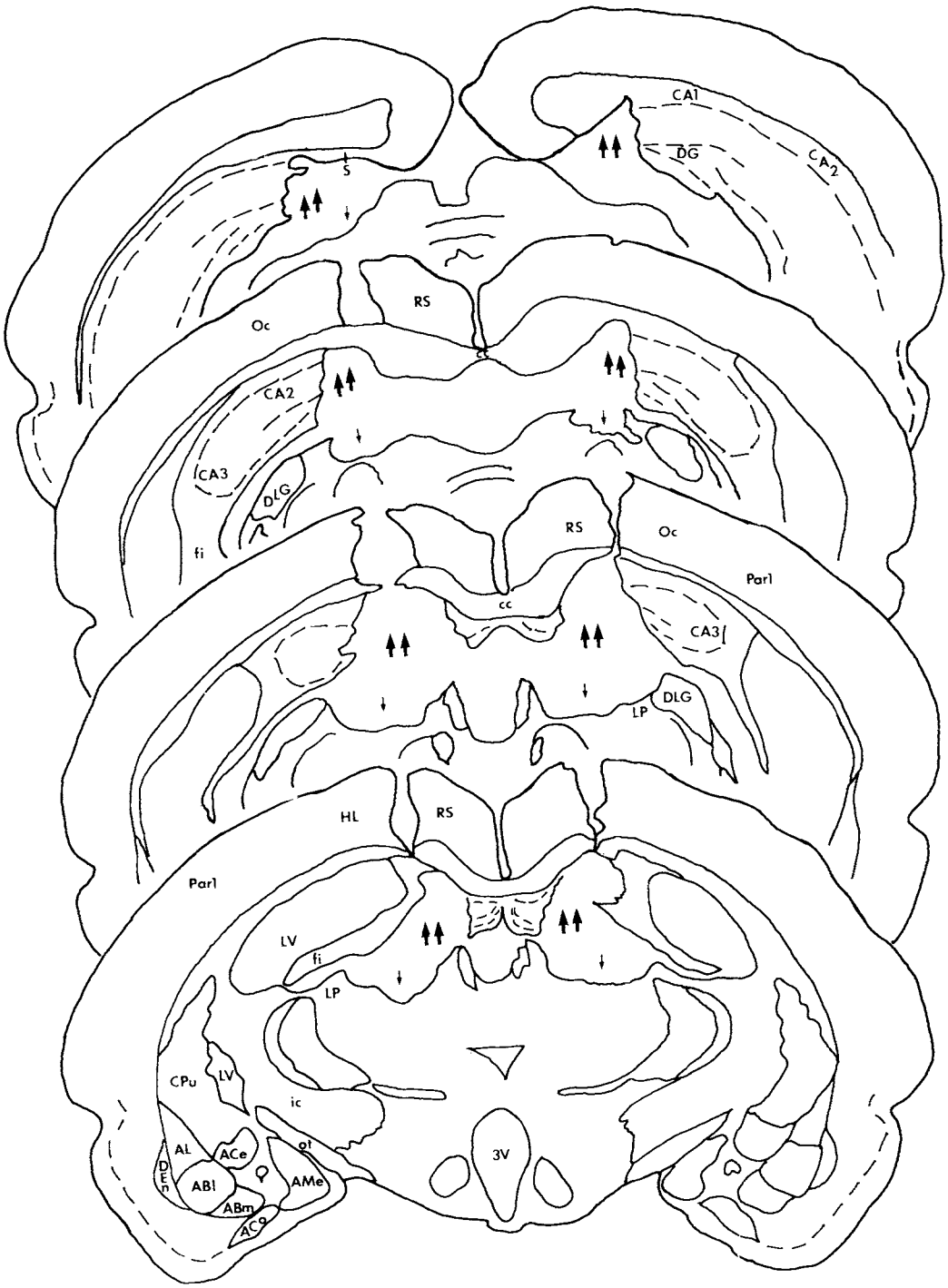

Figure 5. Hippocampal lesions usually transected the dorsal hippocampal formation and produced some damage to all CA fields, the dentate gyrus, and the dorsal subiculum. (Damage to the hippocampus is indicated by bold double arrows. Incidental damage to the underlying thalamus and overlying cortex is indicated by small single arrows. ABl = basolateral nucleus, amygdala; ABm = basomedial nucleus, amygdala; ACe = central nucleus, amygdala; ACo = cortical nucleus, amygdala; AL = lateral nucleus, amygdala; AMe = medial nucleus, amygdala; CA1–CA3 = fields of Ammon's horn; cc = corpus callosum; CPu = caudate putamen; DEn = dorsal endopiriform nucleus; DG = dentate gyrus; DLG = dorsolateral geniculate; fi = fimbria; HL = hindlimb area of cortex; ic = internal capsule; LP = lateral posterior thalamic nucleus; LV = lateral ventricle; Oc = occipital cortex; ot = optic tract; Parl = parietal cortex; RS = retrosplenial cortex; S = subiculum; 3V = third ventricle.)

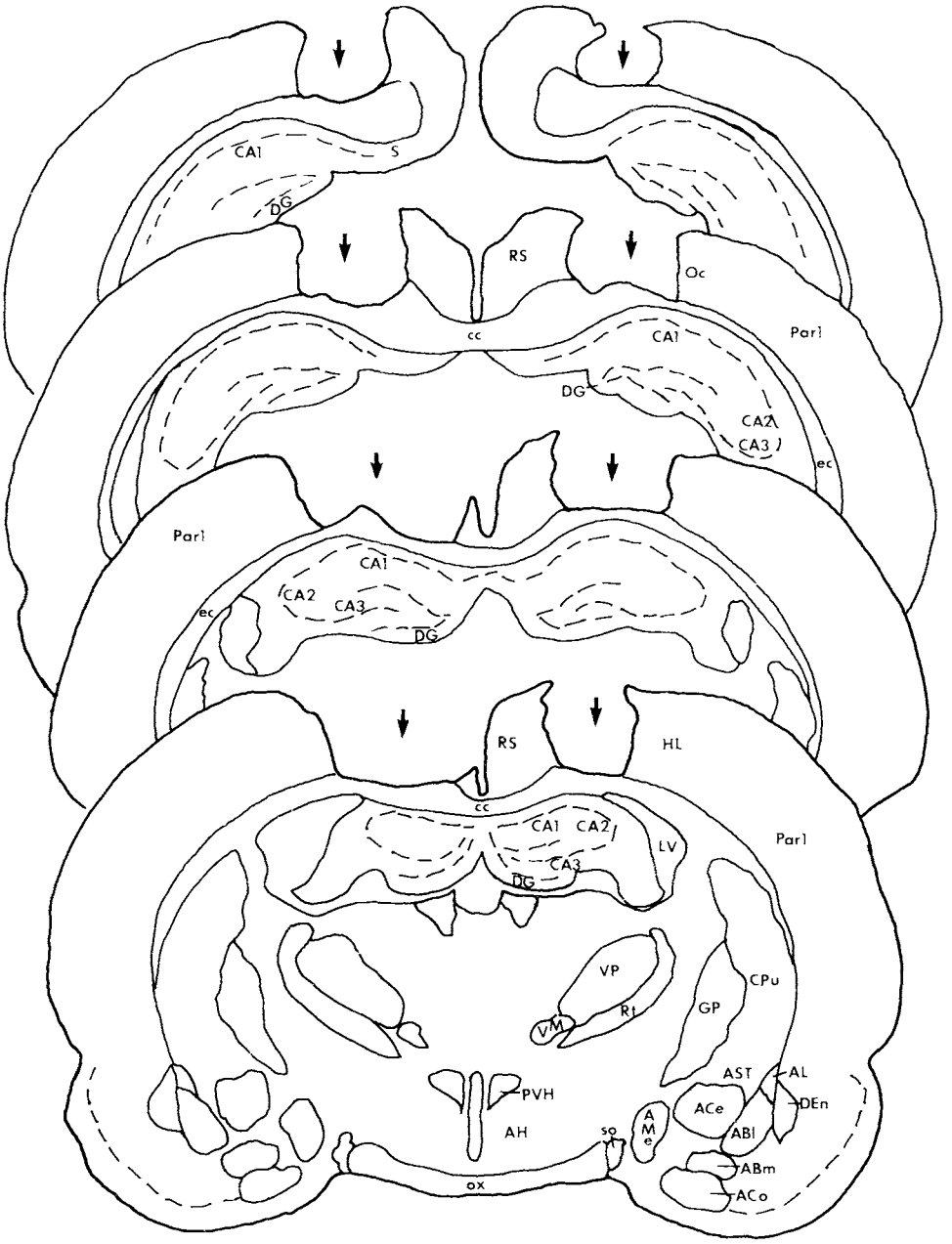

Figure 6. Cortical lesions included the sensorimotor region overlying the dorsal hippocampus. (The lesioned area is indicated by single arrows. ABl = basolateral nucleus, amygdala; ABm = basomedial nucleus, amygdala; ACe = central nucleus, amygdala; ACo = cortical nucleus, amygdala; AH = anterior hypothalamus; AL = lateral nucleus, amygdala; AMe = medial nucleus, amygdala; AST = amygdalostriatal transition area; CA1–CA3 = fields of Ammon's horn; cc = corpus callosum; CPu = caudate putamen; DEn = dorsal endopiriform nucleus; DG = dentate gyrus; ec = external capsule; GP = globus pallidus; HL = hindlimb area of cortex; LV = lateral ventricle; Oc = occipital cortex; ox = optic chiasm; Parl = parietal cortex; PVH = paraventricular hypothalamus; RS = retrosplenial cortex; Rt = reticular thalamic nucleus; S = subiculum; so = supraoptic nucleus; VM = ventromedial thalamic nucleus; VP = ventral posterior thalamic nucleus.)

cal areas that integrate inputs from several different modalities, and the hippocampal formation (Amaral, 1987; Herzog & van Hoesen, 1975; Jones & Powell, 1970; LeDoux, Cicchetti, Xagoraris, & Romanski, 1990; LeDoux, Ruggiero, & Reis, 1985; Ottersen, 1982; Turner, Mishkin, & Knapp, 1980). For modality-specific CSs, either thalamic or cortical inputs to the amygdala suffice as transmission routes (Romanski & LeDoux, 1991). For more complex stimuli involving more than one

modality, projections from multimodal areas of the neocortex to the amygdala are likely to be necessary. For the most complex stimuli, particularly those for which spatial organization is important, the hippocampus and the projection from the subiculum to the amygdala may be required. In this scheme, the hippocampus contributes to fear conditioning not as an associative structure but much the same as other CS processing channels (sensory thalamus and sensory cortex) that relay sensory inputs to the amygdala. The hippocampal projections simply transmit more complex kinds of signals. Interestingly, thalamic, neocortical, and hippocampal (subicular) inputs to the amygdala converge in the lateral amygdaloid nucleus (Amaral, 1987; Herzog & van Hoesen, 1975; LeDoux, Farb, & Ruggiero, 1990; LeDoux et al., 1987; Russchen, 1982; Turner et al., 1980), which may be the afferent gateway to the emotional functions organized through the amygdala (LeDoux, 1990; LeDoux, Cicchetti, Xagoraris, & Romanski, 1990). Although hippocampal projections to the amygdala are, in general, not as robust as amygdaloid projections to the hippocampus (Amaral, 1987), we have recently examined the projection from the subiculum to the amygdala in the rat with Pha-L and found a substantial input to the lateral and basolateral nuclei (Phillips & LeDoux, 1991). There is no anatomical justification for rejecting the notion that hippocampal inputs to the amygdala are involved in contextual conditioning.

Our behavioral experiment, in which we examined the effects of variations in US intensity on conditioning to explicit and contextual stimuli, indicated that contextual conditioning is not a necessary aspect of fear conditioning. At low intensities of the US, conditioning only developed to the explicit CS. At intermediate intensities, conditioned freezing developed to both the explicit CS and the context, but contextual conditioning required a greater number of exposures to the US. As the intensity of the US increases, the organism becomes more sensitive to a wider range of stimulus factors in the environment. The hippocampus may play some role in selecting which of the many available environmental stimuli are particularly relevant to the immediate situation, a view that is consistent with attentional theories of hippocampal function (Moore & Stickney, 1980; Solomon, 1977). It remains to be determined whether the increase in stimulus selection produced by higher intensities of the US represents a kind of nonspecific supersensitivity to the environment or whether associative processes are at work.

Classical conditioning is usually thought of as involving tight temporal coupling of the CS and US. However, contextual CSs are continuously present and, therefore, do not predict the occurrences of the US. Nevertheless, they are clearly part of the stimulus ensemble that is associated with the US (Fanselow, 1986, 1990; Helmstetter & Fanselow, 1989). Winocur and Olds (1978) suggested that contextual stimuli may serve as retrieval cues. In this sense they may set the stage for the expression of conditioned responses in the presence of stimuli more explicitly related to the US. However, the results of our parametric experiments suggest that contextual cues can elicit conditioned freezing before the onset of the tone CS, especially when relatively intense shocks are used. Numerous studies have similarly shown that contextual stimuli elicit conditioned freezing responses when there is no explicit CS (D. C. Blanchard & R. J. Blanchard, 1972; Fanselow & Tighe, 1988; Helmstetter & Fanselow, 1989; Hirsh, 1974). Contextual stimuli therefore need to be considered both in terms of their association with the US and in terms of their ability to modulate the association of an explicit CS with the US. The latter, it would seem, must be dependent on the former. Although projections from the hippocampal formation to the amygdala may be involved in the association of context with the US, the ability of contextual stimuli to facilitate retrieval may depend on complex interactions between the hippocampus, amygdala, and sensory neocortical areas.

The amygdala and hippocampus have long been viewed as closely interrelated structures (Amaral, 1987; Maclean, 1949, 1952; Mishkin, 1982; Pribram, 1967). Although the exact nature of hippocampal–amygdaloid interactions are poorly understood at present, the fear conditioning procedures used in this study may offer a new approach to the problem of understanding how these two brain areas interact in the mediation of the cognitive and emotional functions of the brain.

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