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RESEARCH ARTICLE

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Amygdala, hippocampus, and unconditioned fear

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Abstract Embedded within contemporary views of emotional learning is a well-founded agreement that the amygdala plays a pivotal role in the formation and consolidation of aversive memories formed during fear conditioning. However, it is important to determine whether observed deficits are reflective of a memory impairment or whether they are simply attributable to a deficit in the performance of unconditioned fear responses such as freezing. Within the neurobiology of learning and memory literature, there is an ongoing debate concerning the potential role of the amygdala in the performance of unconditioned fear responses. A view put forth by Vazdarjanova and McGaugh (1998) suggests that the amygdala is not required for the formation and consolidation of the aversive memories formed during fear conditioning, but is essential in the performance of unconditioned fear responses. Data provided by Maren (1999) counter this view by positing that the amygdala is not required for the performance of fear responses, but its role is of a mnemonic nature in the conditioning of fear to neutral cues. To clarify the amygdala's participation in these two processes, a useful approach would involve a situation where animals with amygdala damage were examined for their unconditioned fear responses in reaction to footshock as well as the conditioning of these reactions to previously neutral cues paired with the aversive event. We have previously reported that rats with amygdala or hippocampal damage are impaired in discriminative fear conditioning to context. In the present experiment, we report the initial unconditioned fear responses to footshock by these same animals as well as the conditioned responses during testing. In both groups, the fear responses assessed (freezing, urination, defecation, and locomotion) were not impaired and did not differ from those expressed by the sham animals. The impairment of discriminative fear conditioning to context, in combination with the present experiment, represents a dissociation where damage to specific memory structures (amygdala or hippocampus) debilitates the mnemonic processes involved in fear conditioning, but not the performance of the fear responses per se.

Keywords Fear · Conditioning · Multiple measures of fear · Amygdala · Hippocampus

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Introduction

Fear plays an important part in the life of many organisms as it involves the nervous system's ability to detect danger and produce defensive responses critical for survival. In many species, a common pattern of behavioral responses includes withdrawal (avoidance or escape) from the danger, somatomotor immobility (freezing), a host of autonomic adjustments, such as changes in arterial pressure and heart rate (Blanchard and Blanchard 1972; Iwata and LeDoux 1988) as well as the release of stress hormones and hypoalgesia. A good example of an aversive event that elicits these behavioral and physiological responses is footshock. Areas of the central nervous system that control the unconditioned emergence of fear responses include regions of the brainstem involved in the mediation of cardiovascular responses (Hopkins and Holstege 1978; Holstege 1996). Areas of the hypothalamus have been implicated in the production of ultrasonic vocalizations in stressful and potentially dangerous situations (Brudzynski and Bihari 1990). The amygdala participates in the conditioning of autonomic fear responses through its projections to the hypothalamus, which in turn project to brainstem areas and spinal premotor neurons of the autonomic nervous system. The amygdala also mediates the conditioning of behavioral fear responses through its projections to the midbrain central gray (LeDoux et al. 1988).

Lesions of the amygdala eliminate or attenuate the fear elicited in response to a stimulus formerly pai-red with footshock (Blanchard and Blanchard 1972; Hitchock and Davis 1986; LeDoux et al. 1990; Davis 1992; Fanselow and Kim 1994).

The idea that the impairment reflects a change in emotionality rather than a learning deficit emerged from the observation that monkeys with lesions of the amygdala were insensitive to stimuli that normally evoked intense fear (Kluver and Bucy 1939; Weiskrantz 1956). Along the same lines, researchers examined the effects of amygdala lesions on emotional responses to conditioned and unconditioned threat stimuli (Blanchard and Blanchard 1972). The presentation of a cat served as the innate fear stimulus, and the change of the rat's reaction to the threat stimulus, i.e., approach and contact, served to illustrate that amygdala lesions resulted in the alteration of species-typical defensive reactions (Blanchard and Blanchard 1972).

The use of different fear responses used to index innate fear have produced results that are inconsistent with the notion that amygdala lesions result in a change of reaction to threatening stimuli. Large amygdala lesions have been found to reduce open field activity, a behavior that is indicative of reduced fear (Grossman et al. 1975). However, handling (Kemble et al. 1979) as well as the age of the organism when the lesion was performed can eliminate this effect (Eclancher and Karli 1979). Similarly, latency to begin eating in a novel environment, a measure of neophobia, does not change consistently with amygdala lesions (cf. Aggleton et al. 1989). Defecation, a fear response that occurs in reaction to a fearful event such as a footshock has been shown to condition to a fearful environment (Vanderwolf et al. 1988; Sutherland and McDonald 1990; Avanzi et al. 1998; Antoniadis and McDonald 1999). Unconditioned defecation to footshock has been previously examined in control rats as well as in rats with amygdala and hippocampus lesions (Sutherland and McDonald 1990). Interestingly, all three groups showed a similar increase in defecation over baseline, indicating that amygdala lesions do not necessarily alter reactions to aversive stimuli, at least when the fear response assessed is defecation.

In addition, the participation of the hippocampus in unconditioned fear has also been examined and reports have also been discrepant. In one study, hippocampal lesions produced a decrement in defensive immobility reactions, a marker of altered emotionality, while the avoidance response to a conditioned stimulus remained intact (Blanchard et al. 1970). That unconditioned defecation was not affected by hippocampal lesions in the aforementioned experiment seems to suggest that the hippocampus is not involved in defensive reactions (Sutherland and McDonald 1990).

Taken together, these contrasting findings are not clear about the participation of the amygdala and hippocampus in fear reactions. These inconsistencies encouraged us to examine the role that the amygdala and the hippocampus play in unconditioned fear, with the simultaneous assessment of multiple measures of fear including freezing, urination, defecation, and locomotion. These measures have been shown to condition to a fearful context (Antoniadis and McDonald 1999) and may help to clarify the participation of the hippocampus and the amygdala in unconditioned fear. The results presented in this paper are from the training phase of a fear conditioning to context experiment, and some of the testing data has appeared in a recently published paper by Antoniadis and McDonald (2000). Therefore, this provides an assessment of fear responses in the same animals within the three groups (amygdala or hippocampus lesions and shams) at the training phase (unconditioned responses) and the testing phase (conditioned responses).

Materials and methods

Subjects

Twenty-four male Long-Evans rats were used. The animals were housed individually in single Plexiglas cages (24 cm long × 22 cm wide × 20 cm high) and were maintained on a 12:12-h light-dark cycle. The rats weighed approximately 300–325 g at their arrival and were given free access to food and water. The principles of laboratory animal care (NIH Publication no. 86–23, revised 1985) were upheld.

Apparatus

A white square prism (41 cm long × 41 cm wide × 29 cm high) and a black triangle prism (61 cm long × 61 cm wide × 30 cm high) served as the shock-chambers. Isoamyl acetate served as the olfactory cue in the black triangle prism and eucalyptus served as the olfactory cue in the white square. A camera placed 2 feet in front of the mirror allowed the experimenter to video tape ongoing behavior. The training phase was conducted in two different rooms within two different laboratories. Animals experienced the shock chamber in the "shock room" and the safe chamber in the "no-shock room". The entire apparatus including the chambers, the shock generator, the video camera and the mirror were transported back and forth on a trolley. For the testing phase, the shock chamber was referred to as the "paired context", and the safe chamber was referred to as the "unpaired context". In order to assess conditioning to the chamber and not any fear acquired by the room or any part of the procedure, all testing took place in the "no-shock room" for the testing phase of the experiment. As such, greater fear in the paired context during testing expressed by one or many of the measures assessed can only be attributed to the aversive properties acquired by the paired context.

Surgery

All rats undergoing surgery were first injected with 0.2 ml of atropine to facilitate respiration and were subsequently anaesthetized with sodium pentobarbital (65 mg/kg i.p.). The rats were randomly assigned to one of the three treatment groups: amygdala damage, hippocampus damage, and sham lesion. Eight animals were assigned to each group. Bilateral neurotoxic lesions were made using the Paxinos and Watson atlas to locate all coordinates. (Paxinos and Watson 1982) Lesions were stereotaxically placed and the coordinates were measured in relation to bregma and the skull surface. Neurotoxic lesions of the hippocampus were made with injections of NMDA infused through 30-gauge stainless-steel cannulae over 3 min. The injection coordinates were: 3.1 mm posterior, 1 mm lateral, and 3.6 mm ventral; 3.1 mm posterior, 2.0 mm lateral, and 3.6 mm ventral; 4.1 mm posterior, 2.0 mm lateral, and 4.0 mm ventral; 4.1 mm posterior, 3.5 mm lateral, and 4.0 mm ventral; 5.0 mm posterior, 3.0 mm lateral, and 4.1 mm ventral; 5.0 mm posterior, 5.2 mm lateral, and 5.0 mm ventral; 5.0 mm posterior, 5.2 mm lateral, and 7.3 mm ventral; 5.8 mm posterior, 4.4 mm lateral, and 4.4 mm ventral; 5.8 mm posterior, 5.1 mm lateral, and 6.2 mm ventral; 5.8 mm posterior, 5.1 mm lat-

eral, and 7.5 mm ventral. The total volume injected in the hippocampus was 2.10 µl. The amygdala was also damaged by NMDA infusion. The coordinates were: 2.3 mm posterior, 4.8 mm lateral, and 9.4 mm ventral; 3.3 mm posterior, 4.6 mm lateral, and 9.4 mm ventral. The total volume injected in the amygdala was 1.6 µl. Prior to NMDA injections all animals received 0.1 ml valium to prevent the occurrence of any seizures. Upon completion, the incision was closed using stainless-steel wound clips. Hibotane was applied to the incision site. Following the lesion, animals were allowed to recover for 7 days before the transmitter implantation operation.

Histology

Subsequent to the completion of behavioral testing, all animals were anesthetized with somnotol and perfused cardially with 0.9% saline and 4% paraformaldehyde. Brains were removed and stored in 20% sucrose, 4% paraformaldehyde overnight and cut at –17° C with a cryostat. Coronal sections (40 µm) were cut from tissue of the hippocampus and amygdala lesioned groups. These sections were mounted on gelatin-coated slides and stained with cresyl violet. Lesions were then examined under a light microscope and reconstructed on rat brain atlas sections from (Paxinos and Watson 1982).

Freezing

Freezing was defined as a total absence of body or head movement, except that associated with breathing. Video tapes were scored by the experimenter at the end of the experiment, and this measure of fear was quantified in amount of time spent freezing in seconds.

Urination

The experimenter counted the number of emissions by each animal and confirmed the initial assessment by scoring the video tapes.

Locomotion

Equi-distant lines (2 inches) perpendicular to the chamber grids were drawn on the Plexiglas table and were used to quantify locomotor behavior. The number of times that the animal crossed any of the lines served as an index of the amount of locomotion. This measure was also assessed by scoring the video tapes.

Defecation

The experimenter counted the number of feces produced by each animal and confirmed initial assessment by scoring the video tapes.

Procedure

Handling

Animals were handled individually by the experimenter for 3 min within each room (the shock room and the no-shock room) for 4 consecutive days. This was done in an attempt to render the room and the experimenter neutral. The order in which the animals experienced the rooms was counterbalanced.

Pre-exposure

This phase consisted of pre-exposure to the entire apparatus for 2 consecutive days (10 min/day). This pre-exposure occurred in the "no-shock room" for both days. Animals were placed in the middle alley and were given free access to the experimental apparatus for a total period of 10 min each day.

Training

During the training phase of the experiment, each daily trial lasted 5 min. On day one of each training session, half of the animals within each group were confined in their assigned shock chamber, i.e., the to-be "paired context", and received one set of three footshocks (1 mA) at the 2-, 3-, and 4-min mark of the training session. For all the measures assessed, comparisons were established before the beginning of the shock treatment, i.e., from the 0- to 2-min mark, and after the beginning of the shock administration, i.e., from the 3- to 5-min mark. The other half of the rats within each group were confined in their assigned safe chamber, i.e., the to-be "unpaired context", in the "no-shock room". The chamber that served as the shock-chamber was counterbalanced so that half the animals experienced the aversive event in the black triangle and the other half experienced the aversive event in the white square. All animals experienced the shock chamber in the shock room. The order in which the animals experienced each context was also counterbalanced so that half the animals were confined in their shock chamber on day one for 5 min and confined in the safe chamber on day two for 5 min. Animals always experienced the shock chamber in the "shock room" and the safe chamber in the "no-shock room". We only report the first conditioning session because it seems to be the only instance that is reflective of unconditioned fear responding.

Testing

As an attempt to capture the amount of fear conditioned to the chamber as opposed to any fear that may have generalized to the room, all testing took place in the "no-shock room". Testing occurred on two consecutive days. Half of the animals were individually confined in the paired context on day one of the session, and the other half experienced the paired context on day two of the session. Each test lasted for 20 min. The conditioning and testing sequence was repeated three times.

Results

Histology

Figure 1 shows reconstructions of the smallest (darkened area) and the largest (striped area) lesions of the amygdala. As can be seen, large portions of both the anterior and posterior amygdala were damaged. Neurotoxic lesions of the amygdala produced extensive cell loss and gliosis in the central, basolateral, and lateral nuclei of the amygdala. One of the rats in the amygdala-lesion group also sustained damage to the piriform cortex unilaterally. Based on the lesion sites, three rats were removed from the group. One rat sustained unilateral damage to the dorsal amygdala that extended to the caudate putamen. The second rat sustained posterior hippocampal damage unilaterally, and the third rat had unilateral damage to the amygdala.

Figure 2 shows reconstructions of the smallest (darkened area) and the largest (striped area) lesions of the hippocampus. The animals included in this group had large lesions of both dorsal and ventral portions of the hippocampus. Neurotoxic lesions of the hippocampus produced substantial cell loss and gliosis in the hippo-

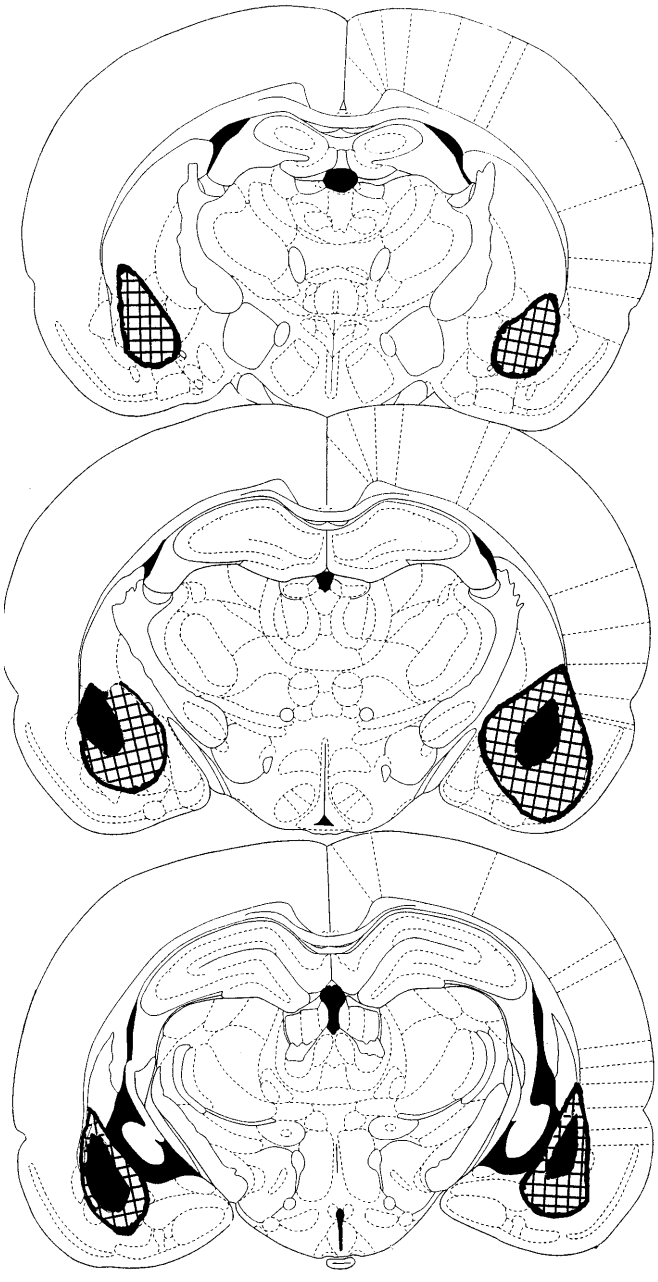

Fig. 1 Drawings of neurotoxic amygdala lesions. The *darkened area* represents the minimum extent of all lesions for all rats and the *striped area* represents the maximum extent of all lesions for all rats

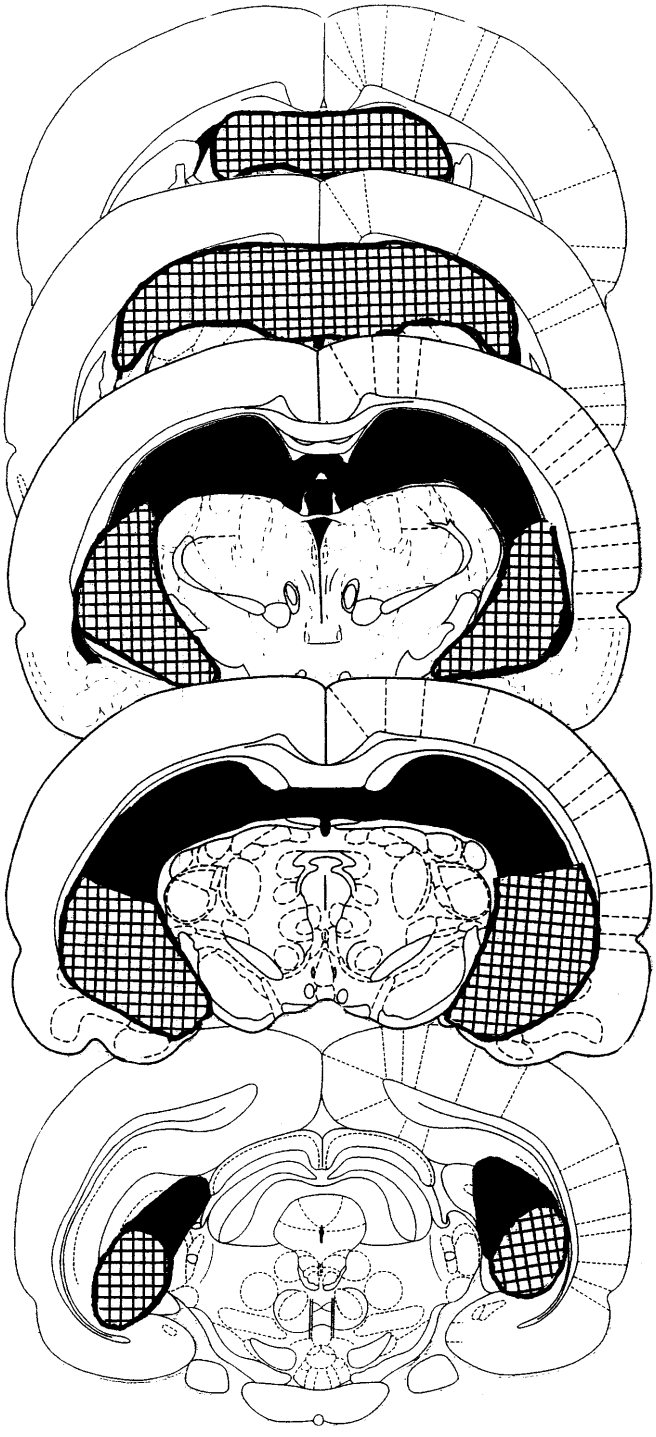

Fig. 2 Drawings of neurotoxic lesions of the hippocampus. The *striped areas* represent the maximum extent of all lesions, and the *darkened areas* represent the minimum extent of all lesions

campus. All of the lesions in the hippocampal group were virtually identical in extent of damage, except for one animal that had some bilateral sparing of the ventral portions of the hippocampus.

Training phase

Freezing

Figure 3 (top left) shows the mean amount of time that the rats in the three different groups spent freezing in their respective shock chamber before (2 min) and after (3 min) the footshock. The graph shows that the three groups spent more time freezing subsequent to the shock administration. A 3 (group) × 2 (time: pre-shock versus post-shock) ANOVA revealed a non-significant group effect [$F(2,21)=0.13$, $P>0.05$], a significant time effect [$F(1,21)=235.46$, $P<0.05$], and a non-significant group ×

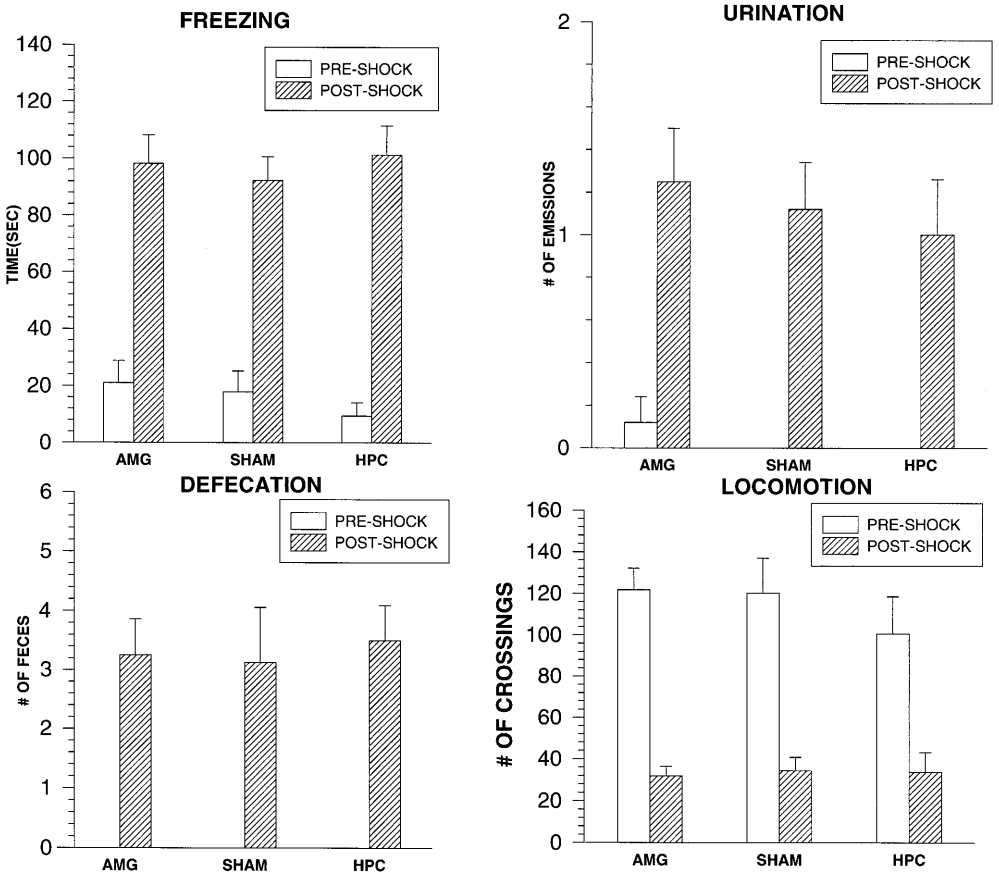

Fig. 3 *Top left* Mean amount of time spent freezing (in seconds) before (pre-shock) and after (post-shock) shock administration for the animals with amygdala (*AMG*) or hippocampus (*HPC*) damage and the sham group (*SHAM*). *Top right* Mean number of emissions of urine before (pre-shock) and after (post-shock) shock administration for the animals with amygdala or hippocampus damage and the sham group. *Bottom left* Mean amount of fecal matter (number of feces) emitted before (pre-shock) and after (post-shock) shock administration for the animals with amygdala or hippocampus damage and the sham group. *Bottom right* Mean number of line crossings before (pre-shock) and after (post-shock) shock administration for the animals with amygdala or hippocampus damage and the sham group

time interaction [$F(2,21)=1.04$, $P>0.05$]. Planned comparisons indicated a significant difference in the amount of time spent freezing before and after the shock in all three groups ($P<0.05$)

Urination

Figure 3 (top right) shows the mean amount of emissions for the three groups of rats in the shock chamber. The graph illustrates that more emissions were produced subsequent to the shock administration for all groups. A 3 (group) × 2 (time) ANOVA indicated a non-significant group effect [$F(2,21)=0.70$, $P>0.05$], a significant time effect [$F(1,21)=43.01$, $P<0.05$], and a non-significant interaction [$F(2,21)=0.06$, $P>0.05$]. Planned comparisons on each group revealed a significant difference in emissions between the two time points for the all groups ($P<0.05$).

Defecation

Figure 3 shows the mean amount of feces produced in the shock chamber and illustrates that all three groups produced more fecal matter subsequent to the shock administration. A 3×2 ANOVA revealed a non-significant group effect [$F(2,21)=.06$, $P>0.05$], a significant time effect [$F(1,21)=60.42$, $P<0.05$], and a non-significant interaction [$F(2,21)=.14$, $P>0.05$]. Planned comparisons revealed that the difference in defecation between the two time points was significant for all groups ($P<0.05$). These results suggest that unconditioned defecation is a measure of fear that is not affected by either lesion examined.

Locomotion

Figure 3 (bottom right) shows the mean level of locomotion expressed in number of crossings. A 3×2 ANOVA revealed a non-significant group effect [$F(2,21)=0.38$, $P<0.05$], a significant time effect, [$F(1,21)=76.72$, $P<0.05$], and a non-significant interaction, [$F(2,21)=0.60$, $P>0.05$]. Planned comparisons revealed that the lack of locomotion after shock onset, relative to the high level of locomotion before shock administration, reflects the level of fear that was unaffected by either lesion examined.

Testing phase

Freezing

Figure 4A shows the mean amount of time the rats in the three different groups spent freezing in the paired

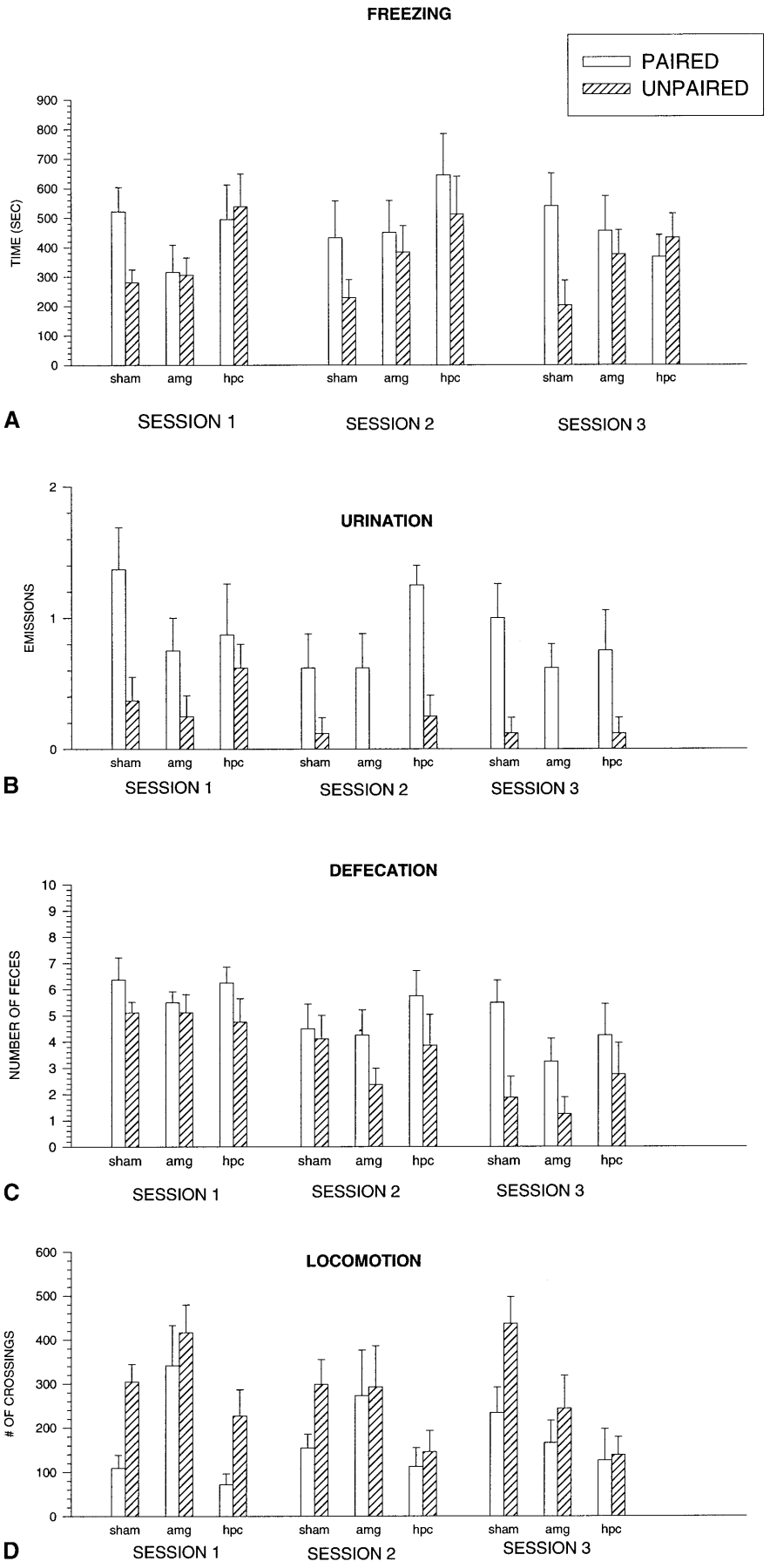

Fig. 4 **A** Mean amount of time (in seconds) spent freezing in the paired and unpaired chambers during test sessions 1, 2, and 3 by rats with amygdala (*amg*) or hippocampus (*hpc*) lesions and the sham animals (*sham*). **B** Mean number of emissions of urine in the paired and unpaired context during test session 1, 2, and 3 by rats with amygdala or hippocampus lesions and the sham animals. **C** Mean amount of fecal matter (number of feces) emitted in the paired and unpaired context during test session 1, 2, and 3 by rats with amygdala or hippocampus lesions and the sham animals. **D** Mean level of locomotion (number of crossings) in the paired and unpaired context during test sessions 1, 2, and 3 for rats with amygdala or hippocampus lesions and the sham animals

and unpaired contexts during the three testing sessions. The graph shows that the sham animals spent more time freezing in the paired than in the unpaired context at all three testing sessions [$F(1,21)=5.93$, $P<0.05$; $F(1,21)=5.93$, $P<0.05$; $F(1,21)=41.45$, $P<0.05$; respectively]. The amygdala- and hippocampus-lesioned animals showed no notable discrimination in their freezing behavior at either session ($P>0.05$).

Urination

Figure 4B shows the mean level of urination for the three groups across the three testing sessions. For the sham group, urination was consistently greater in the paired context at test session 1 [$F(1,21)=6.58$, $P<0.05$], session 2 [$F(1,21)=6.58$, $P<0.05$], and session 3 [$F(1,21)=8.40$, $P<0.05$]. For the amygdala-lesioned group, the level of urination only became higher in the paired context at session 3 [$F(1,21)=5.83$, $P<0.05$], while the hippocampus lesioned group demonstrated this discrimination at test session 2 [$F(1,21)=7.03$, $P<0.05$] and test session 3 [$F(1,21)=5.83$, $P<0.05$]. These results suggest that conditioned urination is not impaired following hippocampal or amygdala lesions and suggests that another memory system may participate in the conditioning of this fear response.

Defecation

Figure 4C shows the mean amount of feces emitted in each chamber by the three groups throughout the three testing sessions. For the sham group, the level of defecation was similar in the paired and unpaired context at test session 1 [$F(1,21)=1.55$, $P>0.05$] and test session 2 [$F(1,21)=0.16$, $P>0.05$], but became considerably higher in the paired context at test session 3 [$F(1,21)=16.24$, $P<0.05$]. The level of defecation for the amygdala lesioned group was similar between the two chambers in test session 1 and 2, but became notably higher in the paired chamber at test session 3 [$F(1,21)=4.94$, $P<0.05$]. The hippocampus lesioned group demonstrated no such discrimination, as level of defecation was similar between the two contexts across all three testing sessions ($P>0.05$). These results suggest that conditioned defecation is a measure of fear that was impaired in animals with hippocampus lesions, but remained intact in sham and amygdala lesioned animals.

Locomotion

Figure 4D shows the level of locomotion in both chambers for the three groups. As can be seen, there was a higher level of locomotion in the unpaired than in the paired chamber in the sham group across test session 1 [$F(1,21)=9.50$, $P<0.05$], session 2 [$F(1,21)=19.20$, $P<0.05$], and test session 3 [$F(1,21)=21.21$, $P<0.05$]. For the amygdala-lesioned group, the level of locomotion between the two contexts did not differ significantly across all test sessions. The hippocampus lesioned group showed a higher level of locomotion in the paired chamber at test session 1 [$F(1,21)=6.01$, $P<0.05$], but this extinguished at session 2 [$F(1,21)=0.86$, $P>0.05$] and test session 3 [$F(1,21)=.079$, $P>0.05$].

Discussion

The assessment of multiple measures of fear resulted in contrasting findings to prominent views of the amygdala and the hippocampus in unconditioned fear. Lesions to the amygdala or hippocampus did not impair the expression of unconditioned fear responses assessed in the present study. Footshock elicited an increase in freezing, defecation, and urination in all groups. These are responses that emerge in reaction to an aversive and stressful event, and the present results suggest that an intact amygdala or hippocampus is not required for the experience of unconditioned fear (Antoniadis and McDonald 2000).

Locomotion reflects the level of total body motion and is an additional measure of fear. Results indicate that the level of locomotion was higher before shock onset than after shock onset and did not differ between the groups, reflecting a similar level of fear. This experiment revealed that the observational period designed to assess the immediate fear reaction to an aversive event, i.e., immediately following the onset of footshock, seems to be an appropriate one for assessing the participation of different neural structures in the mediation of unconditioned fear responses. Findings from the present experiment indicate that the amygdala is not critical for the initial experience of fear. However, during the testing phase, both amygdala- and hippocampus-lesioned animals were impaired in the discriminative conditioning of freezing and locomotion. Conditioned defecation was not impaired in the amygdala-lesioned group, but impaired in the hippocampus-lesioned group. Evidence suggests that the hippocampus mediates conditioned defecation to a fearful context (Vanderwolf et al. 1988; Sutherland and McDonald 1990). Indeed, amygdala lesions do not interfere with the acquisition of conditioned defecation (Vanderwolf et al. 1988; Sutherland and McDonald 1990), but hippocampus lesions do interfere with the conditioning of this fear response (Sutherland and McDonald 1990). Conditioned urination was not impaired in either the hippocampus- or amygdala-lesioned groups, results that have been taken to suggest that another memory system may participate in the conditioning of this fear response (Antoniadis and McDonald 2000). The selective impairment of conditioned defecation in the hippocampus-lesioned group reflects one of the specific contributions of this structure in discriminative fear conditioning to context. The selective impairment of conditioned heart rate in the amygdala-lesioned group reflects the specific contribution of the amygdala in dis-

criminative fear conditioning to context. The common impairment in conditioned freezing and locomotion reflects the synergistic contribution of both memory structures in this form of emotional learning. None of the fear responses assessed in the training phase were impaired in either group. The comparison between the training and testing phase is interesting in regard to the possible roles of the amygdala and hippocampus in fear responding and fear conditioning to context. Animals with amygdala damage showed a similar level of fear to that of the sham animals during the training phase of the experiment. However, in the testing phase of the experiment, the group with amygdala damage was impaired in the conditioning of some fear responses. The same outcome occurred in the group with hippocampus lesions. This dissociation provides information for understanding the contribution of each memory structure in fear processes. Indeed, this dissociation suggests that the memory structures examined participate in a mnemonic process that encompasses the formation of an association between a contextual representation and an aversive event, as well as the conditioning of different fear responses. Importantly, given that for both lesioned groups the level of fear was similar to that of the sham animals, the data also suggest that these memory structures are not critical for the mediation of unconditioned fear responding to the footshock.

Current theories: the role of the amygdala in fear conditioning

The amygdala: an unconditioned fear system

One view of the role of the amygdala in fear conditioning suggests that the amygdala is selectively involved in the mediation of unconditioned fear responses. Amygdala lesions have been associated with a general taming effect in different species (Goddard 1964) as well as a reduction in species-specific defensive reactions (Blanchard and Blanchard 1972). Electrical stimulation of the amygdala produces a constellation of behavioral and physiological measures of fear, by virtue of its connections to target sites in the hypothalamus, brain stem, and midbrain central gray, areas that are important in the emergence of reactions to noxious stimuli. Blanchard and Blanchard's (1972) findings support this view. They examined rats' defensive reactions in response to a cat. In their experiment 1, their task involved exposure to a sedated cat, and rats (both controls and rats with amygdala lesions) came into contact with the cat (more than once) during a period of 5 min. The observed lack of fear in the rats with amygdala lesions may have been due to the possibility of repeated contact with a sedated, nonthreatening cat during a prolonged period (5 min), which may have resulted in the extinction of unconditioned fear evoked by the cat. In their experiment 3, the rat was placed in a runway with a cat that was immediately removed from the runway as soon as it came in mild contact with the rat (touching rat gently with the nose). The rat was given five such cat-approach trials, each separated by 60 s, which may have again been sufficient for any fear evoked by the presently non-threatening cat to have extinguished.

In contrast, our procedure involved the assessment of the immediate fear responses to three successive footshocks (2 s each) for a total period of 3 min. The level of fear during that period was in turn compared with the level of fear prior to the shock onset. The significant increase in the responses expressed in all three groups suggests that the brainstem and midbrain sites that are involved in the emergence of behavioral and autonomic fear symptoms can support these responses to an aversive event in the absence of an intact amygdala or hippocampus (see Canteras et al. 1997).

Post-shock freezing has a delayed onset and is long in duration (minutes), in contrast to the view of a typical unconditioned response that is immediate and short-lived (Fanselow 1980). These probabilistic and temporal properties of freezing have been taken to indicate, according to a view put forth by Fanselow, that post-shock freezing is not an unconditioned response to the footshock, but rather a conditioned response to the contextual cues that surrounded the occurrence of the aversive event (Fanselow 1980). Although we agree with this view in principle, based on the data reported here it seems that there is a limited temporal window for detecting unconditioned fear responses.

Freezing in response to footshock was assessed in a recent active avoidance experiment, and although for both amygdala lesioned and sham animals there was an increase in time spent freezing with increasing number of footshocks, overall level of freezing was significantly greater in the sham group. This result is taken to illustrate that amygdala lesions impair the expression of unconditioned fear (Vazdarjanova and McGaugh 1998). Results from the present study do not replicate this finding, given that the level of fear in the amygdala-lesioned group was comparable to that of the sham and hippocampus-lesioned group in all measures of fear assessed. Amygdala lesions have been reported to block stress-induced freezing, defecation, and ultrasonic vocalizations (Goldstein et al. 1996). The data that served to depict the amygdalar blockade of stress-induced responses is derived from observations during the testing day when animals were presented only with tones (i.e., without footshocks). Responses from this session most probably reflect the conditioning of fear to the tone and the surrounding context and cannot be used as an index of unconditioned fear responses to footshock. As assessed in the present study, the responses immediately following shock onset seem to reflect unconditioned fear.

The amygdala: a learning and memory system

The other view posits that the amygdala is a learning and memory system involved in the formation and consolida-

tion of fearful memories that occur during fear conditioning. The amygdala is involved in a conditioning process, and amygdala lesions should impede the expression of conditioned associative freezing in the presence of the conditioned cues (Sutherland and McDonald 1990; Selden et al. 1991; Helmstetter 1992; Kim and Fanselow 1992; Phillips and LeDoux 1992; Kapp et al. 1982, 1990). In the present experiment, animals with amygdala damage expressed a similar level of post-shock freezing to that of hippocampus-lesioned and sham animals. However, they were subsequently impaired in discriminative fear conditioning to context used to assess the amount of fear conditioned to the chamber in which the shock occurred (paired context) in comparison to a chamber in which shock was never administered (unpaired context) (Antoniadis and McDonald 2000). These results suggest that post-shock freezing and the other fear responses assessed are mediated by a process other than that of discriminative fear conditioning to contextual cues, but are rather an unconditioned reaction in response to shock. Similarly, the rats with hippocampal lesions were impaired on the discriminative conditioning of fear, as expressed by the amount of freezing in the paired versus the unpaired context (Antoniadis and McDonald 2000). However, their immediate reaction to shock expressed in freezing and the other fear responses assessed in the present experiment were not affected. The learning/performance issue is of great relevance in the present context with respect to the amygdala's participation in the expression of fear, be it conditioned or unconditioned. There is no evidence to date that lesions of the amygdala impair conditioned fear (freezing), while leaving unconditioned fear (freezing) intact (see Cahill et al. 1999). This evidence is required to delineate the participation of the amygdala in the conditioning of fear and exclude the possibility that the suspected memory impairment is simply due to the impaired performance of unconditioned fear responses. In the present experiment, animals from the two lesioned groups showed no impairment in their expression of unconditioned fear responses. When subsequently tested for fear conditioning to the paired context, the same group of animals with amygdala lesions were selectively impaired in the discriminative conditioning of heart rate, while animals with hippocampal lesions were selectively impaired in the conditioning of body temperature and defecation (Antoniadis and McDonald 2000). Both groups were impaired at conditioned freezing, locomotion ultrasonic vocalizations, and preference. In combination, these two experiments represent a dissociation, whereby damage to specific memory structures (amygdala or hippocampus) impaired fear conditioning as expressed by multiple measures of fear. Damage to the same structures left the unconditioned expression of fear intact. This dissociation has been expressed differently in a study by LeDoux et al. (1990), whereby a group of amygdala-lesioned animals that received random CS-US presentations showed a similar level of freezing to that seen in a nonassociative sham group. Freezing to the CS was, thus, not reduced in the amygdala-lesioned group; if anything, amygdala-lesioned rats showed a higher level of freezing. In the same study, amygdala-lesioned rats that received the paired treatment were impaired at fear conditioning to the tone. These results seem to illustrate that lesions of the amygdala impair the conditioning of fear responses, but not the expression of fear to the US. (LeDoux et al. 1990). In support of the idea that amygdala lesions do not impair the performance of fear responses, Maren (1999) recently reported that animals with amygdala damage showed initial neophobia to a novel chamber, suggesting that in these rats unconditioned freezing evoked by a novel environment, albeit low, is intact.

Multiple measures of fear

In previous experiments that involved testing the fear elicited by a context previously paired with footshock in comparison to a context previously paired with safety, we assessed multiple measures of fear, including freezing, heart rate, locomotion, body temperature, defecation, urination, ultrasonic vocalizations, and preference (Antoniadis and McDonald 1999, 2000). Results from both experiments showed that heart rate and body temperature are fear responses that require expanded testing windows (20 min) for a difference to be detected between a fearful and a non-fearful environment. Indeed, it is only in the last 10 min of a 20 min testing session that a discriminatively higher level of heart rate emerges in the paired than in the unpaired context. Similarly, it is only after two testing sessions of 20 min each that body temperature is discriminatively lower in the paired than in the unpaired context during the third session (Antoniadis andMcDonald 2000). Accordingly, in the present experiment, we did not assess these two physiological measures, given that our observation period was only 5 min long. Both fear conditioning studies mentioned above involved multiple training and testing sessions. The emission of ultrasonic vocalizations was characterized as a "slow" measure of conditioned fear, given that it required three conditioning sessions before a discriminatively higher level of vocalizations was produced in the chamber previously paired with footshock (Antoniadis and McDonald 1999). Interestingly, although some animals (all animals are controls) vocalized at the first, second, and third shock session, others only started vocalizing at the second shock session. Since, in the present experiment, we only assessed fear responses during the first shock session, we do not report that measure of fear because at that point in time it is not reflective of unconditioned vocalizations for all animals. It seems that the threshold to begin vocalizing is different between animals. Indeed, individual differences have been observed in treatments that evoke vocalizations in animals, such as footshock (Tonoue et al. 1986; Cuomo et al. 1988). Importantly, once the animals start vocalizing in response to footshock, they emit the same re-

sponse when placed in the chamber where the shock administration occurred and do so to a greater extent in that context than in a context where shock was never experienced (Antoniadis and McDonald 1999).

All in all, the results from the present study suggest that the post-shock responses of fear, assessed within a limited temporal window (5 min), are unconditioned reactions of fear that are not affected in rats with amygdala or hippocampus damage.

References

Aggleton JP, Blindt HS, Rawlins JNP (1989) Effect of amygdaloid and amygdaloid-hippocampal lesions on object recognition and spatial working memory in rats. Behav Neurosci 103: 962–974

Antoniadis EA, McDonald RJ (1999) Discriminative fear conditioning to context expressed by multiple measures of fear in the rat. Behav Brain Res 101:1–13

Antoniadis EA, McDonald RJ (2000) Amygdala, hippocampus and discriminative fear conditioning to context. Behav Brain Res 108:1–19

Avanzi V, Castilho VM, Andrade TGCS de, Brandao ML (1998) Regulation of contextual conditioning by the median raphe nucleus. Brain Res 70:174–184

Blanchard RJ, Blanchard DC (1972) Innate and conditioned reactions to threat in rats with amygdaloid lesions. J Comp Physiol Psychol 372:281–290

Blanchard RJ, Blanchard DC, Fial RA (1970) Hippocampal lesions in rats and their effect on activity avoidance and aggression. J Comp Physiol Psychol 71:92–102

Brudzynski SM, Bihari F (1990) Ultrasonic vocalization in rats produced by cholinergic stimulation in the brain. Neurosci Lett 209:222–226

Cahill L, Weinberger NM, Roozendaal B, McGaugh JL (1999) Is the amygdala a locus of "conditioned fear"? Some questions and caveats. Neuron 23:227–228

Canteras NS, Chiavegatto S, Ribeiro Do Valle LE, Swanson LW (1997) Severe reduction of rat defensive behavior to a predator by discrete hypothalamic chemical lesions. Brain Research Bulletin 44:297–305

Cuomo V, Cagiano R, DeSalvia MA, Restani P, Galimberti R, Colonna S, Racagni G, Galli CL (1988) Ultrasonic vocalization in rat pups as a marker of behavioral development: an investigation of the effects of drugs influencing brain opioid system. Neurotoxicol Teratol 10:465–469

Davis M (1992) The role of the amygdala in conditioned fear. In: Aggleton JP (ed) The amygdala: neurobiological aspects of emotion, memory, and mental dysfunction. Wiley, New York, pp 255–305

Eclancher F, Karli P (1979) Effects of early amygdaloid lesions in the development of reactivity in the rat. Physiol Behav 22: 1123–1134

Fanselow MS (1980) Conditional and unconditional components of post-shock freezing. Pavlovian J Biol Sci 15:177–182

Fanselow MS, Kim JJ (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. Behav Neurosci 108:210–212

Goddard GV (1964) Functions of the amygdala. Psychol Bull 62:89–109

Goldstein LE, Rasmusson AM, Bunney BS, Roth RH (1996) Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. J Neurosci 16:4787–4798

Grossman SP, Grossman L, Walsh L (1975) Functional organization of the rat amygdala with respect to avoidance behavior. J Comp Physiol Psychol 88:829–850

Helmstetter FJ (1992) Contribution of the amygdala to learning and performance of conditioned fear. Physiol Behav 51:495–504

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. Behav Neurosci 100:11–22

Holstege G (1996) Descending motor pathways and the spinal motor system: limbic and non-limbic components. Prog Brain Res 107:307–421

Hopkins DA, Holstege G (1978) Amygdaloid projections to the mesencephalon, pons and medulla oblongata in the cat. Exp Brain Res 32:529–547

Iwata J, LeDoux JE (1988) Dissociation of associative and nonassociative concommitants of classical fear conditioning in the freely behaving rat. Behav Neurosci 102:66–76

Kapp BS, Gallagher M, Underwood MD, McNAll CL, Whitehorn D (1982) Cardiovascular responses elicited by electrical stimulation of the amygdala central nucleus in the rabbit. Brain Res 234:251–262

Kapp BS, Wilson A, Pascoe JP, Supple W, Whalen PJ (1990) A neuroanatomical system analysis of conditioned bradycardia in the rabbit. In: Gabriel M, Moore J (eds) Neurocomputation and learning: foundations of adaptive networks. Bradford Books, MIT Press, pp 53–90

Kemble ED, Studelska DR, Schmidt MK (1979) Effects of central amygdaloid nucleus on ingestion taste reactivity exploration and taste aversion. Physiol Behav 22:789–793

Kim JJ, Fanselow MS (1992) Modality-specific retrograde amnesia of fear. Science 256:675–677

Kluver H, Bucy PC (1939) Preliminary analysis of functions of the temporal lobes in monkeys. Arch Neurol Psychiatry 42:979–1000

LeDoux JE, Iwata J, Cicchetti P, Reis DJ (1988) Differential projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. J Neurosci 8:2517–2529

LeDoux JE, Cicchetti P, Xagoraris A, Romanski LM (1990) The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. J Neurosci 10:1062–1069

Maren S (1999) Neurotoxic basolateral amygdala lesions impair learning and memory but not performance of conditional fear in rats. J Neurosci 19:8696–8703

Paxinos G, Watson C (1982) The rat brain in stereotaxic coordinates. Academic Press, Sydney

Phillips RG, Ledoux JE (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106:274–285

Selden NRW, Everitt BJ, Jarrard LE, Robbins TW (1991) Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. Neuroscience 42:2335–2350

Sutherland RJ, McDonald RJ (1990) Hippocampus, amygdala, and memory deficits. Behav Brain Res 34:57–79

Tonoue T, Ashida Y, Makino H, Hata H (1986) Inhibition of shock-elicited ultrasonic vocalization by opioid peptides in the rat: a psychotropic effect. Psychoneuroendocrinology 11:177–184

Vanderwolf CH, Kelly ME, Kraemer P, Streather A (1988) Are emotion and motivation localized in the limbic system and nucleus accumbens? Behav Brain Res 27:45–58

Vazdarjanova A, McGaugh JL (1998) Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. Proc Natl Acad Sci USA 95:15003–15007

Weiskrantz L (1956) Behavioral changes associated with ablation of the amygdaloid nucleus in monkeys. J Comp Physiol Psychol 49:381–391