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) debilitating 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

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 paired with footshock (Blanchard and Blanchard 1972; Hitchcock and Davis 1986; LeDoux et al. 1990; Davis 1992; Fanselow and Kim 1994).

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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 coordinates. (Paxinos and Watson 1982) Lesions were stereotactically 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-