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HIPPOCAMPAL AND AMYGDALAR INVOLVEMENT IN DISCRIMINATORY PLACE LEARNING

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Abstract—A conflict task was developed that simultaneously examines place aversion learning and fear-motivated context discrimination. The task superimposed Pavlovian discriminative fear conditioning on an appetitively motivated instrumental response (alternation). Rats were trained to alternate along a high-walled, diamond-shaped runway between two chambers for food. On half of the trials, a tone CS signaled the fact that a fixed section at the apex of the runway was electrified. Both the tone and the shock were turned on at the beginning of, and remained on for the duration of, each tone trial. A new trial was initiated at the time the animal entered the subsequent food chamber. Therefore, during a tone trial, in order to attain additional food reinforcement, the animal had to cross over the electrified region at the runway apex. Behavioral performance of rats with small lesions of the amygdala or dorsal hippocampus (DH) was compared with that of sham-operated controls. All groups displayed significant discriminative responding, hesitating more on tone trials while in areas of the runway adjacent to the shock region. Animals with lesions of the DH were similar to controls with respect to the tone-mediated discrimination, yet were delayed in the initial expression of a location-specific fear response. Conversely, amygdala lesions did not affect place learning; however, these animals were impaired in their suppression of the fear response following repeated unpaired trials.

Key words: fear conditioning, context discrimination, extinction, dorsal hippocampus, amygdala, spatial navigation.

The role of the medial temporal lobe (mTL) in memory and emotion has been a subject of empirical research for almost a century (Aggleton, 1992, 2000; Amaral and Witter, 1995; Klüver and Bucy, 1937; MacLean, 1952; Papez, 1937; Scoville and Milner, 1957; Squire, 1992; Weiskrantz, 1956). Contextual fear conditioning has been used extensively to elucidate the mnemonic functions of structures within the mTL (see Maren, 2001b). Associative memory for a previously neutral conditioned stimulus (CS) paired with an aversive unconditioned stimulus (US) is dependent upon the amygdala, while neuronal plasticity within the amygdala develops in parallel with behavioral expression of conditioned fear (Cheng et al., 2003; Collins and Pare, 2000; Davis, 2000; LaBar et al., 1998; Quirk et al., 1995; Rogan et al., 1997; Tsvetkov et al., 2002). Furthermore, afferents from widespread regions of the brainstem, thalamus, and neo-cortex, as well as the hippocampal system, converge in the amygdala (Amaral et al., 1992; Pitkänen, 2000). Therefore, the amygdala appears to be a site within the mTL where the Pavlovian associations underlying fear memory are formed (Fanselow and LeDoux, 1999).

The amygdala has also been shown to be critically involved in appetitive conditioning in both rodents and humans (Childress et al., 1999; Everitt et al., 2000, 2003; Gottfried et al., 2003). However, there remains some disagreement as to the precise functional roles of different amygdalar sub-nuclei with respect to conditioned responding on appetitive tasks (see Gabriel et al., 2003 for discussion). Additionally, the amygdala can modulate vigilance and attentional processes (Davis and Whalen, 2001; Holland and Gallagher, 1999; Kapp et al., 1992), and an amygdalar role in the extinction of fear memory has been demonstrated (Davis et al., 2003; Falls et al., 1992; Royer and Pare, 2002; Walker et al., 2002).

Another structure in the mTL, the hippocampus, is a main component of the episodic, or declarative memory system (Cohen and Eichenbaum, 1994; Squire, 1992; Tulving, 1983). It has been suggested that ongoing experience is represented within the entorhinal–hippocampal networks via location and/or context dependent firing-rate modulation of place cells (Eichenbaum et al., 1999; Ferbinteanu and Shapiro, 2003; Markus et al., 1995; O'Keefe and Nadel, 1978; Oler and Markus, 2000; Redish, 1999; Wilson and McNaughton, 1994). A considerable body of evidence demonstrates a hippocampal role in the performance of spatial memory tasks both in rodents (Jarrard, 1993; Morris et al., 1982; Olton and Samuelson, 1976) and in primates (Ekstrom et al., 2003; Maguire et al., 1997; Parkinson et al., 1988). Sutherland and Rudy (1989) proposed that the hippocampus is crucial in memory tasks that require learning the conjunctive, or configural relationships between environmental stimuli, with spatial processing being only one such example. The behavioral task used in the present experiment requires the learning of conjunctive relationships between spatial and non-spatial stimuli.

During fear conditioning, damage to the hippocampus selectively affects memory for the classically conditioned context (shock chamber) without disturbing the association made between a discrete CS (usually a tone) and the shock US (Anagnostaras et al., 2001; Kim and Fanselow,

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Abbreviations: CS, conditioned stimulus; DH, dorsal hippocampus; mTL, medial temporal lobe; US, unconditioned stimulus; VH, ventral hippocampus.

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1992; Maren et al., 1995; Phillips and LeDoux, 1992; Ward et al., 1999). The selectivity of these deficits points to the complementary yet functionally distinct participation by both the hippocampus and the amygdala during the acquisition of conditioned fear. Thus, the hippocampal and amygdalar systems appear to work synergistically and in parallel to direct learning of this associative task (Maren and Fanselow, 1995; Richter-Levin and Akirav, 2000).

A key step toward the full appreciation of the neural basis of learning is a better understanding of the synthetic interactions that take place among different memory systems. To this end, the current behavioral task was developed to further demonstrate the dissociable roles of the hippocampal and amygdalar memory systems within a single learning paradigm. Traditionally, fear conditioning studies have examined the modification of simple reflexive behaviors, employing species-specific responses (freezing, enhanced startle, etc.) to infer an animal's memory for an aversive event (Fendt and Fanselow, 1999). Here however, we report on a novel fear conditioning task, where hippocampus-reliant place learning and amygdala-reliant conditioned discrimination can together be dissociated and simultaneously assessed.

The task involved the superimposition of Pavlovian discriminative fear conditioning on an appetitively motivated instrumental response (alternation task). Rats were trained to use a tone CS to differentiate between "dangerous" (tone) and "safe" (no-tone) trials while alternating on a runway for food reinforcement. Performance of animals with small lesions of the amygdala or dorsal hippocampus (DH) was compared with that of sham controls. Small lesions were employed to prevent total impairment, and to allow lesioned animals to acquire all stages of the task. There were four main hypotheses regarding the experimental results:

1) Lesions of the CA1 region of DH would impair learning the location of the shock region.
2) The DH lesions would also produce a configural deficit. Once the spatial task was acquired the superimposition of a non-spatial, discriminative contingency to the spatial task would also be impaired.
3) Lesions of the amygdala would affect the ability to acquire both the place aversion, and the discriminative conditioned response.
4) Amygdala lesions would disrupt the extinction/suppression of the fear response under safe conditions.

The results lend support for hypotheses 1 and 4, but not for 2 and 3.

EXPERIMENTAL PROCEDURES

Subjects and apparatus

Twenty-nine adult male Fischer-344 retired breeders (11 months old at the outset of training; Harlan Sprague–Dawley, Indianapolis, IN, USA) were singly housed in tubs, maintained on a 12-h light/dark cycle (lights on 7:30 AM), and food deprived to approximately 80% of their *ad libitum* body weight throughout the experiment. The rats were handled on a regular basis, and fed small amounts of Noyes operant pellets (Research Diets, Inc., New Brunswick, NJ, USA) before training began. Animals were trained to run for food reinforcement in a high-walled, diamond-shaped runway located in a dimly lit room (2.1×2.1 m) containing several visual cues (Fig. 1a, b). Two pellet dispensers (Med-Associates, St. Albans, VT, USA) were located in chambers at opposite ends of the runway. The walls of the runway were made of clear Plexiglas, while its floor and those of the feeding chambers were made of black Plexiglas. The entire floor of the runway was lined with flat 1.4 cm wide stripes of steel; each separated by 1.4 cm. At the entrance to both of the chambers, a photocell and a recessed light-emitting diode were placed opposite one another in the runway walls. As the animal's body came between the photocell and diode, a single 45 mg food pellet was dispensed into a plastic cup inside the chamber (custom written software, A. Kuzin, University of Connecticut, Storrs, CT, USA). Once a pellet had been dispensed in one chamber, the animal was required to traverse the runway and enter the opposing side to attain reinforcement in the same manner. A trial was defined then as a traversal of the runway from one food chamber to the other.

Discrimination training

The animals were given one half-hour training session every other day, for several weeks. An animal was considered proficient at the alternation task if it ran at least 60 trials in 30 min. Once criterion performance was reached, the animals were given full access to food for several days prior to surgery (see below).

Following surgery, the rats were retrained to criterion levels on the alternation task before conditioning sessions began. Throughout discrimination training, the apex of the runway was electrified during "tone trials," which occurred on approximately 50% of the trials, in a pseudo-random order. The tone (75 dB, 300–500 Hz; Radio Shack, Fort Worth, TX, USA) and the shock (Model 82404SS shock generator/scrambler; Lafayette Instruments, Lafayette, IN, USA) were always presented together. Both the tone and the shock were turned on at the beginning of, and remained on for the duration of, a tone trial. Each new trial was initiated at the time the animal entered the subsequent chamber. Therefore, during a tone trial, the rat had to cross over the electrified region at the apex of the runway in order to attain food reinforcement. While the tone remained on for the duration of the trial, the rat only received the shock when crossing the apex of the runway. On each day of discrimination training the session was terminated when the animal either completed 80 alternation trials or 30 min passed.

During the first two training sessions, the animals received a shock of 0.15 mA when crossing over the apex on tone trials. On the third training session, the shock level was lowered to 0.075 mA and the animals were required to run a minimum of 60 trials before moving to the next training session. If the animal did not reach this criterion, it ran the same session again, at the same shock level, on the following training day. At each successive training session, the shock level was increased by 0.025 mA increments, until a maximum of 0.175 mA was reached at training session 7. Once the animals reached the 0.175 mA shock level, they continued to run at a shock level of 0.175 mA and a 40 trial criterion for an additional 16 training sessions. These relatively low current levels were selected on the basis that they were mild enough such that the animals would willingly endure the shock to gain access to the reinforcement, yet sufficient to produce avoidance of the shock region.

On days when the animals were run on the task they were given no additional food following training. This procedure was employed throughout behavioral training, and resulted in high rates of responding, a strategy that maximized food intake while on the runway. The animals were fed larger portions of food the following day to maintain body weight.

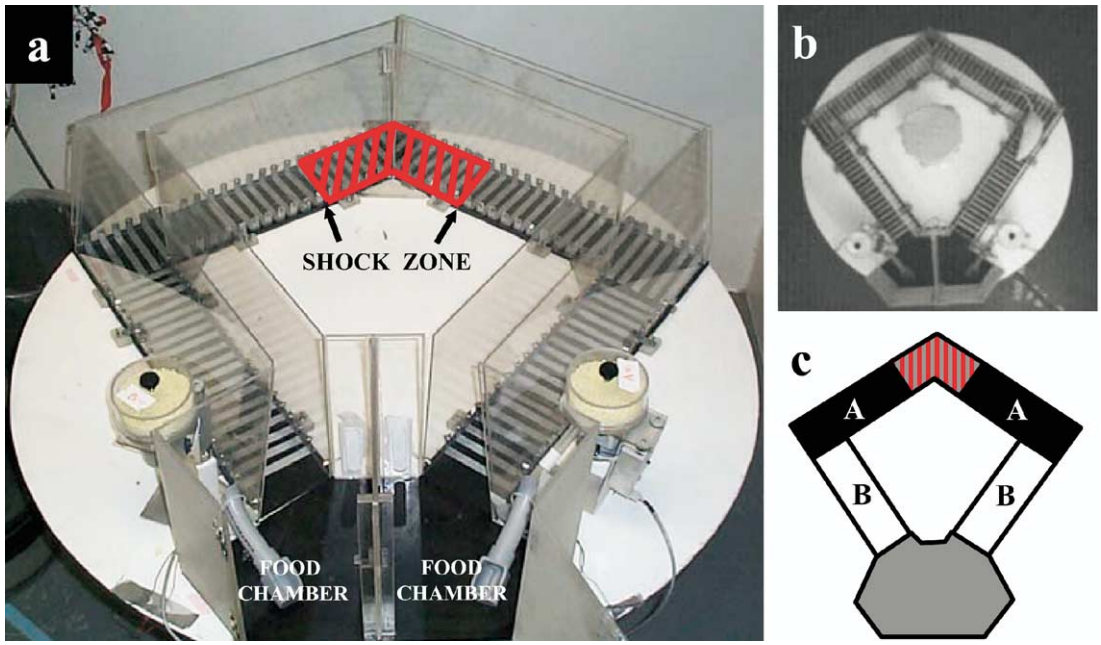

Fig. 1. The behavioral procedure used in this study. (a) Photo of the runway demonstrating the location of the "shock region" and the food chambers. In each training session the rats alternated between food chambers to obtain a single 0.45 mg operant pellet. Each alternation (i.e. running from one food chamber to the next) was considered a single trial. A new trial was initiated at the time the animal entered the subsequent chamber. A maximum of 80 trials or 30 min was allowed per training session. The apex of the runway (shock region) was electrified during "tone trials." Both the tone and the shock were turned on at the beginning of, and remained on for the duration of, each tone trial. Therefore, during a tone trial, the rat had to cross over the electrified region of the runway in order to attain food reinforcement. The tone and shock were presented on 50% of the trials, which were randomly interspersed with "no-tone trials." (b) Frame from the overhead video tracking system that monitored the position of the animals during discrimination training. (c) Diagram of the zones used to analyze the discriminatory behavior. The track was partitioned into several "zones." The time spent in the different zones during each trial was recorded. The duration of the first visit per trial to each zone of interest was used as the dependent variable to measure place aversion learning and tone discrimination. The spatial analysis compared hesitation in the runway zones adjacent to the shock region (Zone-A) to that in the zones distant from the shock region (Zone-B) with the tone off. The discrimination analysis compared the tone and no-tone trials separately for Zone-A and Zone-B. A videotaped example of the discrimination behavior can be seen by going to http://psychlops.psy.uconn.edu/Markus/DiscriminationTask.html.

Surgery

All experiments were approved by the University of Connecticut animal care committee and conformed to the National Institutes of Health standards for the humane treatment of animals. All efforts were made to minimize the number of animals used and their suffering. The rats were anesthetized with an injection of sodium pentobarbital (Nembutal, 40 mg/kg, i.p.), supplemented with Methoxyflurane (Metofane) if necessary to maintain surgical anesthesia. Once anesthetized, the animals were given additional injections of antibiotic (Ambipen, 0.2 cc, i.m.) to prevent infection, and a peripheral anticholinergic (Glycopyroate, 0.2 cc, i.m.) to block parasympathetic activity, and prevent fluid secretion in the lungs. Body temperature was maintained at 37 °C with a heating pad, the eyes were coated with ophthalmic ointment and covered to prevent drying, and the animals were then mounted into a stereotaxic frame. An incision was made in the scalp, and several small burr holes were drilled for anchor screws to be fastened to the skull. Craniotomies were then made bilaterally centered over either the DH, or the basolateral amygdala. Bilateral electrolytic lesions were made using a UGO Basile lesion maker (Varese, Italy).

For hippocampus lesions, electrodes were lowered into the DH in three parallel locations along a plane at 3.3 mm posterior to Bregma (Paxinos and Watson, 1986). The coordinates were: 1.4 mm, 2.2 mm, and 3.1 mm lateral to the midline, and 3.3 mm, 3.4 mm, and 3.6 mm ventral to the surface of the skull, respectively. A 2.0 mA anodal direct current (DC) current was passed for 20 s through each electrode. The stainless steel electrodes (0.33 mm diameter) were insulated except for approximately 0.5 mm of exposed surface at the tip (A-M Systems Inc., Carlsborg, WA, USA). For amygdala lesions, electrodes were lowered in two parallel locations along a plane at 5.0 mm lateral from the midline. The coordinates were: 2.5 and 3.0 mm posterior to Bregma, and 8.9 mm ventral to the surface of the skull. A 1.0 mA anodal direct current (DC) was passed for 15 s through each electrode. Craniotomies were made, but electrodes were not lowered into the brains of sham-operated rats. These sham-operated animals served as a control for the effects of stress and damage associated with the surgical procedure on behavioral performance.

The craniotomies were sealed with bone wax, and a small screw was fixed to the skull with dental cement. The incision was closed with wound clips, and a topical antibiotic was applied to prevent infection. The rats were postoperatively administered acetaminophen (orally), and an i.p. injection of 0.9% saline to aid in recovery. The animals were given at least 1 week to recover from surgery prior to retraining and conditioning sessions.

Data analysis

During conditioning sessions, a small light emitting diode and 3-V battery were attached to the screw on the animal's head to aid in tracking its position on the runway. The location and direction of the rat's movement was recorded by the S.M.A.R.T. tracking program (Panlab, Barcelona, Spain). The track was partitioned into several "zones" (Fig. 1c). Through observation of well-trained animals it was noted that they would stop just before reaching the shock region, and hesitate for several seconds before crossing

over the electrified apex. Consequently, the duration of the first visit per trial to each zone of interest was used as the dependent variable to measure place learning and conditioned discrimination. It should be noted that on any given trial, only data from when the animal was heading toward the shock region were used for analysis. Because only the initial visit to a zone was measured, possible confounds such as touching the shock grid, or returning to the food chamber were omitted.

Repeated measures ANOVAs examined the effects of group, session, and tone, and relative indices were used as measures of place learning, and discrimination. The spatial analyses compared the average duration in the zones adjacent to (Zone-A) and distant from (Zone-B) the shock region with the tone off (see Fig. 1c). The following formula was used to calculate the spatial discrimination index (R_{place}):

$$R_{place} = \frac{\text{(visit duration in Zone-A)}}{\text{(visit duration in Zone-A + visit duration in Zone-B)}}$$

Thus, a relative score of 0.5 indicates no difference in the time spent in the different regions (hesitation in Zone-A=hesitation in Zone-B). A relative score closer to 1.0 indicates an increase in hesitation as the animal approaches the shock region.

The discrimination analyses compared the tone trials and the no-tone trials separately in the zones adjacent to and distant from the shock region. To calculate the relative index for the tone-context discrimination (R_{tone}) we used the following formula:

$$R_{tone} = \frac{\text{(visit duration with tone)}}{\text{(visit duration with tone + visit duration without tone)}}$$

Thus, a relative score of 0.5 indicates no difference in the time spent in the zone of interest because the average hesitation during tone trials is equal to that during no-tone trials. A relative score closer to 1.0 indicates an increase in hesitation behavior with the tone on.

The advantage of the discrimination index is the fact that it is a within animal analysis, and as such is unaffected by individual differences in running speed. The raw data, depicting raw hesitation times are presented in the learning curve figures.

Probe tests

To ensure that it was the tone that the animals were using as a discriminative stimulus, and not that the animals were somehow sensing the current or the activation of the shock generator, a probe session was conducted on the last day of training (session 24). During the probe session several tone trials were presented without the shock. To analyze the probe data the mean hesitation was calculated separately for the three trial types (no-tone, tone only, tone+shock). Therefore, the possibility that the rats could somehow perceive that the apex was electrified prior to touching it could be ruled out.

Shock sensitivity test

After the last training session the animals were given back full access to food for several days, and subsequently tested for differences in sensitivity to a shock stimulus in a room different from that used during training. The animals were placed in a Skinner box (28×21×21 cm; Med-Associates) and a weak electrical current was passed through the metallic rods that made up its floor. An experimenter, blind to the shock level being used, monitored the animal while the current was gradually increased until the rat removed his forelimbs. This was done five times for each rat with the median used as an index of the animal's shock sensitivity.

Histology

After the last training session, the animals were killed with CO_2 and perfused intracardially with a 10% formalin solution. The brains were removed and placed in formalin and sucrose for at least 24 h. Forty micrometer coronal sections were cut using a cryostat and mounted on a gelatin-coated slide. The tissue was stained using Cresyl Violet and examined microscopically for lesions.

RESULTS

Subjects

The 29 pre-trained animals were divided up into three lesion groups prior to surgery (amygdala: $n=11$, DH: $n=10$, control: $n=8$). Of the 29 animals, the data from eight were excluded from the analysis for various reasons. These included inadequate amygdala lesions ($n=2$), failure to show discrimination at even the highest shock level (one DH and one control animal), and low running rates, which prevented animals from reaching criterion (two DH and one amygdala animal). In addition, one DH animal was excluded because a large pituitary tumor was found during perfusion. All exclusions for anatomical/histological reasons were made blind to the experimental results. For the analysis, data were included from eight amygdala-lesioned ($n=8$), six DH-lesioned ($n=6$), and seven control ($n=7$) animals.

Lesions

Microscopic examination of tissue from animals with DH lesions demonstrated that all animals included in the study had damage to the dorsal CA1 region of hippocampus proper (Fig. 2, left panel). Some animals sustained damage to the CA3 region and the dentate gyrus as well, but to a lesser extent. The coordinates for the lesions of the amygdala targeted its basolateral region, and all animals whose data were included in the study had sustained damage to this region; however, in many cases the lesions encroached other sub-nuclei (i.e. central nucleus; see Fig. 2, right panel). As can be seen in the figure, animals also sustained damage to the cortical regions above the lesion sites. This was a direct result of the heated bone wax used to seal the craniotomies.

Conditioned discrimination

All animals were retrained to criterion levels before discrimination training began, and there were no differences in alternation responding between groups prior to training (ANOVA, $F_{(2,20)}=2.73$, $P<0.092$; note "Pre-conditioning" in Figs. 4, and 5b). The data from the first two training sessions were not included in the analysis because most animals produced very few responses following the initial tone-shock pairing. Once the intensity of the shock was lowered to 0.075 mA on training session three, the animals began to run at higher rates. The animals were required to run a minimum of 40 trials before moving on to the next shock level; therefore, only those data sets with at least 40 trials were included in the analysis. Repeated measures ANOVAs were conducted through training session 19 (two

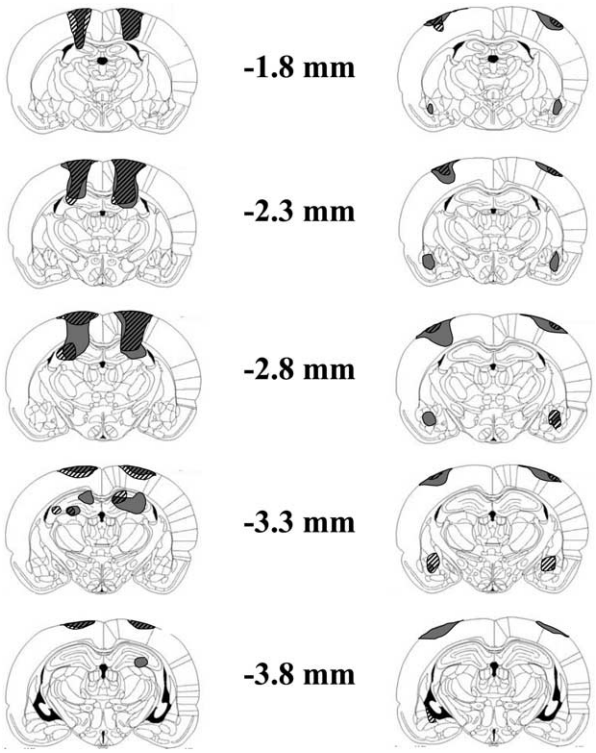

Fig. 2. Representative illustrations demonstrating the largest (gray) and smallest (striped) lesions included in the study at several different anterior-posterior levels. Left, hippocampus lesions. Right, amygdala lesions.

control animals lost their tracking-diode attachment screw after day 19).

Shock sensitivity

To ensure that any differences in discrimination behavior were not the result of differences in the ability to perceive the shock, a shock sensitivity test was conducted. As can be seen in Fig. 3, there were no differences between experimental groups in sensitivity to a stimulus comparable to that used during conditioning (ANOVA, $F_{(2,18)}=0.005$, $P>0.1$). Therefore, the following results cannot be attributed differences in nociception caused by the lesions.

Effects of lesions on place aversion learning

Place aversion learning was measured by comparing the time spent in the zones adjacent to the shock region with the zones distant from the shock region during no-tone trials. This was done to ensure that tone and shock presentation did not confound the results. Prior to conditioning, there was no difference in the time spent in the zones adjacent to the shock region compared with the zones distant from the shock region (see Fig. 4). The spatial discrimination index showed that prior to conditioning all groups were not significantly different from a R_{place} of 0.5 (*t*-tests, all groups: $P>0.1$).

As can be seen in Fig. 4, with discrimination training, the animals developed a differential response in the zones adjacent to the shock region. The first session where the R_{place} was found to differ significantly from 0.5 was on training session 3 for the control and amygdala lesion groups (control: $P<0.05$; amygdala: $P<0.05$, hippocampus: $P>0.1$), and session 6 for the DH-lesion group (all groups: $P<0.05$). An examination of the total number of trials needed to show spatial discrimination revealed a similar effect. The DH-lesioned rats required almost three times as many trials as the other two groups to show a differential response (DH: 397.5; control: 102.1; amygdala: 98.5 trails). Therefore, DH lesions resulted in the impaired ability to selectively avoid a location where an aversive event had previously been experienced. However, by training session 7 the DH-lesioned animals were showing similar spatial discrimination to controls.

Effects of lesions on conditioned discrimination

Tone discrimination learning was measured by comparing the time spent during the tone trials to that spent during no-tone trials in the zones adjacent to the shock region. This was done based on the empirical observation that the animals were hesitating in this zone while heading toward the shock region. Further demonstration of the fact that the discrimination was unique to the zones adjacent to the shock region is the lack of any difference in hesitation between tone and no-tone trials in the zones distant from the shock region across all training sessions in control animals (compare Fig. 6a and b). All experimental groups displayed discriminative fear conditioning to the tone, spending significantly less time hesitating in the zones adjacent to the shock region on trials when the tone was off (repeated measures ANOVA, all groups: Session: $P<0.05$; Tone: $P<0.05$; Session×Tone: $P<0.05$).

The first training session where R_{tone} was found to differ significantly from 0.5 was on session 9 for the DH-lesioned animals (control: $P>0.1$; amygdala: $P>0.1$; DH: $P<0.05$), session 10 for the control animals (control: $P<0.05$; amygdala: $P>0.1$; DH: $P<0.05$), and session 11 for the amygdala lesion group (all groups: $P<0.05$). Therefore, while DH lesions appeared to result in a slight enhancement in discrimination learning compared with controls, amygdala lesions resulted in a slight delay in discriminative responding.

A tone discrimination deficit unique to the amygdala-lesioned animals is revealed when evaluating the behavior during the no-tone trials. Fig. 5 presents the hesitation data in the zones adjacent to the shock region for the three experimental groups combined, separated by trial type (tone/no-tone). Statistical analysis of the mean hesitation in the zones adjacent to the shock region during tone trials (Fig. 5a) revealed only a significant effect of Session (repeated measures ANOVA, $F_{(16,288)}=25.9$, $P<0.05$), with no effect of Group or an interaction (both: $P>0.1$). However, analysis of the no-tone trials (Fig. 5b) revealed significant effects of Session (repeated measures ANOVA, $F_{(17,306)}=15.3$, $P<0.05$), of Group ($F_{(2,18)}=15.4$, $P<0.05$), and a Session by Group interaction ($F_{(34,306)}=1.58$, $P<0.05$). Post hoc tests revealed that the amygdala lesion group was different from both control and DH-lesioned animals (Sheffe, both: $P<0.05$), while the control and DH-

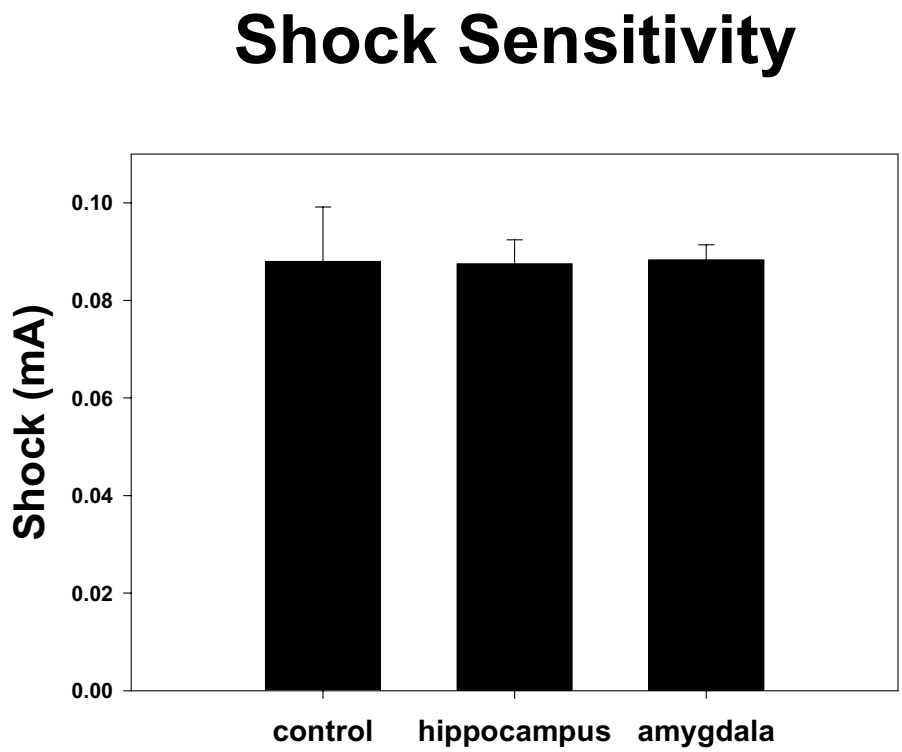

Fig. 3. There were no differences between lesion groups in their sensitivity to the shock stimulus. Note that in all groups, the current required to produce a paw withdrawal response was within the range of the lowest shock levels used during conditioning.

lesioned animals did not differ from one another (Sheffe, $P>0.1$). Therefore, while all of the animals behaved similarly during tone trials (hesitating in the zones adjacent to the shock region before running through it), the amygdala animals displayed impairment in the suppression of a fear response when the tone was not presented.

Analysis of the mean hesitation in the zones distant from the shock region during tone trials (Fig. 6a) revealed only a significant effect of Group (repeated measures ANOVA, $F_{(2,18)}=9.8$, $P<0.05$), with no effect of Session ($P=0.084$) or an interaction ($P>0.1$). Post hoc tests revealed that the DH lesion group was different from both control and amygdala-lesioned animals (Sheffe, both: $P<0.05$), while the control and amygdala-lesioned animals did not differ from one another (Sheffe, $P>0.1$). This effect is the result of slightly greater hesitation for the DH-lesioned animals across several of the training sessions, and further demonstrates the spatial deficit produced by a hippocampus lesion on this task. Thus, even after showing significant learning of the shock location, and after learning the tone discrimination, the DH-lesion group displayed a residual spatial deficit. This was expressed as an increased hesitation in the presence of the tone even in regions distant from the shock zone.

Statistical analysis of the mean hesitation in the zones distant from the shock region during no-tone trials (Fig. 6b) revealed a small but significant effect of Session (repeated measures ANOVA, $F_{(17,306)}=2.3$, $P<0.05$), with no effect of Group ($P>0.1$) or an interaction ($P=0.069$). This effect appears to be a direct result of the incremental shock levels used in the training procedure. On session 7 of the training, the shock intensity reached its maximum level of 0.175 mA. As a consequence, all animals displayed a small increase in hesitation across the board on that session.

Probe tests

During the probe test several tone trials were presented without the shock (Fig. 7). A one-way ANOVA comparing the mean hesitation for the "tone+shock" and "tone only" trials found no effect for any of the experimental groups (control: $F_{(1,9)}=0.15$, $P>0.1$; DH: $F_{(1,11)}=0.14$, $P>0.1$; amygdala: $F_{(1,15)}=0.11$, $P>0.1$). Thus, it can be inferred that the tone served as the discriminative CS.

DISCUSSION

The present study was designed to examine the contributions of the hippocampus and the amygdala to fear-motivated context discrimination. The performance of rats with small lesions of the amygdala or DH was compared with controls on a novel discriminative avoidance/approach task. Discrete rather than complete lesions of both DH and amygdala were used in order to assess impaired behavioral performance through all stages of the task. It was hypothesized that lesions of DH would retard spatial learning. Once the spatial task was acquired, the response to a superimposition of a non-spatial, discriminative contingency to the spatial task would be impaired. It was further hypothesized that lesions of the amygdala would interfere

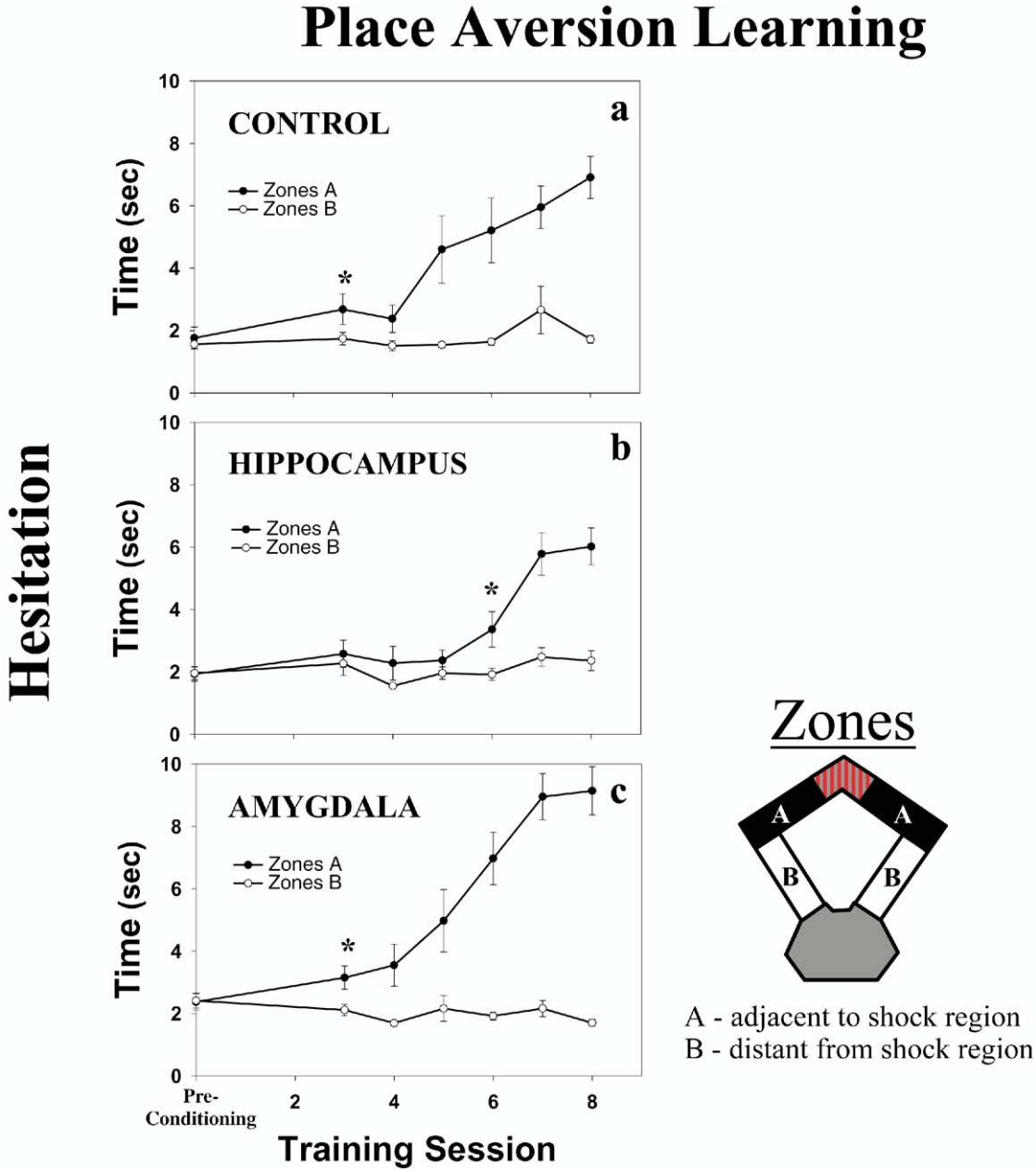

Fig. 4. Comparison of the hesitation response in runway zones adjacent to the shock region (Zone-A) to that in the zones distant from the shock region (Zone-B) during the no-tone trials of the first eight training sessions. Prior to conditioning, there were no differences in the time spent in Zone-A compared with Zone-B. A spatial discrimination index was used to measure place learning (see Experimental Procedures). By session 3, both the control and amygdala groups were displaying significant place learning (control: $t_{(6)}=5.13$, $P<0.05$; amygdala: $t_{(7)}=4.66$, $P<0.05$). The DH group however, did not display discriminative hesitation in the zones adjacent to the shock region until session 6 ($t_{(5)}=6.51$, $P<0.05$).

with the acquisition of both the place aversion and the discrimination, and would likewise disrupt the extinction/suppression of the fear response under safe conditions.

Hippocampus lesion effects

Spatial learning. In the present study, the hippocampus lesions impaired the animals' ability to learn the location where an aversive event took place. This effect was seen in two different ways, an initial learning deficit, and a continuous impairment in performance. First, the DH animals were delayed in exhibiting a differential response along the pathway approaching the shock region (Fig. 4). Second, despite extensive training, during tone trials the DH animals continued to display a small but significant increased hesitation in zones quite distant from the shock region (Fig. 6a). Importantly, this effect was not observed during no-tone trials, and therefore does not appear to simply result from a general decrease in running velocity. These results corroborate many previous studies demonstrating hippocampal involvement in avoidance learning

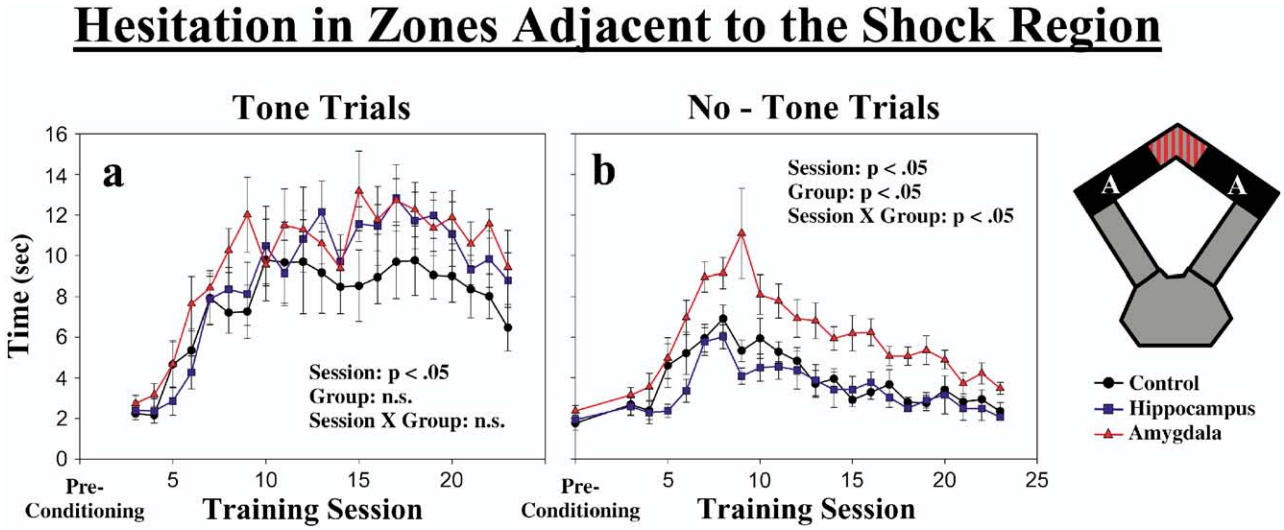

Fig. 5. Group comparison of the hesitation response during (a) the tone trials and (b) the no-tone trials in the zones adjacent to the shock region (Zone-A). (a) The average hesitation in Zone-A during tone trials. All groups hesitated at similar levels in Zone-A when the tone was on. (b) The average hesitation in Zone-A during no-tone trials. Note that in the absence of the tone, the amygdala group displayed a significant impairment in the suppression of the fear response.

(e.g. Farr et al., 2000; Lorenzini et al., 1996), and support the view that the hippocampus is a component of a spatial memory system. However, with enough training the DH animals did develop significant place aversion. This allowed for the examination of conditioned place discrimination in these animals.

Conditional place discrimination. While DH-lesioned animals displayed impairment in spatial learning, they were unimpaired on the tone-mediated discrimination, hesitating in the zones adjacent to the shock region significantly more when the tone was on. Importantly, HD lesions did not affect the ability to use a discriminative CS to guide their behavior. Thus, even though they were delayed in learning the spatial task, these animals were still able to learn the conjunctive relationship between tone and place.

The present results support the "spatial" hypothesis of hippocampal function, and are less compatible with the idea that the hippocampus is necessary to learn associations among configurations of stimuli. The current lesion results are in agreement with single unit recordings from DH during discriminative performance on this task. Neuronal activity was found which was highly responsive to place, and only minimally responsive to the configural aspect of the task (Oler and Markus, unpublished observations). The question of what precise role the hippocampal system plays in this type of task is important, and should be followed up in future studies.

It should also be noted that in the current experiment animals were trained on the task *after* surgery. There is evidence showing that following extensive training, hippocampal animals can exhibit learning on spatial tasks (Anagnostaras et al., 2001; Whishaw, 1998). In the future, training animals prior to producing hippocampal lesions may produce a more severe impairment on this task.

It has previously been shown that rats with partial hippocampus lesions are able to display spatial learning (Moser et al., 1993, 1995). The lesions in the present study did not completely remove the DH (Fig. 2), future experiments employing larger and/or fiber-sparing neurotoxic lesions, may produce greater deficits on learning and performance of this task. Finally, a functional polarity exists along the septo-temporal axis of the rat hippocampus, with the DH primarily dedicated to spatial processing and the ventral hippocampus (VH) related to contextual fear (Bannerman et al., 1999; Hock and Bunsey, 1998; Moser and Moser, 1998; Richmond et al., 1999). Considering that functional differences exist along the longitudinal (septo-temporal) axis, and that it is the VH (rather than DH) which is more highly interconnected with the amygdala (Dolorfo and Amaral, 1998; Pitkänen et al., 2000), future experiments employing selective lesions of the VH may reveal greater involvement of the hippocampal circuitry in the present task.

Amygdala lesion effects

Spatial and conditional learning. The amygdala-lesioned animals developed normal place learning, and only a slight impairment in conditioned discrimination. Considering the well-documented role for the amygdala in mediating Pavlovian associations, it may seem surprising that lesioned animals acquired the task at all. One possible reason for this is the fact that the lesions in the present study were discrete (sparing much of the lateral nucleus), and did not completely destroy the amygdala (see Fig. 2). A second alternative is the possibility that other brain structures were able to compensate for the loss of amygdalar tissue, by acquiring the initial conditioned responses to place and tone. In fact, it has been shown that following complete neurotoxic lesions of basolateral amygdala, animals show spared conditioned fear, although at attenuated levels when compared with intact controls (Cahill et al., 2000; Vazdarjanova and McGaugh, 1998; see how-

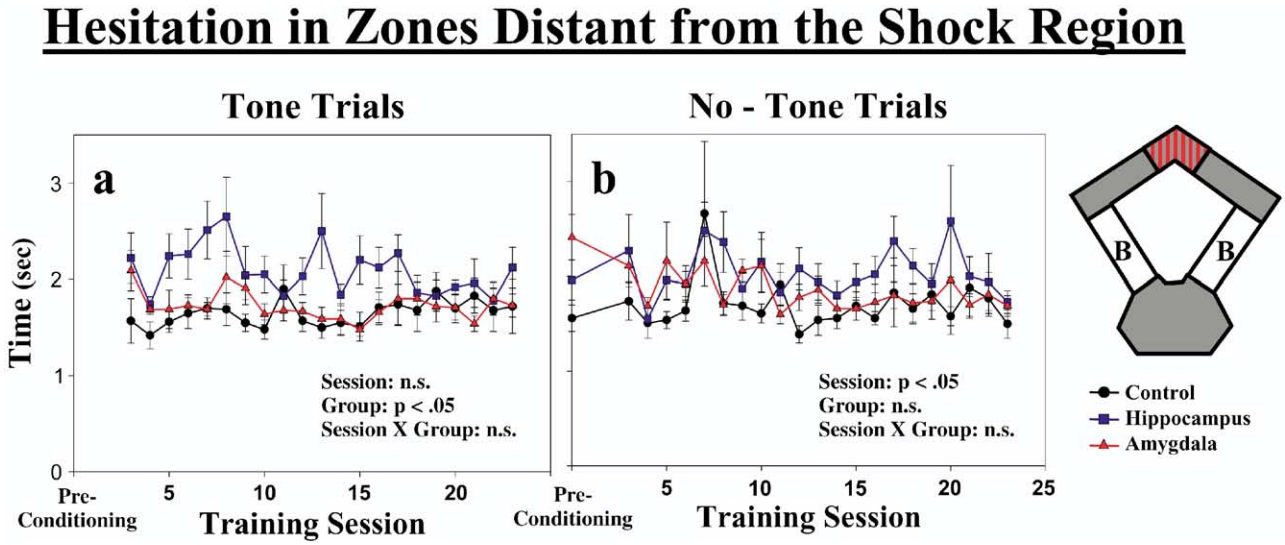

Fig. 6. Group comparison of the hesitation response during (a) the tone trials and (b) the no-tone trials in the zones distant from the shock region (Zone-B). (a) The average hesitation in Zone-B during tone trials. Across several of the training sessions, the DH group displayed slightly greater hesitation in the runway zones that were distant from the shock region when the tone was on. This effect further demonstrates the spatial impairment produced by a hippocampus lesion on this task. (b) The average hesitation in Zone-B during no-tone trials. Note that all three groups displayed a small but significant increase in hesitation on training session 7, which is most likely the result of the incremental shock levels used in the training procedure (see Experimental Procedures). Note the different scale of the *y* axis than that in Fig. 4.

ever Maren, 2001a). Future studies with the present task, employing post-training lesions of the amygdala, may shed light on the question of whether this structure is essential for the acquisition and/or expression of conditioned fear.

A third possible reason for the lack of a learning impairment in amygdala-lesioned rats is the fact that most previous experiments have examined changes in reflexive and/or autonomic function to quantify learning (see Fendt and Fanselow, 1999). The present task however, differs from other aversive tasks in a number of important ways. The task is unlike fear conditioning tasks where one directly examines the conditioned response of an animal in a small inescapable enclosure. Unlike passive avoidance, a large arena is used, the shock region is not clearly demarked, and the shock is continuously present on tone trials. The current experiment also differs from typical conditioned suppression paradigms since it is an *approach–avoidance* conflict task. The rat is placed into a situation where an aversive stimulus is encountered on the route to an appetitive stimulus; thus, we are examining the effects of Pavlovian fear conditioning on an ongoing instrumental behavior (see File, 2000; Miller, 1944).

The type of conflict situation used in the present study is more complex than other fear conditioning paradigms, and may differentially engage the amygdala. In order to dissociate the above possibilities it would be of interest in the future to examine the effects of larger lesions on this type of task, and if possible, to test the same animals on both a classical fear conditioning task as well as the present conflict task.

Suppression of fear response. The main effect of the amygdala lesion was an inability to appropriately suppress the conditioned fear response. This is revealed by the fact that even after the rats displayed the place aversion, and the concomitant differentiation between the tone trials and no-tone trials, an exaggerated conditioned response continued to be expressed in the absence of the tone CS (Fig. 5b). Recently, the role of the amygdala, and its interconnections with medial prefrontal cortex (the infralimbic subregion in particular) in the extinction of fear behavior has received attention (Milad and Quirk, 2002; Quirk et al., 2003). Therefore, the inability of the amygdala-lesioned animals to appropriately suppress the conditioned response may have been the result of disconnection with prefrontal cortex, leading to impaired extinction of fear for the shock region following repeated unpaired (no-tone) trials. In fact, anterograde tracing studies have shown that most of the fibers coming from infralimbic cortex pass through the internal capsule and then course ventrolaterally through the substantia innominata before arriving in the amygdala (McDonald et al., 1996). Upon reexamination of the amygdala lesions produced in the present study (Fig. 2, right panel), it appears likely that some of these corticofugal fibers were damaged.

General discussion

Much of what is currently known regarding the cellular and molecular mechanisms of synaptic plasticity has been the result of studies utilizing Pavlovian conditioning and non-associative forms of learning (e.g. habituation), relatively simple learning paradigms (Cahill et al., 2001; Kandel and Spencer, 1968). While these lines of experimentation have contributed greatly to our understanding of the nature of memory (LeDoux, 2002), a comprehensive appreciation of mammalian memory systems will require the analysis of species-typical (and therefore, inherently more complicated) behavioral paradigms. Several previous studies have used multiple instrumental or Pavlovian measures,

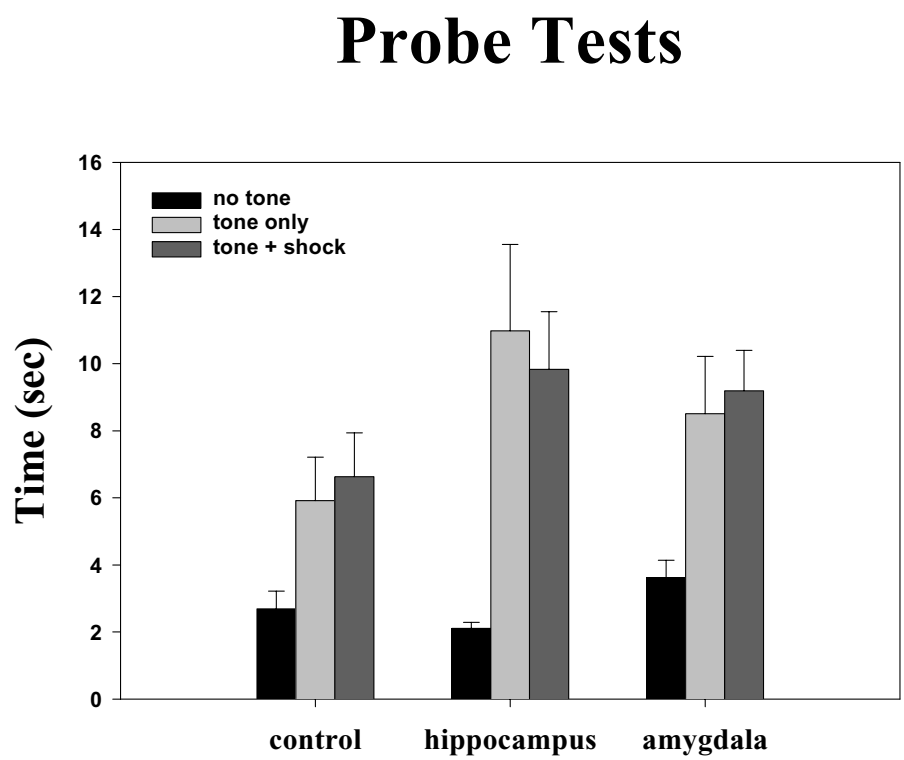

Fig. 7. Hesitation in the zones adjacent to the shock region during the discrimination probe test. Note that for all groups, the average duration increased on tone trials regardless of whether or not the apex of the runway was electrified.

employed either concurrently or sequentially, to investigate the role of these structures in learning (Antoniadis and McDonald, 2000; Selden et al., 1991; Smith et al., 2001). These studies, and others, along with the present results, demonstrate the existence of complementary, synergistic, and even competitive interactions between the hippocampal and amygdalar memory systems.

The time course of acquisition on the current task is much slower than that of classical conditioning experiments, where behavioral expression of conditioned fear can be observed following only a few CS–US pairings (e.g. Oler and Markus, 1998). Furthermore, the animals traverse the environment multiple times; allowing for repeated assessment of the fear related behavior, and an evaluation of the degree of learning. Finally, this task is a variant of the conflict test (File, 1997), since the animal must of its own volition endure the shock if it is to obtain additional reinforcement. Therefore, it could be used to assess the efficacy of anxiolytic drugs, and the effects of stress on the brain, as well as the *in vivo* electrophysiological or neurochemical processes involved in the acquisition and expression of a fear motivated instrumental behavior.

The results of the present experiment support the idea that synergistic interactions among multiple memory systems guide normal learning and expression of complex behavior (Kim and Baxter, 2001). The data support the hypothesis that lesions of the DH would disrupt the learning of a "dangerous" location, but fail to support the second hypothesis that the addition of a non-spatial, conditional component would cause further impairment. Contrary to the third hypothesis, amygdala-lesioned animals were able to acquire both the spatial and discriminative components of the task. However, a role was shown for the amygdala in the suppression of a previously learned and biologically relevant instrumental response.

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