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Temporary inactivation of dorsal hippocampus attenuates explicitly nonspatial, unimodal, contextual fear conditioning

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ABSTRACT

The current study examined the effects of temporary inactivation of the DH on freezing, rearing, ambulating, grooming, and whisking behavior in an explicitly nonspatial contextual fear conditioning paradigm in which olfactory stimuli served as temporally and spatially diffuse contexts. Prior either to training, testing, or both, male Sprague–Dawley rats received bilateral microinfusions of saline or the $GABA_A$ agonist muscimol into the DH. Results indicate that temporary inactivation of DH produced both anterograde and retrograde deficits in contextually conditioned freezing, while sparing the acquisition and expression of freezing to a discrete auditory or olfactory CS. These data suggest that there is a decidedly nonspatial component to the role of DH in contextual conditioning, and that olfactory contextual conditioning is a fruitful means of further exploring this function.

1. Introduction

Pavlovian fear conditioning is among the many behavioral paradigms used to identify the neural substrates of associative learning. In delay fear conditioning procedures, presentations of a neutral conditioned stimulus (CS) overlap and co-terminate with presentations of a noxious unconditioned stimulus (US) such as footshock. Repeated CS–US pairings result in the development of a learned association between the CS and US as well as between the training context and the US; acquisition of these associations is typically assessed by measuring "freezing" responses to subsequent presentations of the CS or training context alone. With respect to the brain areas supporting this form of learning, it is now widely accepted that fear conditioning to both the explicit CS and the context rely heavily on the amygdala (Bailey, Kim, Sun, Thompson, & Helmstetter, 1999; Blanchard & Blanchard, 1972; Helmstetter, 1992; Helmstetter & Bellgowan, 1994), and that the hippocampus is normally not involved in conditioning to an explicit CS (Kim & Fanselow, 1992; Phillips & LeDoux, 1992). However, the role of the hippocampus in contextual conditioning is less clear. In some cases, electrolytic or excitotoxic lesions of dorsal hippocampus (DH) prior to training have been found to disrupt the acquisition of contextual fear conditioning (Kim, Rison, & Fanselow, 1993; Phillips & LeDoux, 1992; Young, Bohenek, & Fanselow, 1994), while other studies report that DH lesions produced prior to training leave the acquisition of contextual fear conditioning intact (Maren, Aharonov, & Fanselow, 1997; Richmond et al., 1999).

Almost without exception, studies investigating the role of DH in contextual learning have defined "context" as the behavioral chamber in which CS–US pairings were presented. However, it is well established that the DH participates in most forms of spatial learning (Bannerman et al., 2002; Eichenbaum, Otto, & Cohen, 1994; Moser, Moser, & Andersen, 1993). Thus, operationalizing "context" as the place where training occurred presents potential interpretive difficulties.

Conceptual definitions of context typically emphasize the nature of the relationship between the US and other temporally and spatially diffuse aspects of the training event (see Balsam & Tomie, 1985). For example, Bannerman, Rawlins, and Good (2006) suggest that the training context is composed of the host of features of an environment or event that are present at the time learning occurs. Previous studies have demonstrated that a variety of stimuli can serve as contextual variables, including the background color of a response key (Thomas, 1985), the presence or absence of unimodal background stimuli (Otto, Cousens, & Rajewski, 1997; Otto & Poon, 2006; Schettino & Otto, 2001), tones of specific pitch (Hulse, Cynx, & Humpal, 1984), and internal states resulting from drug administration or food deprivation (Davidson & Jarrard, 1993; Hock & Bunsey, 1998; Overton, 1964). In light of the evidence that DH may participate in certain aspects of contextual conditioning, this variety within the class of events or stimuli that may function as contextual stimuli underscores the importance of examining neural

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substrates of explicitly nonspatial forms of contextual conditioning.

In the event of permanent damage to a structure, surrounding areas may be compromised, recruited, or otherwise affected, leading some to adopt instead of lesions the reversible technique of temporary inactivation. Since most hippocampal neurons contain the $GABA_A$ receptor (Chan-Palay, 1978), the $GABA_A$ agonist muscimol provides an effective means of examining the role of the hippocampus in learning while avoiding the complications of permanent lesions. Moreover, because the hippocampus can be inactivated either prior to training, prior to testing, or both, this technique provides a means of dissociating the role of the hippocampus in the acquisition versus maintenance or expression of learned associations. Using this technique, several studies have found DH to be critically involved in the acquisition or retrieval of contextual fear memory and context-specific fear expression without affecting contextual discrimination or performance of the freezing response (Corcoran & Maren, 2001; Holt & Maren, 1999; Matus-Amat, Higgins, Barrientos, & Rudy, 2004). While these studies suggest that the DH contributes in some way to acquisition or retrieval of associations between the US and the training chamber, the current studies use this reversible inactivation technique to explore the role of the DH in a nonspatial form of contextual conditioning.

We have recently demonstrated that lesions of the DH dramatically impair the acquisition of associations between an explicitly nonspatial olfactory contextual stimulus and the US (Otto & Poon, 2006). Although these results suggest that DH participates in nonspatial contextual fear conditioning, the permanent nature of the damage to DH precluded examination of the potentially differential role of the hippocampus in the acquisition versus maintenance of olfactory contextual fear memory. To this end, the current study examined the effect of temporary inactivation of DH at various time points before or after training in the same paradigm. Because both inactivation and lesions of the hippocampus often produce an alteration of locomotor behavior that may compete with freezing (see Anagnostaras, Gale, & Fanselow, 2001), the present study examined a number of behavioral responses in addition to the more commonly measured freezing response (Fanselow, 1980; Fendt & Fanselow, 1999). Finally, in order to examine whether inactivation of DH affected either olfactory perception or performance of the freezing response, we examined the effects of temporary inactivation of DH on the acquisition of an olfactory delay fear conditioning paradigm in which an odor served as the explicit CS.

2. General methods

All procedures were approved by Rutgers University's Institutional Animal Care and Use Committee.

2.1. Subjects

Subjects were 75 naïve male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 225–249 g on arrival, and were individually housed in plastic tubs on a 12-h light/dark cycle in an environment-controlled colony room. Animals had access to food and tap water *ad libitum*, and were handled 2 min daily for 5 days prior to surgery. All procedures took place during the light phase of the cycle.

2.2. Apparatus

Fear conditioning was conducted in a behavioral chamber ($30 \times 24 \times 27$ cm) enclosed in an aluminum sound-attenuating box ($56 \times 41 \times 42$ cm). Transparent Plexiglas constituted one pair of opposing walls and the ceiling, and aluminum composed the other pair of opposing walls. The chamber floor consisted of 16 stainless-steel rods (diameter 5 mm) equally spaced by 1.9 cm. These rods were connected to a shock generator (H13-15, Coulbourn Instruments, Allentown, PA) and delivered the scrambled footshock (0.5 mA) US. A sawdust-filled tray was placed under the grid floor. When appropriate, a computer-generated tone (3.9 kHz, 80 dB) was presented through a speaker mounted outside one of the aluminum chamber walls, and a single light bulb (29 V, 0.04A) was located 24.5 cm above the floor. A motion detector (model H24-61, Coulbourn Instruments, Allentown, PA) situated on top of the behavioral chamber allowed detection of movement via a hole drilled through the chamber ceiling.

Olfactory stimuli were presented via ports in the chamber ceiling, using a procedure described previously (Cousens & Otto, 1998; Herzog & Otto, 1997; Herzog & Otto, 1998; Otto & Poon, 2006; Otto et al., 1997). Operation of a solenoid valve directed clean air (1.5 l/min) to a 20-ml bottle containing 3 ml of either 15% pyridine in propylene glycol or strawberry extract (McCormick, Hunt Valley, MD). Odorized air was then directed to the conditioning chamber through Tygon tubing (1/8 in inner diameter) connected to an outlet port in the ceiling of the chamber. An exhaust fan mounted on the sound attenuating enclosure provided ventilation, directing the odorized air out to a vacuum pump; odor was eliminated from the inner behavioral chamber within 20 s of the solenoid closure. Training chambers were cleaned between sessions with commercially available cage cleaner (Research Laboratories Inc.).

The testing apparatus consisted of a separate behavioral chamber in a distal room. While the dimensions of the testing chamber were identical to those of the training chamber, the testing apparatus also contained the distinguishing features of a diagonally striped back wall and a solid black Plexiglas floor. A video camera was positioned in a corner of the sound-attenuating outer chamber and recorded all behavioral and paradigmatic events for later offline analysis. Alcohol wipes were used to clean the inner testing chamber between animals, further differentiating it from the chamber used during training.

2.3. Surgery

Subjects were anesthetized with an i.p. administration of a Ketamine (80 mg/kg)–Xylazine (12 ml/kg) mixture. The subject's head was then shaved and mounted in a stereotaxic frame (Kopf Instruments, Tujunga, CA), and the scalp cleaned with 20% Nolvasan solution. Bupivicaine (0.15 ml) was injected in multiple subcutaneous sites along the scalp midline. The scalp was incised, retracted, and fascia was removed from the skull. Burr holes were drilled bilaterally through the skull at sites overlying the DH (A/P −3.8, M/L ±2.5 from bregma), and four additional burr holes were drilled through the skull nearby; small stainless-steel screws (small parts, MX-0080-02FL-M) were inserted and tightened in these four holes. A double guide cannula (plastics one, Roanoke, VA) was implanted into the two holes above DH, reaching a depth of 2.2 mm ventral to dura. Dental acrylic was applied to both the implanted screws and the cannula to secure the cannula to the skull. The incision was then closed with stainless-steel surgical staples. An obturator was inserted into the guide cannula. After surgery subjects were placed in home cages where they were closely monitored for two days.

3. Experiment 1: effects of temporary inactivation of dorsal hippocampus on explicitly nonspatial, unimodal contextual fear learning

All training and testing procedures are summarized in Table 1, and described in detail below.

Table 1
Experimental design (Experiment 1)

	Training			Test Day One			Test Day Two	
Group	Pre-Training Infusion	Unsafe Contextual Stimulus	24 h	Pre-Testing Infusion	Contextual Stimulus Tested	24 h	Pre-Testing Infusion	Contextual Stimulus Tested
SAL-SAL (N = 11)	Saline	Pyridine or strawberry, counter-balanced within groups		Saline	Unsafe or safe, counter-balanced within groups		Saline	Unsafe or safe, counter-balanced within groups
MUS-SAL (N = 11)	Muscimol			Saline			Saline	
SAL-MUS (N = 11)	Saline			Muscimol			Muscimol	
MUS-MUS (N = 11)	Muscimol			Muscimol			Muscimol	

SAL, saline; MUS, muscimol.

3.1. Drug infusion

Subjects were randomly assigned to one of four groups that received either saline (SAL) or muscimol (MUS) 30 m prior to training, and either saline or muscimol prior to subsequent test sessions on two consecutive days. Two groups received either saline or muscimol prior to training sessions, and saline prior to each testing session (SAL–SAL, $n = 15$, and MUS–SAL, $n = 15$). Two additional groups received either saline or muscimol prior to training sessions, and muscimol prior to testing sessions (SAL–MUS, $n = 15$, and MUS–MUS, $n = 15$).

Between surgery and the onset of behavioral procedures, each subject was brought to the infusion room for 2 min every two days, and the pump was run in the background in order to acclimate subjects to the infusion room and the noise and procedures associated with infusion. Infusions began 10 days after surgery. Subjects were brought individually in clear plastic holding boxes to the infusion room, the obturator was removed and replaced with 30-gauge injection cannula which was attached via polyethylene tubing (PE-10) to 10-μL Hamilton syringes mounted in an infusion pump (Harvard Apparatus, South Natick, MA). Bilateral microinfusion of saline (0.9%, pH = 7.4) or muscimol (1 μg/μL dissolved in 0.9% saline; Sigma Aldrich, St. Louis, MO) occurred over a 1.5-min period. Each bilateral infusion introduced volumes of 0.25 μL saline or muscimol per hemisphere into DH. Rats were held during the infusion period to prevent dislodging the tubing, and held for an additional 2 min to allow diffusion of the muscimol or saline prior to removal of the injection cannula. The dummy cannulae and cap were then replaced, and the subject was returned to the holding box for 28 min before transporting to the training or testing room; thus in all cases, infusions were administered 30 min prior to training or testing sessions.

3.2. Olfactory contextual/auditory delay fear conditioning

Training took place 10 days after surgery in a single session, in one of two identical behavioral chambers (A and B) in the same room. Whether an animal received training in Chamber A or B was counterbalanced for each group. Each subject received an infusion of either saline or muscimol 30 min prior to training as described previously. During the 35-min training session, two olfactory contexts were presented in alternation, each for 5 min and separated from each other by 1-min inter-context-intervals. Thus, each 5 min context was presented three times in alternation. Strawberry extract and pyridine, presented at a flow rate of 1.5 l/min, served as olfactory contextual stimuli and were counterbalanced across groups. During context periods referred to as "safe", no auditory or footshock stimuli were presented; during each presentation of the "unsafe" context, subjects were exposed to three pairings of a 3.9 kHz-pure tone (~80 dB, 20 s) co-terminating with a footshock (2 s, 0.5 mA). Successive CS–US pairings were separated by a 1 min ITI. Therefore, a total of nine CS–US pairings was presented during training, each against the background of an "unsafe" unimodal olfactory contextual stimulus.

3.3. Testing

Testing began 24 h after training in a separate room and chamber, as described previously. Thirty minutes after an infusion of either saline or muscimol, subjects were placed in the testing chamber for 10 min. Testing sessions consisted of a 2 min baseline period followed by a 1 min presentation of one of the contextual stimuli. The tone CS was presented during minute 10. No other stimuli were explicitly presented during the intervening minutes. Responses to unsafe and safe contextual stimuli were assessed on consecutive days and were counterbalanced for each group (see Table 1). That is, for a subject in group SAL–MUS assigned to unsafe test Day 1, muscimol infusion first occurred prior to unsafe context testing on Day 1. Muscimol was again infused 24 h later on test Day 2, during which conditioning to the safe context was tested. Each subject therefore received two testing sessions separated by 24 h, with each test session preceded by the same drug infusion.

3.4. Primary measure of conditioned fear

The primary dependent measure of fear was freezing behavior, scored continuously by a human observer blind to the infusion condition of each subject. The onset and offset of freezing behavior (the adoption of rigid posture unaccompanied by body, extremity, head, or whisker movement except that required for respiration) was recorded via a hand switch that was continuously sampled by the computer. Freezing observations were transformed to the percent time spent freezing during each minute. Simultaneously, an infrared motion detector registered immobility and was sampled by the computer controlling stimulus presentation, yielding the percent time spent immobile during each minute of the testing session.

3.5. Secondary behavioral measures

Previous studies have found a high and positive correlation between freezing behavior assessed by trained human observers and immobility registered by automated apparata (Anagnostaras et al., 2001; Yoon & Otto, 2001). However, alterations of freezing or immobility behavior can potentially be explained by deficits in performance of the behavior of interest independent of an effect on learning *per se* (Anagnostaras et al., 2001; Holt & Maren, 1999). Thus, in addition to freezing and immobility, occurrences of whisking, grooming, ambulating, and rearing behavior were quantified. Data were collected offline from videotapes using a momentary time-sampling technique. Briefly, trained observers unaware of the experimental condition of the subject categorized behavior during each 5-s epoch of a session as one of the following: rearing (standing on hind legs with the upper limbs above midline), ambulating (consecutive gross motor movements that resulted in displacement of the rat in the horizontal plane), whisking (visible movement of vibrissae in the air or contacting surfaces), grooming (body part to body part contact), freezing (ri-

gid and motionless posture, except for respiration-related movement), or nonmovement (non-freezing but motionless posture, such as that exhibited while sleeping). These data were then converted into percentage of time spent engaged in each behavior during relevant testing periods.

3.6. Histology

Following the last testing session, each subject was anesthetized with sodium pentobarbitol (100 mg/kg, i.p.) and perfused transcardially with 0.9% saline and 10% buffered formalin solution. Brains were removed and stored in 30% sucrose solution (wt/vol) for at least 48 h, then frozen and sliced into coronal sections of 50 μm thickness. Slices were mounted on glass slides, stained with cresyl violet, and examined visually via a light microscope for verification of cannula placement in DH.

3.7. Statistical analysis

For analysis, each 10-min testing session was subdivided into four epochs: baseline (the first two minutes during which no stimuli were presented), safe or unsafe context presentation (the third minute during which the olfactory contextual stimuli were presented), pre-CS (the two minutes prior to CS presentation during which no stimuli were presented) and CS (the last minute of testing during which the auditory CS was presented). Freezing behavior during testing sessions was statistically analyzed using two-way repeated measures analyses of variance (ANOVAS), with treatment group as the between-subjects factor and epoch as the within-subjects factor. Subsequent pairwise comparisons were conducted using Student–Newman–Keuls (SNK) post-hoc tests.

3.8. Results

3.8.1. Cannula placement

Fig. 1 illustrates cannula placement in the dorsal hippocampus for each group. Because muscimol is expected to spread at least 1 mm below the site of infusion (Martin, 1991), subjects were retained for statistical analyses only if cannula tips were localized within the DH, above or within (but not below) area CA3. Following histological examination of the location of cannula tracks in DH, four subjects were removed from group SAL–SAL and three subjects were removed from the MUS–SAL, SAL–MUS, and MUS–MUS groups. Three additional subjects were excluded due to post-surgical complications. Thus all groups ultimately included 11 subjects in each condition.

3.8.2. Correlation between hand-scored freezing behavior and computer-detected immobility

Correlational analyses compared freezing data obtained by human observers to the computerized detection of immobility during testing. Two correlation coefficients were obtained for group SAL–SAL. First, the correlation between freezing and immobility during unsafe context presentations was 0.91 ($p < 0.0001$). Second, the correlation between freezing and immobility during safe context presentations was 0.76 ($p = 0.20$). In general, these correlations are similar to those previously reported in the literature comparing human observer scores of freezing.

3.8.3. Effects of particular contextual stimuli and day of unsafe context test

As mentioned previously, the specific odor that was used as the unsafe context was counterbalanced within groups. A *t*-test was conducted on freezing scores of the SAL–SAL group to determine whether performance differed as a function of the specific odor serving as the unsafe context. Results revealed no difference in freezing behavior between-subjects for whom the unsafe contextual stimulus was strawberry, and subjects for whom the unsafe contextual stimulus was pyridine ($t(9) = 0.93$, $p > 0.05$). Therefore, subsequent analyses collapsed data across unsafe contextual stimulus (odor).

Also counterbalanced within groups was the day on which conditioning to the unsafe context was assessed, with some subjects tested for unsafe contextual conditioning on Day 1, and others on Day 2. To evaluate whether the specific day on which unsafe con-

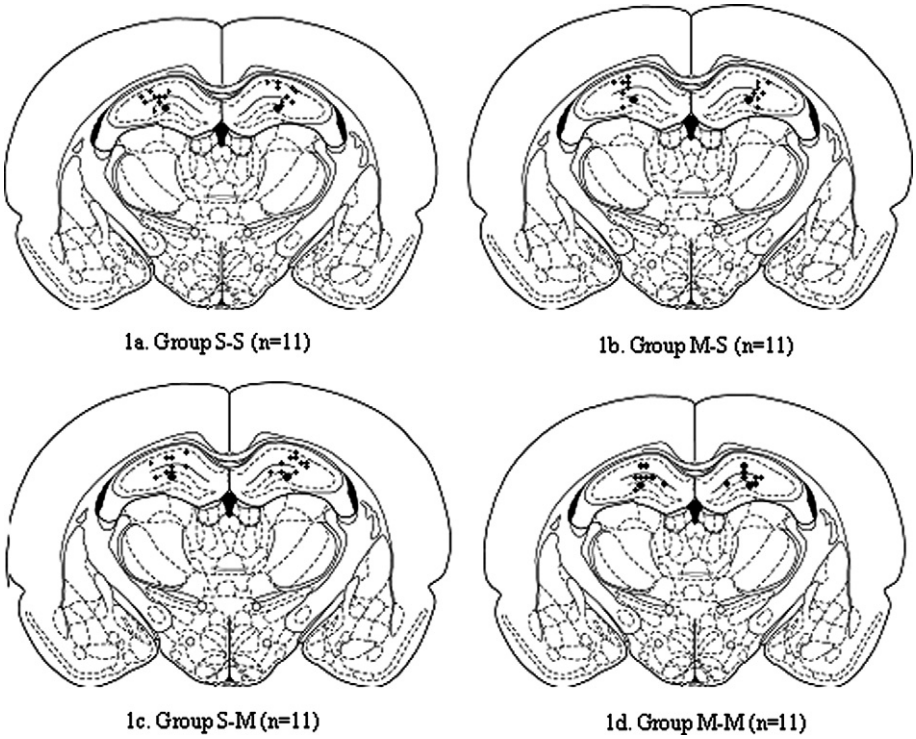

Fig. 1. Cannula placement in dorsal hippocampus for (a) group SAL–SAL, (b) group MUS–SAL, (c) group SAL–MUS, and (d) group MUS–MUS.

textual conditioning was assessed affected performance, a *t*-test compared freezing on unsafe context testing Day 1 versus unsafe context testing Day 2 for group SAL–SAL. This analysis revealed no difference between unsafe context testing days ($t(9) = 0.7$, $p > 0.05$). Data are thus collapsed across unsafe context test day.

3.8.4. Freezing to olfactory contexts and auditory CS

Data from the unsafe context testing session are presented in Fig. 2a. A two-way repeated measures ANOVA on freezing behavior during the unsafe context test revealed a main effect of period ($F(3,120) = 101.12$, $p < 0.0001$), while neither the main effect of group ($F(3,40) = 1.04$, $p > 0.05$) nor the interaction between group and period ($F(9,120) = 1.83$, $p = 0.069$) reached statistical significance. Post-hoc comparisons (Student–Newman–Keuls) were performed to further explore potential differences between groups, and revealed that the groups SAL–MUS, MUS–SAL, and MUS–MUS differed significantly from saline-treated controls (group SAL–SAL) only during the unsafe context period ($p < 0.05$).

Data from the safe context testing session are presented in Fig. 2b; a two-way repeated measures ANOVA on freezing behavior during the safe context test revealed a main effect of period ($F(3,120) = 107.05$, $p < 0.0001$), but no effect of group ($F(3,40) = 0.318$, $p > 0.05$) or group–period interaction ($F(9,120) = 0.439$, $p > 0.05$). Post-hoc comparisons (Student–Newman–Keuls) indicated that none of the groups differed from any other group at any point during the safe context test session.

3.8.5. Other behaviors recorded during test sessions

As described previously, the frequency of several other behaviors was scored off-line by trained observers blind to the infusion condition of subjects using a momentary time-sampling technique.

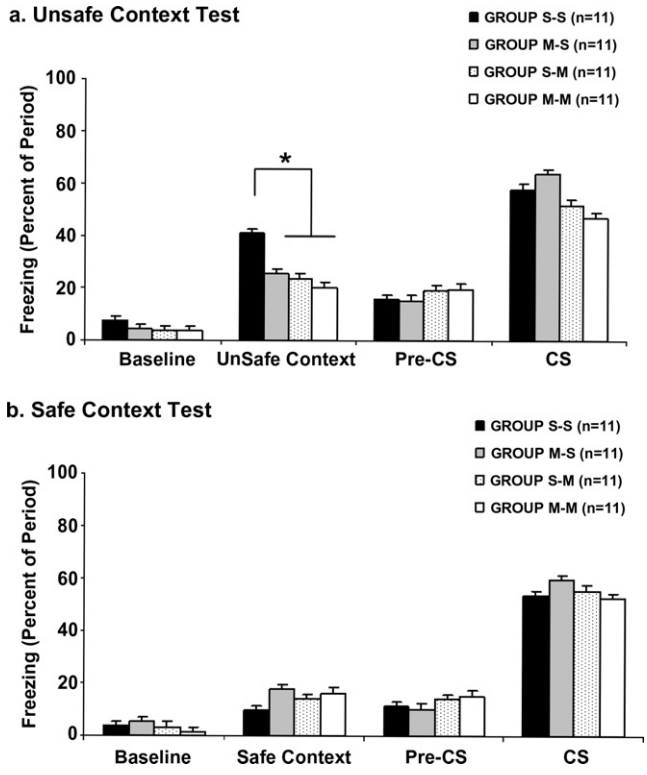

Fig. 2. Freezing behavior during unsafe (a) and safe (b) context test sessions. Data are presented for four periods: baseline (average of minutes 1 and 2 during which no stimuli were presented); unsafe or safe context presentation (minute 3); Pre-CS (average of minutes 8 and 9 during which no stimuli were presented); and CS presentation (minute 10). Asterisk indicates statistically significant differences between groups, as revealed by post-hoc comparisons (SNK).

Specifically, the animal's behavior was observed once every 5-s throughout the 10-min session, and the occurrence of rearing, ambulating, grooming, whisking, freezing, or nonmovement was recorded as described previously. The behavioral data recorded during baseline, unsafe period, pre-CS period, and CS presentation are presented in Fig. 3.

A two-way repeated measures ANOVA on freezing behavior revealed significant main effects of both group ($F(3,30) = 6.32$, $p = 0.0019$) and period ($F(4,120) = 90.54$, $p < 0.0001$), and a significant interaction between them ($F(12,120) = 3.27$, $p = 0.0004$). Although freezing was observed primarily during the unsafe context and the CS presentation, post-hoc comparisons (SNK) indicated that during the unsafe context, the three groups that had received muscimol froze significantly less than saline-treated control subjects ($p < 0.05$). All groups froze robustly during the CS presentation; post-hoc tests further revealed that group MUS–SAL froze significantly more than group SAL–SAL during the CS presentation. Interestingly, no groups differed with respect to the frequency of any other behavior during any of the periods (all Fs < 1.25).

4. Experiment 2: effects of temporary inactivation of dorsal hippocampus on olfactory delay fear conditioning

The results of Experiment 1 suggest that inactivation of DH impairs olfactory contextual conditioning while leaving conditioning to an explicit auditory CS intact. It is possible, however, that temporary inactivation of DH interferes with either olfactory perception or the generation or execution of the freezing response. Although previous data from our laboratory indicate clearly that lesions of DH have no effect on either olfactory perception or freezing behavior (Otto & Poon, 2006), previous studies have not directly tested the effects of temporary inactivation of DH on either of these variables. Thus, an additional experiment was performed to address these possibilities by examining the effect of temporary inactivation of DH on conditioning to an explicit olfactory CS using a delay conditioning procedure.

4.1. Procedure

Animals were trained 30 min after receiving a microinfusion of either saline ($n = 7$) or muscimol ($n = 8$). Training consisted of one delay fear conditioning session in which nine CS–US pairings were presented, separated by 1 min inter-trial intervals. The CS consisted of a 20 s odor presentation (strawberry or pyridine, counterbalanced within groups, delivered at a flowrate of 1.5 l/m) coterminating with a brief footshock (2 s, 0.5 mA). Twenty-four hours later, conditioned responses elicited by the explicit olfactory CS were assessed in a 6 min test session in which no stimuli were presented during minutes 1–2, and the odor CS was presented during minutes 3–6.

4.2. Results

Because the specific odor serving as the discrete conditioned stimulus was counterbalanced for each group, a two-way repeated measures analysis of variance compared freezing behavior to each of the olfactory stimuli for group SAL–SAL. Results revealed a significant main effect of minute ($F(5,25) = 5.52$, $p = 0.0015$), but neither the effect of odor ($F(1,5) = 0.0003$, $p > 0.05$) nor the odor–minute interaction ($F(5,25) = 0.239$, $p > 0.05$) reached statistical significance. Thus, for each group, subsequent data are collapsed across odor.

Fig. 4 illustrates freezing during baseline (the average of minutes 1 and 2) and during CS presentation (minute 3, when CS-elicited freezing is least likely affected by extinction). A two-way repeated measures ANOVA revealed a main effect of minute

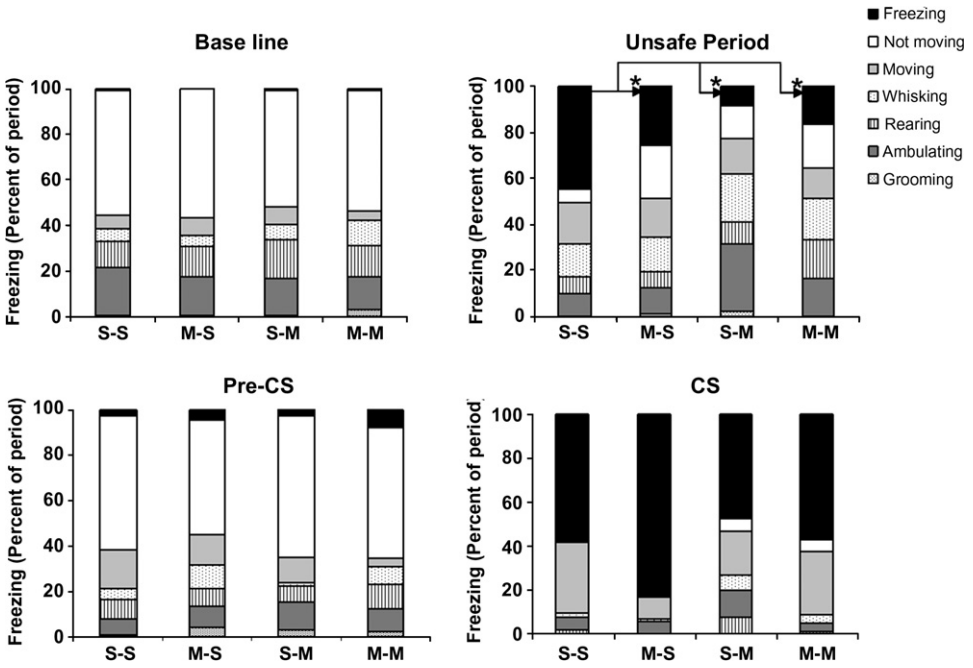

Fig. 3. Behavior allocation during the unsafe contextual stimulus test session. Freezing, rearing, grooming, ambulating, whisking, and other behaviors were recorded during the baseline period, the unsafe period, the pre-CS period, and the CS presentation using a time-sampling method (window of observation, 5 s). Asterisk indicates statistically significant differences between groups revealed by post-hoc comparisons (SNK).

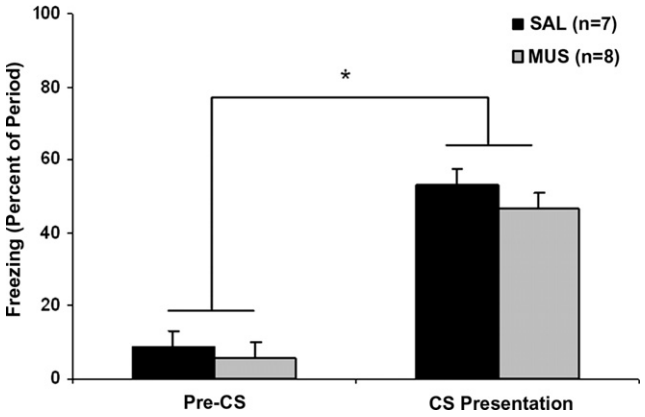

Fig. 4. Freezing behavior to the explicit olfactory CS in Experiment 2. Baseline period: average of minutes 1 and 2; CS presentation: minute 3. For both groups, freezing behavior during the CS presentation differed significantly from that during baseline (asterisk), while there was no difference between SAL and MUS groups.

($F(5,65) = 14.45$, $p < 0.0001$), while neither the main effect of group ($F(1,13) = 0.009$, $p > 0.05$) nor the group by period interaction ($F(5,65) = 0.395$, $p > 0.05$) reached significance.

5. Discussion

The present results suggest that the temporary inactivation of DH, whether prior to training, prior to testing, or both, dramatically impairs olfactory contextual conditioning. By contrast, inactivation of DH spared the acquisition and expression of conditioned freezing to an explicit auditory or olfactory CS, and had no effect on ambulation, grooming, rearing, or whisking behavior. The selective deficit in olfactory contextual conditioning is likely not attributable to stimulus modality, day of testing, or the specific odor used as the "unsafe" contextual stimulus. These data provide further evidence that DH participates in the association of a US with temporally and spatially diffuse contextual stimuli.

The current study employed multiple measures both in order to rule out potential performance accounts of any deficits observed in animals with DH inactivation, and to assess whether behaviors other than freezing were systematically related to contextual fear memory. The data suggest that muscimol has no effect on rearing, ambulating, grooming, or whisking, indicating that the ability of animals to physically execute the responses of interest was not likely compromised by inactivation of DH. This conclusion is strengthened by the fact that even for groups with low levels of freezing behavior during presentation of olfactory contextual stimuli, robust freezing occurred later in the same test session during presentation of the auditory CS, and in Experiment 2, during presentation of an olfactory CS. Thus, consistent with previous data from our laboratory (Otto & Poon 2006), the deficits in olfactory contextual conditioning reported here are likely not due to the inability of animals to perceive the stimuli of interest or to perform the freezing response.

In studies examining the effect of temporary inactivation on contextual conditioning, one precondition for interpreting effects is first to rule out state-dependent learning (Bannerman et al., 2004; Overton, 1964). To this end, the present experiment included a group (MUS–MUS) receiving muscimol prior to both training and testing. If deficits in animals receiving muscimol either prior to training or testing reflected state-dependency, group MUS–MUS would be expected to effectively acquire and express contextual fear conditioning much like that seen in group SAL–SAL. However, the present results indicate a dramatic impairment in group MUS–MUS, suggesting state-dependent learning cannot account for the reduction in freezing behavior observed in the MUS–SAL and SAL–MUS groups.

Although it is widely acknowledged that a variety of stimuli can serve effectively as contextual variables (Otto et al., 1997; Otto & Poon, 2006; Overton, 1964; Randrich & Ross, 1985), studies examining hippocampal participation in contextual fear conditioning typically operationalize the context as the behavioral chamber in which training occurred (Anagnostaras et al., 2001; Chang, Chen, & Liang, 2008; Chen, Kitanishi, Ikeda, Matsuki, & Yamada, 2007;

Hein et al., 2007; Kim et al., 1993; Maren & Fanselow, 1997; Phillips & LeDoux, 1992; Young et al., 1994). The present results are consistent with the notion that spatial and other forms of contextual conditioning may be dissociable processes (Bannerman et al., 2004; Richmond et al., 1999) with overlapping neural substrates (Anderson et al., 2006; Barry et al., 2006).

The results of several studies suggest that DH lesions prior to training impair the acquisition of contextual conditioning (Kim et al., 1993; Maren & Fanselow, 1997; Phillips & LeDoux, 1992; Young et al., 1994), although other studies have reported only retrograde—and not anterograde—impairments in contextual learning after such damage (Anagnostaras et al., 2001; Maren et al., 1997; Richmond et al., 1999). Discrepancies such as these have led some to conclude that the hippocampus is less involved in the acquisition of contextual fear than its subsequent recall (Bannerman et al., 2004; Richmond et al., 1999). Attempting to explain cases in which an anterograde deficit was observed following DH damage, it has been suggested these effects may be due to damage to fibers of passage between dorsal and ventral hippocampus (Bannerman et al., 2004; Maren et al., 1997). The current study speaks specifically to this controversy in that the $GABA_A$ agonist muscimol does not destroy fibers of passage, and yet the reversible inactivation of DH produced both anterograde and retrograde deficits in one form of contextual fear conditioning. The fact that olfactory contextual conditioning is adversely affected by the reversible inactivation of DH, whether occurring prior to or following training, suggests that this region participates in both the acquisition and retention of explicitly nonspatial fear conditioning.

The present data suggest that both the acquisition and retrieval of an association between unimodal contexts and an aversive US depend on neurons within one or more subregions within the dorsal hippocampus. Since muscimol inactivation extends to brain tissue located up to 2 mm from the infusion location (Edeline, Hars, Hennevin, & Cotillon, 2002), our infusion likely inactivated portions of dorsal CA1 and CA3, as well as the dentate gyrus. While accumulating data suggest that there are likely dissociable contributions of these areas to many types of hippocampal-dependent learning (cf. Goodrich-Hunsaker, Hunsaker, & Kesner, 2008), the present data do not permit an examination of this issue. Moreover, although to our knowledge there exists no direct examination of DH neuronal firing patterns within these regions during performance of an olfactory fear conditioning paradigm, recent data suggest that the firing patterns of DH CA1 neurons vary in a context-specific manner during contextual fear learning (Moita, Rosis, Zhou, LeDoux, & Blair, 2004). Thus DH is likely actively engaged during the acquisition of olfactory contextual fear conditioning.

The current paradigm permitted the assessment of conditioned responses to the safe and the unsafe contextual stimuli on separate testing days. Thus, freezing evoked by two separate olfactory stimuli could be compared without potential confounds that might have resulted from evaluating both stimuli in a single test session. Of course, this design raises the possibility that for subjects presented with one contextual stimulus on the first testing day, extinction could account for reduced freezing to the stimulus presented on day two. Importantly, an analysis of freezing to the unsafe contextual stimulus as a function of test day indicated that for group SAL–SAL, freezing was unrelated to day of testing.

There is now widespread agreement that the hippocampus is not critically involved in the acquisition of discrete CS–US associations (Anagnostaras et al., 2001; Lopez-Fernandez et al., 2007; Maren et al., 1997; Otto & Poon, 2006; Phillips & LeDoux, 1992; Schafe, Nader, Blair, & LeDoux, 2001). The results of Experiments One and Two are consistent with this notion, and further suggest that the temporary inactivation of DH has no effect on either the execution of the freezing response *per se* or on olfactory or auditory perception.

Data from a wide range of both neuropsychological and electrophysiological studies suggest that the hippocampus serves mnemonic functions in a variety of behavioral tasks, including spatial learning, contextual fear conditioning, and trace conditioning (Anagnostaras, Maren, & Fanselow, 1999; Beylin et al., 2001; Chang et al., 2008; Da Silva, Bevilaqua, Medina, Izquierdo, & Cammarota, 2008; Dalla, Bangasser, Edgecomb, & Shors, 2007; Hubbard, Nakashima, Lee, & Takahashi, 2007; Jarrard, 1983; Jones, Pearce, Davies, Good, & McGregor, 2007; Otto & Poon, 2006; Solomon, Vander Schaaf, Thompson, & Weisz, 1986; Steele & Morris, 1999; Yoon & Otto, 2007). While it is possible that each of these tasks requires a different "type" of hippocampal processing, a more parsimonious view is that each of these hippocampal-dependent tasks relies on a common aspect of hippocampal function. As we have argued previously (Otto & Poon, 2006; Yoon & Otto, 2007), this process may involve attending to and/or representing spatially and temporally diffuse features of the training event. Generally, performance in paradigms involving either space or a time interval between events or stimuli require the hippocampus while those in which CS and US or other behavioral or paradigmatic events overlap do not (Moyer, Deyo, & Disterhoft, 1990; Solomon et al., 1986; Yoon & Otto, 2007). The potential role of the hippocampus in representing events with diffuse or imprecise temporal qualities is consistent with the predictions of several biologically realistic models of hippocampal function (Levy, 1996; Wallenstein, Eichenbaum, & Hasselmo, 1998; Wallenstein & Hasselmo, 1997). Specifically, Wallenstein and Hasselmo (1997) suggest that relationships of this sort are mediated by sparsely interconnected networks of pyramidal cells in hippocampal layer CA3. According to this model, these "context fields" underlie the representation of stimuli whose temporal relationship falls outside the parameters normally required for the induction of synaptic plasticity. Thus, the association among temporally discontiguous events or items may be preserved by such context fields and accessed when relevant. The data reported here are consistent with this notion, and further suggest that the neural processes underlying other hippocampal-dependent tasks are similar to those underlying olfactory contextual conditioning.

While a number of studies have concluded that selective damage of DH impairs either the acquisition or retention of contextual conditioning when the context is defined as the chamber in which training occurred (Anagnostaras et al., 2001; Kim et al., 1993; Maren et al., 1997; Maren & Fanselow, 1997; Phillips & LeDoux, 1992; Richmond et al., 1999; Young et al., 1994), compelling recent evidence suggests that space is not the only contextual variable encoded by the hippocampus (Anderson et al., 2006; Otto & Poon, 2006). Results of the present study support this notion, and further suggest that theories of hippocampal function need to account for forms of explicitly nonspatial contextual learning. The time-course and precise characterization of dorsal hippocampal participation in these explicitly nonspatial forms of contextual learning remains to be determined.

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References

Anagnostaras, S. G., Gale, G. D., & Fanselow, M. S. (2001). The hippocampus and contextual fear conditioning: Recent controversies and advances. *Hippocampus, 11*, 8–17.

Anagnostaras, S. G., Maren, S., & Fanselow, M. S. (1999). Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: Within-subjects examination. *Journal of Neuroscience, 19*, 1106–1114.

Anderson, M. I., Killing, S., Morris, C., O'Donoghue, A., Onyiagha, D., Stevenson, R., Verriotis, M., & Jeffrey, K. J. (2006). Behavioral correlates of the distributed coding of spatial context. *Hippocampus, 16*, 730–742.

Bailey, D. J., Kim, J. J., Sun, W., Thompson, R. F., & Helmstetter, F. J. (1999). Acquisition of fear conditioning in rats requires the synthesis of mRNA in the amygdala. *Behavioral Neuroscience, 113*, 276–282.

Balsam, P. D., & Tomie, A. (1985). *Context and learning*. Hillsdale, NJ: Erlbaum.
Bannerman, D. M., Deacon, R. M. I., Offen, S., Friswell, J., Grubb, M., & Rawlins, J. N. P. (2002). A double dissociation of function within the hippocampus: Spatial memory and hyponeophagia. *Behavioral Neuroscience, 116*, 884–901.
Bannerman, D. M., Rawlins, J. N. P., & Good, M. A. (2006). The drugs don't work-or do they? Pharmacological and transgenic studies of the contribution of NMDA and GluR-A-containing AMPA receptors to hippocampal-dependent memory. *Psychopharmacology, 188*, 552–566.
Bannerman, D. M., Rawlins, J. N. P., McHugh, S. B., Deacon, R. M. J., Yee, B. K., Bast, T., Zhang, W.-N., Pothuizen, H. J., & Feldon, J. (2004). Regional dissociations within the hippocampus-–memory and anxiety. *Neuroscience and Biobehavioral Reviews, 28*, 273–283.
Barry, C., Lever, C., Hayman, R., Hartley, R., Burton, S., O'Keefe, J., Jeffrey, K. J., & Burgess, N. (2006). The boundary vector cell model of place cell firing and spatial memory. *Reviews in Neuroscience, 17*, 71–97.
Beylin, A. V., Gandhi, C. C., Wood, G. E., Talk, A. C., Matzel, L. D., & Shors, T. J. (2001). The role of the hippocampus in trace conditioning: Temporal discontinuity or task difficulty? *Neurobiology of Learning and Memory, 76*, 447–461.
Blanchard, D. C., & Blanchard, R. J. (1972). Innate and conditioned reactions to threat in rats with amygdaloid lesions. *Journal of Comparative Physiology and Psychology, 81*, 281–290.
Chang, S. D., Chen, D. Y., & Liang, K. C. (2008). Infusion of lidocaine in to the dorsal hippocampus before or after the shock training phase impaired conditioned freezing in a two-phase training task of contextual fear conditioning. *Neurobiology of Learning and Memory, 89*, 95–105.
Chan-Palay, V. (1978). Quantitative visualization of gamma-aminobutyric acid receptors in hippocampus and area dentata demonstrated by 3H muscimol autoradiography. *Proceedings of the National Academy of Science, 75*, 2516–2520.
Chen, J., Kitanishi, T., Ikeda, T., Matsuki, N., & Yamada, M. K. (2007). Contextual learning induces an increase in the number of hippocampal CA1 neurons expressing high levels of BDNF. *Neurobiology of Learning and Memory, 88*, 409–415.
Corcoran, K. A., & Maren, S. (2001). Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *Journal of Neuroscience, 21*, 1720–1728.
Cousens, G., & Otto, T. (1998). Both pre- and post-training lesions of the basolateral amygdala abolish the expression of olfactory and contextual fear conditioning. *Behavioral Neuroscience, 112*, 1092–1103.
Da Silva, W. C., Bonini, J. S., Bevilaqua, L. R. M., Medina, J. H., Izquierdo, I., & Cammarota, M. (2008). Inhibition of mRNA synthesis in the hippocampus impairs consolidation and reconsolidation of spatial memory. *Hippocampus, 18*, 29–39.
Dalla, C., Bangasser, D. A., Edgecomb, C., & Shors, T. J. (2007). Neurogenesis and learning: Acquisition and asymptotic performance predict how many new cells survive in the hippocampus. *Neurobiology of Learning and Memory, 88*, 143–148.
Davidson, T. L., & Jarrard, L. E. (1993). A role for the hippocampus is the utilization of hunger signals. *Behavioral and Neural Biology, 59*, 167–171.
Edeline, J., Hars, B., Hennevin, E., & Cotillon, N. (2002). Muscimol diffusion after intracerebral microinjections: A reevaluation based on electrophysiological and autoradiographic quantifications. *Neurobiology of Learning and Memory, 78*, 100–124.
Eichenbaum, H., Otto, T., & Cohen, N. J. (1994). Two functional components of the hippocampal memory system. *Behavioral and Brain Sciences, 17*, 449–517.
Fanselow, M. (1980). Conditional and unconditional components of post shock freezing in rats. *Pavlovian Journal of Biological Science, 15*, 177–182.
Fendt, M., & Fanselow, M. S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neuroscience and Biobehavioral Reviews, 23*, 743–760.
Goodrich-Hunsaker, N. J., Hunsaker, M. R., & Kesner, R. P. (2008). The interactions and dissociations of the dorsal hippocampus subregions: How the dentate gyrus, CA3, and CA1 process spatial information. *Behavioral Neuroscience, 122*, 16–26.
Hein, A. M., Stutzman, D. L., Bland, S. T., Barrientos, R. M., Watkins, L. R., Rudy, J. W., & Maier, S. F. (2007). Prostaglandins are necessary and sufficient to induce contextual fear learning impairments after interleukin-1 beta injections into the dorsal hippocampus. *Neuroscience, 150*, 754–763.
Helmstetter, F. J. (1992). Contribution of the amygdala to learning and performance of conditional fear. *Physiology and Behavior, 51*, 1271–1276.
Helmstetter, F. J., & Bellgowan, P. S. (1994). Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. *Behavioral Neuroscience, 108*, 1005–1009.
Herzog, C., & Otto, T. (1997). Odor-guided fear conditioning. II. Lesions of anterior perirhinal cortex disrupt fear conditioned to the explicit CS but not to the training context. *Behavioral Neuroscience, 111*, 1265–1274.
Herzog, C. D., & Otto, T. (1998). Contributions of anterior perirhinal cortex to olfactory and contextual fear conditioning. *Neuroreport, 9*, 1855–1859.
Hock, B. J., & Bunsey, M. D. (1998). Differential effects of dorsal and ventral hippocampal lesions. *Journal of Neuroscience, 18*, 7027–7032.
Holt, W., & Maren, S. (1999). Muscimol inactivation of the dorsal hippocampus impairs contextual retrieval of fear memory. *Journal of Neuroscience, 19*, 9054–9062.
Hubbard, D. T., Nakashima, B. R., Lee, I., & Takahashi, L. K. (2007). Activation of basolateral amygdala corticotropin-releasing factor 1 receptors modulates the consolidation of contextual fear. *Neuroscience, 150*, 818–821.
Hulse, S. H., Cynx, J., & Humpal, J. (1984). Absolute and relative pitch discrimination in serial pitch perception by birds. *Journal of Experimental Psychology: General, 113*, 38–54.
Jarrard, L. E. (1983). Selective hippocampal lesions and behavior: Effects of kainic acid lesions on performance of place and cue tasks. *Behavioral Neuroscience, 97*, 251–259.
Jones, P. M., Pearce, J. M., Davies, V. J., Good, M. A., & McGregor, A. (2007). Impaired processing of local geometric features during navigation in a water maze following hippocampal lesions in rats. *Behavioral Neuroscience, 121*, 1258–1271.
Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256*, 657–677.
Kim, J. J., Rison, R. A., & Fanselow, M. S. (1993). Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. *Behavioral Neuroscience, 197*, 1093–1098.
Levy, W. B. (1996). A sequence predicting CA3 is a flexible associator that learns and uses context to solve hippocampal-like tasks. *Hippocampus, 6*, 579–591.
Lopez-Fernandez, M., Montaron, M., Varea, E., Rougon, G., Venero, C., Abrous, D. N., & Sandi, C. (2007). Upregulation of polysialylated neural cell adhesion molecule in the dorsal hippocampus after contextual fear conditioning is involved in long-term memory formation. *Journal of Neuroscience, 27*, 4552–4561.
Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behavioral Brain Research, 88*, 261–274.
Maren, S., & Fanselow, M. S. (1997). Electrolytic lesions of the dorsal hippocampus, fimbria-fornix, or entorhinal cortex produce anterograde deficits in contextual fear conditioning in rats. *Neurobiology of Learning and Memory, 67*, 142–149.
Martin, J. H. (1991). Autoradiographic estimation of the extent of reversible inactivation produced by microinjection of lidocaine and muscimol in the rat. *Neuroscience Letters, 127*, 160–164.
Matus-Amat, P., Higgins, E. A., Barrientos, R. M., & Rudy, J. (2004). The role of the dorsal hippocampus in the acquisition and retrieval of context memory representations. *Journal of Neuroscience, 24*, 2431–2439.
Moita, M. A., Rosis, S., Zhou, Y., LeDoux, J. E., & Blair, H. T. (2004). Putting fear in its place: Remapping of hippocampal place cells during fear conditioning. *Journal of Neuroscience, 24*, 7015–7023.
Moser, E., Moser, M. B., & Andersen, P. (1993). Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but it is hardly present following ventral lesions. *Journal of Neuroscience, 13*, 3916–3925.
Moyer, J. R., Jr., Deyo, R. A., & Disterhoft, J. F. (1990). Hippocampectomy disrupts trace eye-blink conditioning in rabbits. *Behavioral Neuroscience, 104*, 243–252.
Otto, T., Cousens, G. A., & Rajewski, K. (1997). Odor-guided fear conditioning in rats: Acquisition, retention, and latent inhibition. *Behavioral Neuroscience, 111*, 1257–1264.
Otto, T., & Poon, P. (2006). Dorsal hippocampal contributions to unimodal contextual conditioning. *The Journal of Neuroscience, 26*, 6603–6609.
Overton, D. A. (1964). State-dependent or "dissociated" learning produced with pentobarbital. *Journal of Comparative and Physiological Psychology, 5*, 3–12.
Phillips, R., & LeDoux, J. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience, 106*, 274–285.
Randrich, A., & Ross, R. T. (1985). Contextual stimuli mediate the effects of pre- and post-exposure to the unconditioned stimulus on conditioned suppression. In P. D. Balsam & A. Tomie (Eds.), *Context and learning* (pp. 105–132). Hillsdale, NJ: Erlbaum.
Richmond, M. A., Yee, B. K., Pouzet, B., Veenan, L., Rawlins, J. N. P., Feldon, J., & Bannerman, D. M. (1999). Dissociating context and space within the hippocampus: Effects of complete, dorsal, and ventral excitotoxic hippocampal lesions on conditioned freezing and spatial learning. *Behavioral Neuroscience, 113*, 1189–1203.
Schafe, G. E., Nader, K., Blair, H. T., & LeDoux, J. E. (2001). Memory consolidation of Pavlovian fear conditioning: A cellular and molecular perspective. *Trends in Neuroscience, 24*, 540–546.
Schettino, L. F., & Otto, T. (2001). Patterns of Fos expression in the amygdala and ventral perirhinal cortex induced by training in an olfactory fear conditioning paradigm. *Behavioral Neuroscience, 115*, 1257–1272.
Solomon, P. R., Vander Schaaf, E. R., Thompson, R. F., & Weisz, D. J. (1986). Hippocampus and trace conditioning of the rabbit's classically conditioned nictitating membrane response. *Behavioral Neuroscience, 100*, 729–744.
Steele, R. J., & Morris, R. G. (1999). Delay-dependent impairment of a matching-to-place task with chronic and intrahippocampal infusion of the NMDA-antagonist D-AP5. *Hippocampus, 9*, 118–136.
Thomas, D. R. (1985). Contextual stimulus control of operant responding in pigeons. In P. D. Balsam & A. Tomie (Eds.), *Context and learning* (pp. 295–321). Hillsdale, NJ: Erlbaum.
Wallenstein, G. V., Eichenbaum, H., & Hasselmo, M. E. (1998). The hippocampus as an associator of discontiguous events. *Trends in Neuroscience, 21*, 317–323.
Wallenstein, G. V., & Hasselmo, M. E. (1997). GABAergic modulation of hippocampal population activity: Sequence learning, place field development, and the phase precession effect. *Journal of Neurophysiology, 78*, 393–408.
Yoon, T., & Otto, T. (2001). Contributions of the dorsal and ventral hippocampus to trace fear conditioning in rats. *Society for Neuroscience Abstracts, 89*, 14.
Yoon, T., & Otto, T. (2007). Differential contributions of dorsal vs. ventral hippocampus to auditory trace fear conditioning. *Neurobiology of Learning and Memory, 87*, 464–475.
Young, S. L., Bohenek, D. L., & Fanselow, M. S. (1994). NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: Immunization against amnesia by context preexposure. *Behavioral Neuroscience, 108*, 19–29.