Differential contributions of dorsal vs. ventral hippocampus to auditory trace fear conditioning

Taejib Yoon, Tim Otto *

Program in Behavioral Neuroscience, Department of Psychology, Rutgers University, New Brunswick, NJ 08854, USA

Abstract

The effect of excitotoxic lesions of dorsal vs. ventral hippocampus on the acquisition and expression of auditory trace fear conditioning was examined in two studies. In Experiment 1, animals received excitotoxic lesions of either the dorsal or ventral hippocampus or sham surgeries one week prior to conditioning, and were tested 24 h later. In Experiment 2, animals received excitotoxic lesions of either the dorsal or ventral hippocampus or sham surgeries 24 h after training, and were tested one week after surgery. Both pre- and post-training lesions of ventral hippocampus impaired the acquisition and expression, respectively, of auditory trace fear conditioning. Pre-training lesions of dorsal hippocampus had no effect on the acquisition of trace fear conditioning, while post-training lesions of dorsal hippocampus dramatically impaired expression during subsequent testing. Although in some cases animals with lesions of ventral hippocampus exhibited locomotor hyperactivity, it is unlikely that the pattern of observed deficits can be attributed to this effect. Collectively these data suggest that the dorsal and ventral hippocampus may contribute differentially to the mnemonic processes underlying fear trace conditioning.
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1. Introduction

Converging lines of evidence suggest that different regions within the hippocampus can play functionally distinct roles in memory (Moser & Moser, 1998; Richmond et al., 1999). Specifically, findings from recent studies indicate that the hippocampus is a heterogeneous structure, with both anatomical (Burwell & Amaral, 1998; Pitkanen, Pikkarainen, Nurminen, & Ylinen, 2000; Risold & Swanson, 1996) and functional (Bannerman et al., 1999; Maren & Holt, 2004; Richmond et al., 1999; Rogers, Hunsaker, & Kesner, 2006) dissociations along its septotemporal axis, supporting the view that the dorsal and ventral hippocampus may be involved in discrete processes that separately mediate some forms of memory (Moser & Moser, 1998).

The results of a number of studies suggest that the hippocampus plays a particularly prominent role in some forms of trace conditioning, a Pavlovian conditioning paradigm in which the offset of the conditioned stimulus (CS) precedes the onset of the unconditioned stimulus (US). Specifically, hippocampal damage has been shown to impair performance in both trace eyeblink (Moyer, Deyo, & Disterhoft, 1990; Solomon, Schaaf, Thompson, & Weisz, 1986) and trace fear conditioning (Chowdhury, Quinn, & Fanselow, 2005; McEchron, Bouwmeester, Tseng, Weiss, & Disterhoft, 1998; Quinn, Oommen, Morrison, & Fanselow, 2002) paradigms. With respect to trace fear conditioning, acquisition of hippocampal-dependent CS–US associations necessarily relies on interactions between the hippocampus and the amygdala, a structure known to be critically involved in both the acquisition and expression of fear conditioning in general (LeDoux, 2000). Anatomically, the ventral, but not the dorsal, hippocampus makes direct connections to the amygdala

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* Corresponding author. Fax: +1 732 445 2263.
  E-mail address: totto@rci.rutgers.edu (T. Otto).

(Pitkanen et al., 2000). Specifically, the CA1 region within the ventral hippocampus projects to the basal, accessory basal, and amygdalohippocampal transition area of the amygdala (Canteras & Swanson, 1992; Pitkanen et al., 2000), while the dorsal hippocampus projects to the amygdala only via the ventral hippocampus (Pitkanen et al., 2000). These anatomical considerations suggest that trace fear conditioning may depend more on the ventral hippocampus than on the dorsal hippocampus. However, although prior studies have examined the effect of either complete aspirative hippocampal lesions prior to conditioning (McEchron et al., 1998), or post-conditioning excitotoxic lesions of the dorsal hippocampus (Chowdhury et al., 2005; Quinn et al., 2002) only one study to date has systematically examined the effects of pre-training, excitotoxic lesions of dorsal vs. ventral hippocampus on the acquisition of trace fear conditioning (Rogers et al., 2006); the results of this study indicate that neither dorsal nor ventral hippocampal lesions affected the acquisition of trace fear conditioning, while ventral damage alone impaired the retention of trace fear conditioning over a 48 h retention interval. These data are consistent with the notion that the dorsal and ventral subfields may participate differentially in some aspects of trace fear conditioning.

The goal of the present experiments was to further explore the extent to which the dorsal and ventral hippocampus are differentially involved in trace fear conditioning to a discrete auditory CS. Specifically, separate experiments examined the effects of pre-training and post-training excitotoxic lesions of the dorsal or ventral hippocampal subfields on the acquisition and expression of CS–US associations. Because hippocampal lesions often produce hyperactivity, a behavior in competition with the freezing response used to assess conditioning, lesion-induced hyperactivity was also examined. Collectively, the data suggest that while lesions of the ventral, but not dorsal, hippocampus dramatically impair the acquisition of auditory trace fear conditioning, both ventral and dorsal hippocampal lesions after training impair the expression of trace conditioning during testing. This pattern of deficits is not likely to be due to lesion-induced hyperactivity.

2. General methods

Methods common to both experiments are described in detail below. All procedures have been approved by Rutgers University's Institutional Animal Care and Use Committee.

2.1. Subjects

Sixty-nine naive male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing between 225 and 275 g at the time of surgery were used as subjects. All subjects were housed individually in hanging metal wire mesh cages in a colony on a 12-hr light/dark cycle with lights on at 7 a.m. All behavioral procedures occurred during the light cycle. Subjects were provided with ad libitum access to food and water. Subjects were handled for 3 min daily for 5 days prior to surgical procedures and behavioral training.

2.2. Apparatus

2.2.1. Fear conditioning and testing chambers

Auditory trace fear conditioning was conducted in two identical behavioral chambers ($30 \times 24 \times 27$ cm) from BRS/LVE (Beltsville, MD). Each chamber was enclosed within an aluminum sound-attenuating enclosure ($56 \times 41 \times 42$ cm). A pair of opposing walls and the ceiling of the conditioning chamber were made of transparent Plexiglas; another pair of opposing walls were made of aluminum. The floor of the chamber consisted of 16 stainless steel rods (5 mm in diameter), equally spaced from each other by 1.9 cm. These rods were connected to a shock generator (model H13-15, Coulbourn Instruments, Allentown, PA), and served to deliver the scrambled footshock US. A tray filled with sawdust was placed under the grid floor. Each chamber was equipped with a computer-activated tone generator (3.9 kHz, 80 dB) and speaker mounted 14 cm above the floor outside one of the aluminum walls. A single light bulb (28 V, 0.04 A) was situated 10.5 cm above where the tone generator was mounted.

An infrared motion detector with fresnel lens, dual element differential detector (13 nM infrared radiation), and 90-deg viewing angle (model H24-61, Coulbourn Instruments, Allentown, PA) was mounted on the ceiling of each chamber. Relative changes in detected energy produced by movement provided a continuous output for the entire duration of that movement or consecutive movements with an inter-event interval of less than 400 ms. This output was directed to the computer that also controlled all paradigmatic events, which sampled the detector at 1 Hz and recorded both movement and immobility throughout the entire session.

Testing (either 24 h or 8 days following conditioning) was conducted in a separate chamber located in a different experimental room. The testing chambers had the same configuration as those used for conditioning except that the floor of the testing chamber was covered with black Plexiglas, and the transparent Plexiglas walls were modified with alternating black and white stripes in order to provide the testing chamber with different visual cues from the conditioning chambers. As during conditioning, movement and immobility were recorded throughout the entire session.

2.2.2. Open-field chamber

Locomotor activity was measured in a walled square chamber ($85 \times 85 \times 30$ cm) made of black Plexiglas. White lines on the floor divided the chamber floor into thirty-six 14 cm squares. The chamber was placed on a table (73 cm high) in the center of a novel room relative to those used for conditioning and testing. The room was lit with a light fixture (65 W) placed 90 cm above the center of the chamber. An experimenter unaware of the experimental condition of the animal was seated approximately 1 m from the open-field chamber and manually recorded locomotor activity.

2.3. Procedure

2.3.1. Surgery

Subjects were treated with atropine (0.4 mg/kg, ip) before surgery to suppress respiratory secretion. Subjects were then anesthetized with sodium pentobarbital (50 mg/kg, ip). The subject's head was shaved and mounted in a stereotaxic frame (Kopf Instruments, Tujunga, CA). The shaved area was cleaned with 20% Nolvasan solution and the local anesthetic bupivicaine (0.1 ml, 0.25%) was injected at several locations below the scalp. The scalp was then incised and retracted, and six small burr holes (three per hemisphere) were drilled into the skull over the intended lesion sites. Lesions were produced by infusions of NMDA (20 μg/ μl; Sigma, St. Louis, MO), dissolved in 0.1 M phosphate-buffered saline (pH 7.4). The NMDA was loaded in a 10- μl Hamilton syringe that was mounted on a microinjector (Kopf), which was, in turn, attached to a standard stereotaxic arm (Kopf). Infusion of NMDA was performed over 4 min (0.05 μl/min, 0.2 μl per site) at three sites per hemisphere in the dorsal hippocampus (AP: −2.8, ML: ±1.6, DV: −3.3; AP: −4.2, ML ±2.6, DV: −3.0; AP: −5.3, ML: ±4.0, DV: −3.3), or in the ventral hippocampus (AP: −4.4, ML: ±4.6, DV: −6.4; AP: −5.2, ML: ±4.6, DV: −6.5; AP −5.6, ML: ±4.6, DV: −6.0 mm). All DV coordinates were relative to dura. After infusion at each site, the syringe was left in position for 5 min to allow for drug diffusion. On completion of all of the infusions, the incision was closed

with stainless steel surgical staples. Antibiotic cream was applied to the sutured area. Sham-operated subjects received the same surgical procedures, except that the tip of the syringe was lowered only to 1.5 mm or 4.5 mm ventral to dura as controls for dorsal and ventral hippocampal lesion groups, respectively, and no infusions were made. After surgery, subjects were housed in holding cages for 1–2 days for close monitoring before returning to their home cages.

2.3.2. Auditory trace fear conditioning and testing

Auditory trace fear conditioning took place in a single session consisting of 10 trials of forward pairings of an auditory CS and a footshock US. The conditioning session began with a 4-min intertrial interval (ITI). For each trial, a 20-s CS (3.9 kHz, 80 dB) was followed by a 30-s trace interval and then by 2-s presentation of the US (0.6 mA). Acquisition of trace fear conditioning with a 30-s trace interval has previously been shown to depend on hippocampal processing (Misane et al., 2005). Trials were separated by 4-min intertrial intervals (ITIs). Behavioral measures (see below) were recorded throughout the entire conditioning session.

2.3.3. Testing

Post-training expression of conditioned fear was assessed in a single test session either 24 h (Experiment 1) or 7 days (Experiment 2) after the conditioning session. The test session employed the same protocol as that for the conditioning session, except that no footshocks were presented, and the session consisted of 6 trials instead of 10. As during conditioning, behavioral measures (see below) were recorded throughout the entire testing session.

2.3.4. Behavioral measure

To eliminate the subjectivity of manual assessment of freezing behavior exhibited by rats, immobility registered by an infrared motion detector (H24-61, Coulbourn Instruments, Allentown, PA; see General Methods: Apparatus for more details) was used as the dependent measure of fear. Consistent with reports from other laboratories using automated assessments of activity in fear conditioning studies (Anagnostaras, Josselyn, Frankland, & Silva, 2000; Misane et al., 2005; Pouzet, Zhang, Richmond, Rawlins, & Feldon, 2001), we have previously found a strong and significant positive correlation between immobility recorded by this motion detector and the manual assessment of freezing behavior ($r = .72$, $p < .05$; Yoon & Otto, 2001). Briefly, the computer controlling the onset and offset of stimuli also sampled the motion detector at 1 Hz. Data were subsequently converted to percent time spent immobile for each of the three periods of interest for each trial: 4 min ITI, 20-s CS, and 30-s trace interval.

2.3.5. Locomotor activity

Previous studies have found that lesions of the ventral hippocampus often increase locomotor activity (Lipska, Jaskiw, Chrapusta, Karoum, & Weinberger, 1992; Maren, 1999; Richmond et al., 1999). Because locomotor hyperactivity could potentially interact with the immobility measure used to assess conditioning, we examined the level of ambulation and rearing during a single 6-min session approximately 24 h after the testing session. The level of locomotor activity was measured in an open-field chamber. A subject was placed in one corner of the chamber to start the session. The amount of ambulation, defined as the crossing of all four legs from one square to another, and the amount of rearing, defined as taking front two legs off of the floor, were recorded by an experimenter unaware of the lesion conditions of subjects.

2.3.6. Histology

At the end of the behavioral experiments, subjects were deeply anesthetized with sodium pentobarbital (100 mg/kg, ip) and perfused transcardially with 0.9% saline, followed by 10% buffered formalin solution. The brain was removed and post-fixed in 10% formalin solution for approximately 24 h before being transferred to 10% formalin-30% sucrose solution (wt/vol) for at least four days. The brain was later frozen and sliced in coronal sections at 50-μm thickness. Brain slices were mounted on glass microscope slides, stained with thionin, and coverslipped for visual examination of the extent of lesioned areas.

2.3.7. Statistical analysis

Statistical analysis for both trace fear conditioning and testing utilized separate two-way repeated measures analysis of variance (ANOVA). Treatment group was the between-subjects factor and trial was the within-subjects factor. Post hoc comparisons were conducted using Tukey's honestly significant difference (HSD) test ($\alpha = .05$). Statistical analysis during the testing session was limited to data from the first three trials because the behavior at the beginning of testing was thought to best represent the conditioned fear response to tone, and was least affected by extinction. Locomotion and rearing data were analyzed using a one-way ANOVA and Tukeys post hoc HSD test.

3. Experiment 1—The effect of pre-training lesions of dorsal vs. ventral hippocampus on the acquisition of auditory trace fear conditioning

Subjects received surgery prior to the behavioral experiments. Following a 7- to 10-day post-surgical recovery period, behavior during trace fear conditioning and testing (24 h after conditioning) was examined. One day after the completion of testing, the level of locomotor activity was assessed in an open-field chamber during a single 6-min session.

3.1. Results

3.1.1. Lesion placement

Following histological examination, four animals in the ventral lesion group and six animals in the dorsal lesion group were excluded from statistical analyses due to sparing of the respective hippocampal areas. Thus, the final group sizes for the ventral (VH-PRE), dorsal (DH-PRE), and sham (SH-PRE) lesion groups were 8, 9, and 12 subjects, respectively. Fig. 1 illustrates the extent of the largest and smallest lesioned areas for groups VH-PRE and DH-PRE reconstructed from serial coronal sections stained with thionin. All subjects in group DH-PRE consistently sustained extensive bilateral damage throughout the dorsal hippocampal region. Most subjects in group DH-PRE sustained partial disruption of dorsal subiculum as well. All subjects in group VH-PRE sustained extensive bilateral damage to the ventral hippocampus. Four subjects in group VH-PRE sustained partial damage to the tip of CA3 region of the very posterior end of the dorsal hippocampus, but the extent of damage was minimal. None of the subjects in group VH-PRE sustained damage to the entorhinal cortex or perirhinal cortex. For all subjects in groups DH-PRE and VH-PRE, the amygdala remained intact.

3.1.2. Auditory trace fear conditioning

Data from the dorsal and ventral sham lesion groups were combined because there was no statistical difference in their performance.

3.1.3. Acquisition of trace fear conditioning

Data from the acquisition phase of auditory trace fear conditioning is illustrated in Fig. 2. Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 4-min ITIs are shown in Fig. 2a. Ventral hippocampal lesions significantly attenuated the level of immobility across
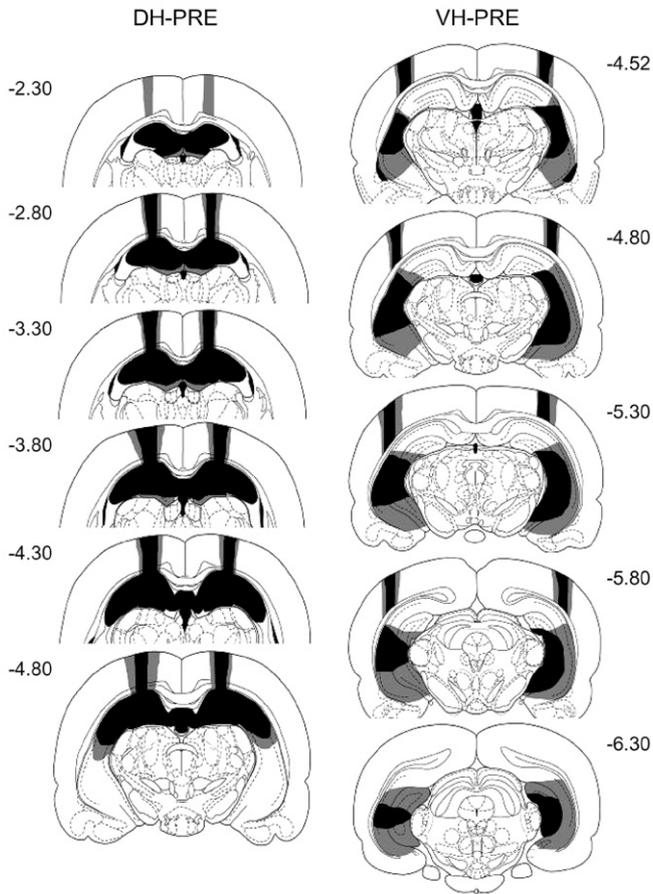
Fig. 1. Schematic representation of coronal sections showing the smallest (black) and largest (gray) lesion extent for groups DH-PRE (*left*) and VH-PRE (*right*) after pre-conditioning NMDA infusions directed at the dorsal and ventral hippocampus, respectively in Experiment 1. The numerical values indicate the distance in millimeters relative to bregma in the anterior–posterior plane.

trials. A two-way ANOVA with trial as the within-subjects factor and lesion condition as the between-subjects factor revealed significant main effects of lesion condition,

$F(2, 26) = 17.21$, $p < .001$, and trial, $F(9, 234) = 42.66$, $p < .001$, and a significant interaction between trial and lesion condition, $F(18, 234) = 4.51$, $p < .001$. Subsequent post hoc analyses (Tukey) revealed that group VH-PRE exhibited a significantly lower level of immobility than groups DH-PRE and SH-PRE during each of trials 2–10 but not during trial 1. No significant difference was found between groups DH-PRE and SH-PRE during any of the 10 trials.

The mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 20-s tone presentations are shown in Fig. 2b. A two-way ANOVA with trial as the within-subjects factor and lesion condition as the between-subjects factor revealed significant main effects of lesion condition, $F(2, 26) = 10.32$, $p < .001$, and trial, $F(9, 234) = 6.40$, $p < .001$, and a significant interaction between trial and lesion condition, $F(18, 234) = 2.51$, $p < .001$. Subsequent post hoc analyses (Tukey) revealed that group VH-PRE exhibited a significantly lower level of immobility than group SH-PRE during each of trials 2–8 and trial 10 but not during trials 1 and 9. No significant differences were found between groups VH-PRE and DH-PRE, or SH-PRE and DH-PRE.

The mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 30-s trace intervals are shown in Fig. 2c. As illustrated, ventral hippocampal lesions significantly attenuated the level of immobility. A two-way ANOVA with trial as the within-subjects factor and lesion condition as the between-subjects factor, revealed significant main effects of lesion condition, $F(2, 26) = 19.54$, $p < .001$, and trial, $F(9, 234) = 22.52$, $p < .001$, and a significant interaction between trial and lesion condition, $F(18, 234) = 2.65$, $p < .001$. Subsequent post hoc analyses (Tukey) revealed that group VH-PRE exhibited a significantly lower level of immobility than groups DH-PRE and SH-PRE during each of trials 2–10 but not during trial 1. No significant difference was found between groups DH-PRE and SH-PRE during any of the 10 trials.
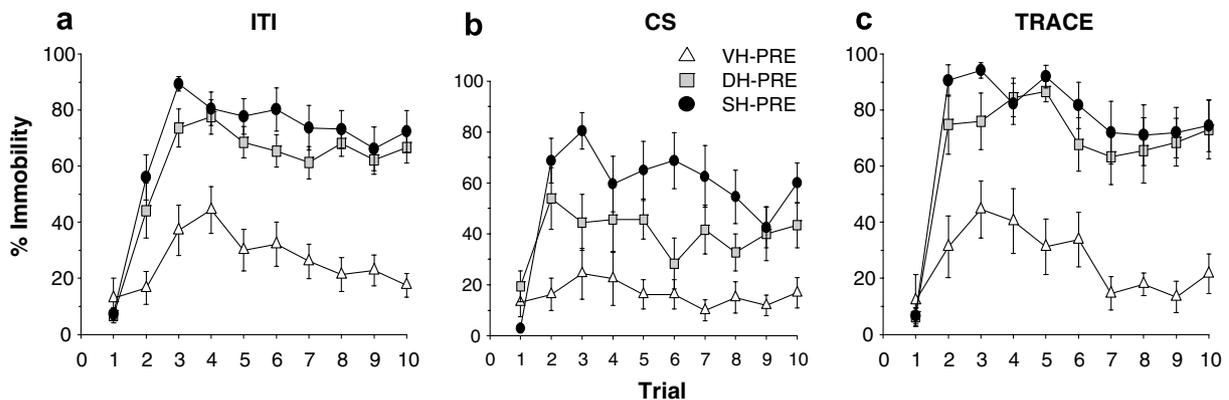

Fig. 2. Levels of immobility during the conditioning session exhibited by different lesion groups that received pre-conditioning bilateral NMDA infusions in the ventral (VH-PRE, $n = 8$), or dorsal (DH-PRE, $n = 9$) hippocampus in Experiment 1. Control group (SH-PRE, $n = 12$) received sham surgery with no drug infusions. (a) Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 4-min ITI across the 10 trials of the conditioning session. (b) Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 20-s tone presentation across the 10 trials of the conditioning session. (c) Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 30-s trace interval after tone presentation across the 10 trials of the conditioning session.

3.1.4. Expression of trace fear conditioning during testing

For statistical analysis during testing, data from only the first three trials were used because the behavior at the beginning of testing was least affected by extinction. Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 4-min ITIs are shown in Fig. 3a. As illustrated, ventral hippocampal lesions significantly attenuated the level of immobility across trials. A two-way ANOVA with trial as the within-subjects factor and lesion condition as the between-subjects factor revealed significant main effects of lesion condition, $F(2, 26) = 20.58$, $p < .001$, and trial, $F(2, 52) = 38.40$, $p < .001$, and a significant interaction between trial and lesion condition, $F(4, 52) = 3.42$, $p = .02$. Subsequent post hoc analyses (Tukey) revealed that group VH-PRE exhibited a significantly lower level of immobility than groups DH-PRE and SH-PRE during each of trials 1–3. No significant difference was found between groups DH-PRE and SH-PRE during any of the first three trials.

The mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 20-s tone presentations are shown in Fig. 3b. As illustrated, ventral hippocampal lesions significantly attenuated the level of immobility across trials. A two-way ANOVA with trial as the within-subjects factor and lesion condition as the between-subjects factor revealed a significant main effect of lesion condition, $F(2, 26) = 10.76$, $p < .001$, but failed to reveal a significant main effect of trial, $F(2, 52) = .27$, $p = .76$, or a significant interaction between trial and lesion condition, $F(4, 52) = 2.49$, $p = .054$. Subsequent post hoc analyses (Tukey) revealed that groups VH-PRE and SH-PRE exhibited a significantly lower level of immobility than group DH-PRE, but no significant difference was found between groups VH-PRE and SH-PRE.

The mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 30-s trace intervals are shown in Fig. 3c. As illustrated, ventral hippocampal lesions significantly attenuated the level of immobility across trials. A two-way ANOVA with trial as the within-subjects factor and lesion condition as the between-subjects factor, revealed a significant main effect of lesion condition, $F(2, 26) = 53.41$, $p < .001$, but failed to reveal a significant main effect of trial, $F(2, 52) = 2.12$, $p = .13$, or a significant interaction between trial and lesion condition, $F(4, 52) = .14$, $p = .97$. Subsequent post hoc analyses (Tukey) revealed that group VH-PRE exhibited a significantly lower level of immobility than groups DH-PRE and SH-PRE, but no significant difference was found between groups DH-PRE and SH-PRE.

3.1.5. Locomotor activity

Mean ambulation counts ($\pm SEM$) exhibited by different lesion groups are shown in Fig. 4a. A one-way ANOVA revealed a significant main effect of lesion condition, $F(2, 26) = 8.13$, $p = .002$. Subsequent post hoc analyses (Tukey) revealed that group VH-PRE exhibited a significantly higher level of ambulation than groups DH-PRE and SH-PRE, but no significant difference was found between groups DH-PRE and SH-PRE. Mean rearing counts ($\pm SEM$) exhibited by different lesion groups during the session are shown in Fig. 4b. A one-way ANOVA revealed a significant main effect of lesion condition, $F(2, 26) = 3.66$, $p = .04$. Subsequent post hoc analyses (Tukey) revealed that group VH-PRE exhibited a significantly higher level of rearing than group DH-PRE, but no significant differences were found between groups DH-PRE and SH-PRE, or VH-PRE and SH-PRE. Thus, consistent with previous findings, ventral hippocampal lesions significantly increased locomotor activity.

The significantly elevated level of locomotor activity produced after lesions of the ventral hippocampus leaves open the possibility that the lower level of immobility
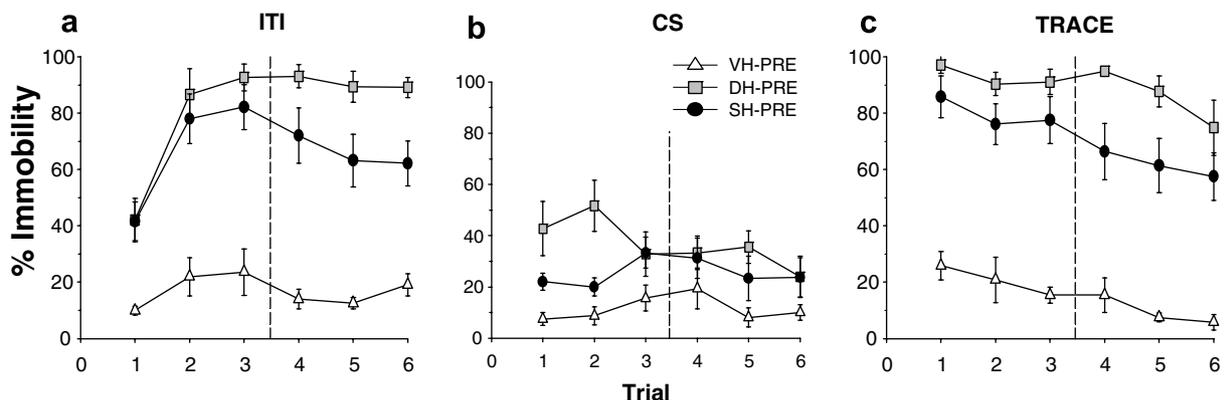

Fig. 3. Levels of immobility during the testing session exhibited by different lesion groups that received pre-conditioning bilateral NMDA infusions in the ventral (VH-PRE, $n = 8$), or dorsal (DH-PRE, $n = 9$) hippocampus in Experiment 1. The control group (SH-PRE, $n = 12$) received sham surgery with no drug infusions. Testing was conducted 1 day after trace fear conditioning. (a) Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 4-min ITI across the 6 trials of the testing session. (b) Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 20-s tone presentation across the 6 trials of the testing session. (c) Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 30-s trace interval after tone presentation across the 6 trials of the testing session. Statistical analyses were conducted using the data from the first three trials.
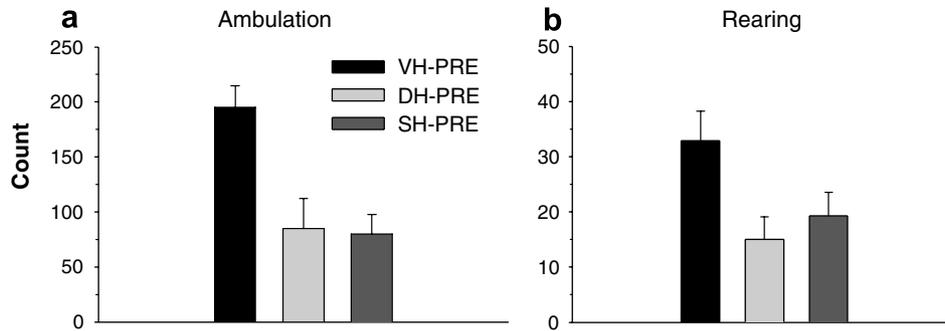
Fig. 4. Locomotor activity during the open-field test exhibited by the ventral (VH-PRE, $n = 8$), dorsal (DH-PRE, $n = 9$), and sham (SH-PRE, $n = 12$) hippocampal lesion groups in Experiment 1. The level of locomotor activity was examined in an open-field chamber during a single 6-min session 1 day after the tone testing. (a) Mean ($\pm SEM$) counts of ambulation exhibited by different lesion groups. (b) Mean ($\pm SEM$) counts of rearing exhibited by different lesion groups.

exhibited by group VH-PRE during conditioning and testing sessions may be a performance effect related to locomotor hyperactivity rather than an actual impairment in the acquisition of CS–US associations. In order to address this possibility, the Pearson product–moment correlation (r) between locomotor activity and the level of immobility exhibited by group VH-PRE during the 30-s trace intervals during trials 2–6 in conditioning and trials 1–3 in testing sessions (cf. Maren, 1999) was examined; these data are illustrated in Fig. 5. The correlation between immobility and ambulation failed to reach statistical significance
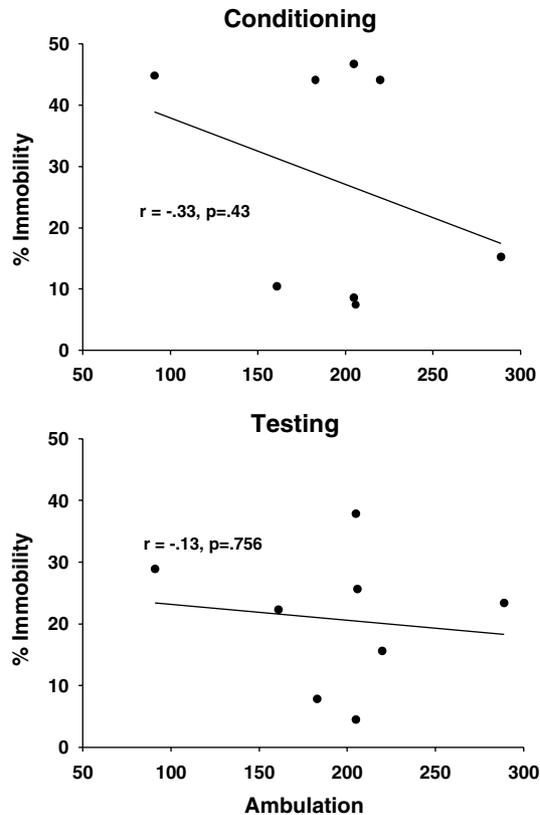

Fig. 5. Correlation between levels of locomotor activity observed during the open-field test and levels of immobility observed during the conditioning (top) and testing (bottom) sessions for animals with ventral hippocampal lesions. Neither of the correlations reached statistical significance.
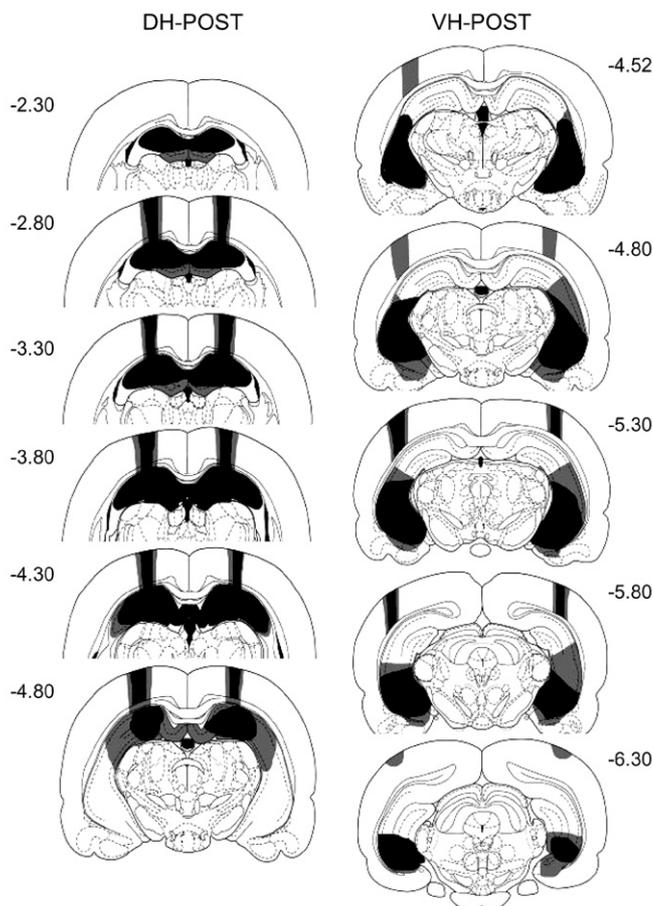
during conditioning, $r = -.33$, $p = .43$, or testing, $r = -.132$, $p = .756$. In addition, the correlations between locomotor activity and immobility were not statistically significant for any of the other relevant periods (CS, ITI).

4. Experiment 2—The effect of post-training lesions of dorsal vs. ventral hippocampus on the subsequent expression of auditory trace fear conditioning

Subjects received surgery 24 h after the acquisition of trace fear conditioning. Subjects were randomly assigned to one of the groups that received bilateral excitotoxic lesions of either dorsal or ventral, or sham hippocampal surgery. Testing was conducted following a 7-day post-surgical recovery period. One day after testing, the level of locomotor activity was assessed in an open-field chamber during a single 6-min session.

4.1. Results

4.1.1. Lesion placement

Following histological examination, three animals in the ventral lesion group and four animals in the dorsal lesion group were excluded from statistical analyses due to sparing of the respective hippocampal areas. Thus, the final group sizes for the ventral (VH-POST), dorsal (DH-POST), and sham (SH-POST) lesion groups were 9, 6, and 8 subjects, respectively. Fig. 6 illustrates the extent of the largest and smallest lesioned areas for groups VH-POST and DH-POST reconstructed from serial coronal sections stained with thionin. All subjects in group DH-POST consistently sustained extensive bilateral damage throughout the dorsal hippocampal region with minimal damage to surrounding areas. Most subjects in group DH-POST sustained partial disruption of dorsal subiculum. All subjects in group VH-POST sustained extensive bilateral damage to the ventral hippocampus. In 5 subjects, there was partial damage extending to the ventral subiculum (bilateral damage in 4 subjects). Five subjects in group VH-POST sustained partial damage to the tip of CA3 region of the very posterior end of the dorsal hippocampus, but the extent of damage was minimal. None of the subjects in group VH-POST

Fig. 6. Schematic representation of coronal sections showing the smallest (black) and largest (gray) lesion extent for groups DH-POST (*left*) and VH-POST (*right*) after post-conditioning NMDA infusions directed at the dorsal and ventral hippocampus, respectively in Experiment 2. The numerical values indicate the distance in millimeters relative to bregma in the anterior–posterior plane.

sustained damage to the entorhinal cortex or perirhinal cortex. For all subjects in groups DH-POST and VH-POST, the amygdala remained intact.

4.1.2. Auditory trace fear conditioning

Data from the dorsal and ventral sham lesion groups were combined because there was no statistical difference in their performance.

4.1.3. Acquisition of trace fear conditioning

The acquisition of the tone (CS)–footshock (US) association exhibited during trace fear conditioning is illustrated in Figs. 7a–c. Because the animals did not receive surgery until after the conditioning session, group differences were neither expected nor found.

4.1.4. Expression of trace fear conditioning during testing

Expression of trace fear conditioning was examined 7 days after surgery (8 days post-training). For statistical analysis during testing, data from only the first three trials were used because the behavior at the beginning of testing was least affected by extinction. Expression of learned fear exhibited during the testing session is illustrated in Fig. 8. The mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 4-min ITIs is shown in Fig. 8a. As illustrated, both dorsal and ventral hippocampal lesions significantly attenuated the level of immobility across trials. A two-way ANOVA with trial as the within-subjects factor and lesion condition as the between-subjects factor revealed significant main effects of lesion condition, $F(2,20) = 5.80$, $p = .01$, and trial, $F(2,40) = 18.27$, $p < .001$, and a significant interaction between trial and lesion condition, $F(4,40) = 4.42$, $p = .01$. Subsequent post hoc analyses (Tukey) revealed that group VH-POST exhibited a significantly lower level of immobility than group SH-POST during trials 2–3 but not during trial 1. The low levels of immobility during minute 1 suggest that the animals were not generalizing the training and testing chambers. In addition, group DH-POST exhibited a significantly lower level of immobility than group SH-POST during trial 3. No significant difference was found between groups DH-POST and VH-POST during any of the first three trials.
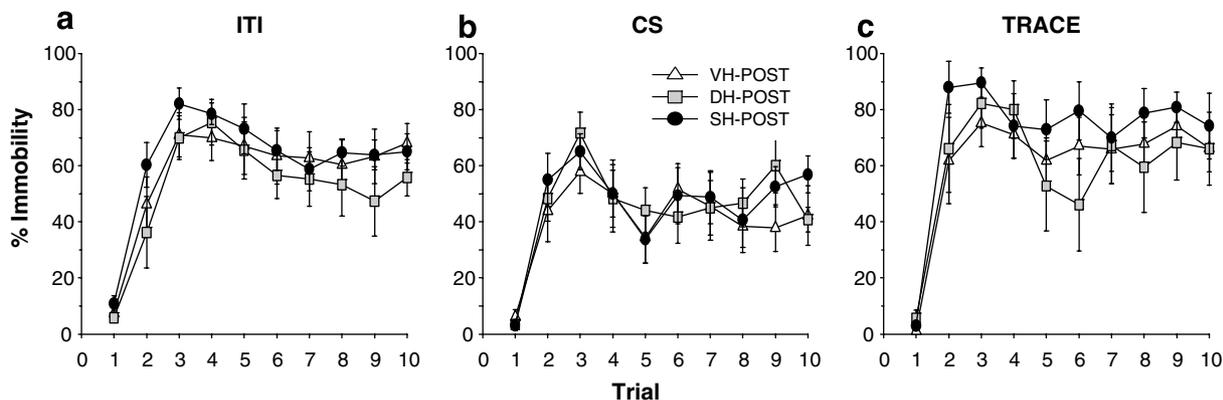

Fig. 7. Levels of immobility during the conditioning session exhibited by different lesion groups that received post-conditioning bilateral NMDA infusions in the ventral (VH-POST, $n = 9$), or dorsal (DH-POST, $n = 6$) hippocampus in Experiment 2. The control group (SH-POST, $n = 8$) received sham surgery with no drug infusions. Conditioning was conducted 1 day prior to lesion surgery. (a) Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 4-min ITI across the 10 trials of the conditioning session. (b) Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 20-s tone presentation across the 10 trials of the conditioning session. (c) Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 30-s trace interval after tone presentation across the 10 trials of the conditioning session.

The mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 20-s tone presentation is shown in Fig. 8b. As illustrated, ventral and dorsal hippocampal lesions slightly attenuated the level of immobility across trials. A two-way ANOVA with trial as the within-subjects factor and lesion condition as the between-subjects factor failed to reveal a significant main effect of lesion condition, $F(2, 20) = 1.65$, $p = .22$, or trial, $F(2, 40) = 2.30$, $p = .11$, but revealed a significant interaction between trial and lesion condition, $F(4, 40) = 2.82$, $p = .04$. Subsequent post hoc analyses (Tukey) failed to reach statistical significance among lesion groups during any of the first three trials.

The mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 30-s trace intervals is shown in Fig. 8c. As illustrated, ventral and dorsal hippocampal lesions significantly attenuated the level of immobility across trials. A two-way ANOVA with trial as the within-subjects factor and lesion condition as the between-subjects factor, revealed a significant main effect of lesion, $F(2, 20) = 11.14$, $p < .001$, and a significant inter-action between trial and lesion condition, $F(4, 40) = 2.73$, $p = .04$, but failed to reveal a significant main effect of trial, $F(2, 40) = 1.74$, $p = .19$. Subsequent post hoc analyses (Tukey) revealed that group VH-POST exhibited a significantly lower level of immobility than group SH-POST during each of trials 1–3, and that group DH-POST exhibited a significantly lower level of immobility than group SH-POST during trials 1 and 2. No significant difference was found between groups DH-POST and VH-POST during any of the first three trials.

4.1.5. Locomotor activity

The level of ambulation and rearing were examined during a single 6-min session approximately 24 h after the testing session. Mean ambulation counts ($\pm SEM$) exhibited by different lesion groups are shown in Fig. 9a. A one-way ANOVA failed to reveal a significant main effect of lesion condition, $F(2, 20) = .03$, $p = .97$.

Mean rearing counts ($\pm SEM$) exhibited by different lesion groups during the session are shown in Fig. 9b. A
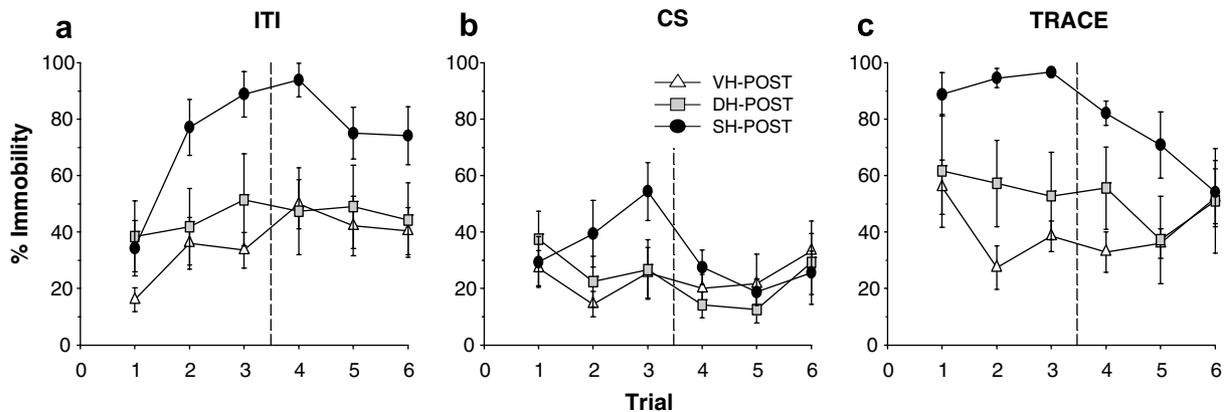

Fig. 8. Levels of immobility during the testing session exhibited by different lesion groups that received post-conditioning bilateral NMDA infusions in the ventral (VH-POST, $n = 9$), or dorsal (DH-POST, $n = 6$) hippocampus in Experiment 2. The control group (SH-POST, $n = 8$) received sham surgery with no drug infusions. Testing was conducted 7 days after surgery. (a) Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 4-min ITI across the 6 trials of the testing session. (b) Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 20-s tone presentation across the 6 trials of the testing session. (c) Mean ($\pm SEM$) percentage of immobility exhibited by different lesion groups during the 30-s trace interval after tone presentation across the 6 trials of the testing session. Statistical analyses were conducted using the data from the first three trials.
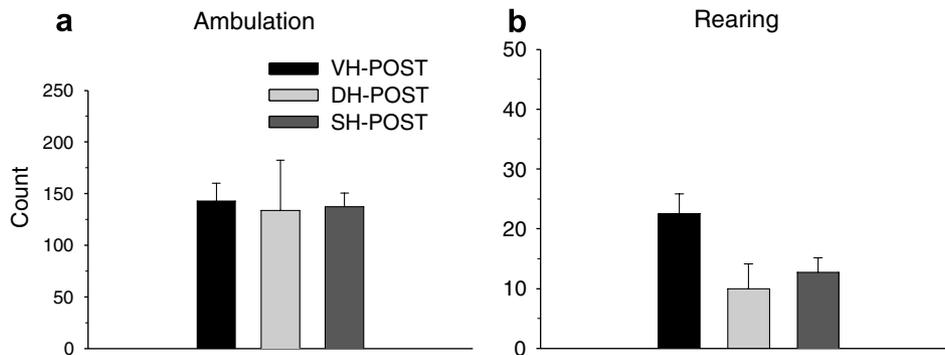

Fig. 9. Locomotor activity during the open-field test exhibited by the ventral (VH-POST, $n = 9$), dorsal (DH-POST, $n = 6$), and sham (SH-POST, $n = 8$) hippocampal lesion groups in Experiment 2. The level of locomotor activity was examined in an open-field chamber during a single 6-min session 1 day after the tone testing. (a) Mean ($\pm SEM$) counts of ambulation exhibited by different lesion groups. (b) Mean ($\pm SEM$) counts of rearing exhibited by different lesion groups.

one-way ANOVA revealed a significant main effect of lesion condition, $F(2,20) = 4.27$, $p = .03$. Subsequent post hoc analyses (Tukey) revealed that group VH-POST exhibited a significantly higher level of rearing than group DH-POST, but no significant differences were found between groups DH-POST and SH-POST, or VH-POST and SH-POST.

In order to address the potentially confounding factor of locomotor activity in the present study, we examined the Pearson product–moment correlation (r) between rearing activity and the level of immobility exhibited by group VH-POST during the 30-s trace intervals during the testing session. Rearing was chosen as the activity measure to be correlated with immobility because rearing values, unlike ambulation values, were significantly greater in animals with ventral hippocampal lesions. As illustrated in Fig. 10, the correlation between immobility and rearing during the first three minutes of testing failed to reach statistical significance, $r = -.34$, $p = .37$. The correlation between rearing and behavior during the conditioning session was not examined because the animals did not undergo surgical procedures until after conditioning was completed.

4.2. Discussion

The contribution of the hippocampus to the acquisition and expression of auditory trace fear conditioning was examined by producing bilateral excitotoxic lesions of the dorsal or ventral hippocampus either before or after conditioning. The pre-training lesion data indicate that acquisition of trace fear conditioning is critically dependent on the integrity of ventral, but not dorsal, hippocampus. Our post-training lesion findings further indicate that both the dorsal and ventral hippocampus are significantly involved in maintaining the CS–US association acquired during trace
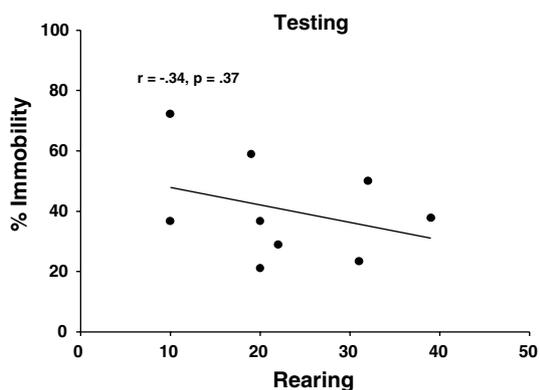

Fig. 10. Correlation between levels of rearing behavior observed during the open-field test and levels of immobility observed during testing for animals with ventral hippocampal lesions. Rearing was chosen as the activity variable because in Experiment 2 only rearing values were elevated for animals with ventral hippocampal lesions. The correlation between rearing and performance during the conditioning session is not shown because the animals did not undergo surgical procedures until 24 h after acquisition.

conditioning. These findings are discussed more fully below.

4.2.1. Ventral, but not dorsal, hippocampus is necessary for the acquisition of trace fear conditioning

Pre-training excitotoxic lesions of ventral hippocampus dramatically impaired both the acquisition and subsequent expression of trace fear conditioning. By contrast, pre-training excitotoxic lesions of dorsal hippocampus had no effect on either the acquisition of trace fear conditioning nor its expression 24 h later. These data support the notion that dorsal and ventral hippocampus subserve functionally distinct processes in this paradigm.

With respect to pre-training lesions of dorsal hippocampus, the results of the present study are consistent with those reported by Rogers et al. (2006), who found that pre-training lesions limited to dorsal CA1 had no effect on either the acquisition of trace fear conditioning or its expression when assessed 48 h after acquisition. The present data are, however, partially inconsistent with the effects of ventral hippocampal damage reported by Rogers et al. (2006). Specifically, unlike Rogers et al., we found a significant deficit during the initial conditioning session following pre-training lesions of ventral hippocampus. There are several reasons that may account for this apparent discrepancy. First, the trace intervals differed between the two studies, (30-s in the present study, 10-s in Rogers et al.). A recent study by Misane et al. (2005) suggests that hippocampal participation in trace conditioning likely diminishes with trace intervals less than 15-s. A second important difference between these two studies is that the lesions used by Rogers et al. (2006) included only the CA1 hippocampal subfield, whereas the lesions in the present study included CA1, CA3, and dentate gyrus. With this in mind, it is possible that ventral hippocampal subfields CA3 and the dentate gyrus participate critically in the acquisition of trace fear conditioning, while CA1 alone may not. Whether the findings reported here are due to simply more extensive hippocampal damage or alternatively point to a specific role of CA3 or the dentate gyrus is unclear. However, Wallenstein, Hasselmo, and Eichenbaum (1998; see also Levy, 1996 and Wallenstein and Hasselmo, 1997) provide a theoretically compelling and biologically realistic model suggesting that the ability of the hippocampus to associate temporally discontiguous stimuli is mediated by the activity among asymmetrically and sparsely interconnected networks of CA3 pyramidal cells. According to this model, CA3 can mediate associations between stimuli whose temporal disparity or relationship falls outside the temporal parameters normally required for the induction of synaptic plasticity. This notion is consistent with the effect of hippocampal lesions on trace conditioning and with the lack of effect of hippocampal lesions on delay conditioning, and suggests that the apparent discrepancy between the present findings and those reported in Rogers et al. (2006) may be due to CA3 damage in the study reported here.

As with the data reported by Rogers et al. (2005), the present findings that excitotoxic damage of dorsal hippocampus had no effect on the acquisition of auditory trace fear conditioning are inconsistent with those reported by Fendt, Fanselow, and Koch (2005) and by Burman, Starr, and Gewirtz (2006), who found that dorsal hippocampal lesions impaired the acquisition of trace fear conditioning when assessed using a fear-potentiated startle paradigm. With respect to the study reported by Burman et al. (2006), at least three paradigmatic differences could potentially account for the discrepancy with the data reported here. First, unlike the present studies that employed excitotoxic lesions of the hippocampus, those reported by Burman et al. (2006) examined the effects of electrolytic lesions of the hippocampus, which damage not only hippocampal principal cells but fibers of passage as well. Second, Burman et al., use a 3 s trace interval, whereas the present studies employed a 30 s trace interval. A recent study reported by Misane et al. (2005) has revealed that the nature of hippocampal involvement in trace fear conditioning likely interacts with the length of the trace interval. Finally, previous work has revealed a number of inconsistencies in hippocampal contributions to the acquisition of fear conditioning as assessed by freezing vs. fear-potentiated startle (McNish, Gewirtz, & Davis, 1997). Collectively, these considerations suggest that the role of the dorsal hippocampus in fear conditioning is complex, perhaps paradigm specific, and in need of further characterization.

Maren and Holt (2004) found that electrolytic lesions limited to ventral hippocampus dramatically impaired the acquisition of auditory fear conditioning using a delay conditioning procedure, and that this effect could not be attributed to lesion-induced hyperactivity. The present results extend these findings to both excitotoxic lesions of ventral hippocampus and to auditory trace fear conditioning, and further suggest that the observed deficit is likely not due to lesion-induced hyperactivity. While in the present study animals with pre-training lesions of ventral hippocampus were hyperactive in the open-field, the level of locomotor hyperactivity was not significantly correlated with freezing behavior during either conditioning or testing 24 h later. This interpretation is similar to that provided by Maren (1999). Finally, these results are likely not due to a lesion-induced alteration of US processing (i.e. footshock sensitivity); previous studies have demonstrated that the hippocampus is not likely involved in processing the US (LeDoux, 2000), and all animals in the present study were observed to vocalize and respond to the footshock in a normal manner.

Both the present data and those reported by Rogers et al. (2006) indicate that pre-training dorsal hippocampal lesions have no effect on auditory trace fear conditioning either during training or subsequent testing, suggesting the dorsal hippocampus is not critically involved in the acquisition of CS–US associations in this paradigm. Interestingly, these data are markedly inconsistent with the effect of NMDA receptor antagonism on the acquisition of trace fear conditioning. Specifically, dorsal hippocampal NMDA receptor blockade by APV has been shown to dramatically impair the acquisition of auditory trace fear conditioning in both rats and mice (Misane et al., 2005; Quinn, Loya, Ma, & Fanselow, 2005). Collectively these data suggest that, if intact, the hippocampus participates in learning CS–US associations in trace fear conditioning, but if absent, other brain areas compensate for the lack of hippocampal processing. Moreover, these data raise the intriguing possibility that in the presence of APV the "intact" (i.e. unlesioned) dorsal hippocampus may participate in CS–US processing via uncompromised AMPA receptors, but cannot support the plastic mechanisms required for the acquisition of the associations between those stimuli. This notion is consistent with that proposed by Wiltgen and Fanselow (2003) to account for the differential effects of NMDA receptor antagonism and dorsal hippocampal lesions on contextual conditioning.

4.2.2. Both dorsal and ventral hippocampus participate in the expression of trace fear memories

Post-training excitotoxic lesions of either dorsal or ventral hippocampus dramatically impaired the expression of trace fear conditioning during subsequent testing. These data are consistent with those of prior studies reporting impairments in the expression of trace fear conditioning following post-training dorsal hippocampal lesions (Chowdhury et al., 2005; Quinn et al., 2002), and extend these findings to the ventral hippocampal subfield. Because the lesions were produced soon after training, it is difficult to determine whether the effects observed during testing were due to an impairment in memory consolidation, storage, or retrieval processes. Further study aimed at dissociating these processes will be required for a more complete characterization of hippocampal contributions to trace fear conditioning.

It is interesting to note that the behavior of the sham-operated animals during presentation of the auditory CS was dissimilar to that during either the ITI or the trace interval. Specifically, these animals were considerably more active during presentation of the CS than during the other periods; this effect was particularly prominent during testing. This pattern of effects raises two interesting issues. First, it is possible that during trace conditioning the animals are learning that the tone is a relatively safe period which will *not* be followed immediately by the delivery of footshock. Second, these data highlight the importance of examining behavior during all periods of training and testing as opposed to only during delivery of the explicit CS.

In interpreting these data, it should be pointed out that the training-testing interval in Experiment 1 was 24 h, while in Experiment 2 it was 8 days. This is particularly important to consider in light of the different pattern of effects we observed following pre-vs. post-training lesions of dorsal hippocampus. It is entirely possible that deficits following pre-training lesions may have been observed during testing if the retention period had been longer. While this

experiment awaits completion, we chose in the present studies to keep the surgery-testing interval nearly identical between the two experiments (7 days, Experiment 1; 8 days, Experiment 2) to control for the possible differential effects of hippocampal lesions on hyperactivity across time.

With respect to dorsal hippocampal lesions, the present pattern of effects closely mirrors the effects of pre- vs. post-training hippocampal lesions on spatial contextual conditioning (reviewed in Anagnostaras, Gale, & Fanselow, 2001). Specifically, in both paradigms, pre-training lesions typically have no effect on acquisition (but see Young, Bohenek, & Fanselow, 1994), while post-training lesions dramatically impair the expression of conditioned fear. These findings raise the possibility that while in normal animals dorsal hippocampal processing may be recruited during acquisition and subsequently required for either the maintenance, consolidation, or retrieval of trace fear memory during testing, that processing may not be required for acquisition *per se*. That is, while animals may not need the dorsal hippocampus to acquire trace or spatial contextual conditioning, its integrity is required during testing if it was intact during training. Moreover, and consistent with previous notions (Otto & Poon, 2006; Wallenstein et al., 1998), these data also suggest that contextual and trace conditioning may reflect common properties of hippocampal function.

During testing in Experiment 1, the level of baseline immobility for groups DH-PRE and SH-PRE during the first ITI (prior to the first presentation of tone) was significantly higher than for group VH-PRE; there was a statistically insignificant trend in the same direction in Experiment 2. This suggests that there may have been some level of generalization between the conditioning and testing contexts for animals with sham or dorsal hippocampal lesions, and in turn raises the possibility that the levels of fear elicited by subsequent ITIs, the CS, or the trace interval are confounded with different levels of baseline fear. However, two lines of evidence suggest that these effects are not likely due to a baseline shift. First, relative to animals with ventral hippocampal lesions, animals with post-training lesions of dorsal hippocampus (Experiment 2) did not show elevated fear responses during the ITIs, the CS, or the trace interval despite numerically higher levels of baseline fear (minute 1, ITI). Second, fear responses among animals with pre-training lesions of ventral hippocampus (Experiment 1) were not elevated during minutes 2–6 relative to those during minute 1. Finally, previous studies in our laboratory (Cousens & Otto, 1998; Herzog & Otto, 2002, 1998; Otto & Poon, 2006) have reported levels of conditioned fear (∼80% freezing) that are similar to those of sham and dorsal hippocampal lesion groups in the present study, but in the absence of significant levels of baseline fear. These data suggest that while it is possible that animals with sham lesions or lesions of dorsal hippocampus may have exhibited some level of context generalization, the conditioned fear responses reported here are not importantly confounded by differentially enhanced levels of baseline fear.

Misane et al. (2005) recently reported that hippocampal involvement in trace fear conditioning is critically dependent on the length of the trace interval. Specifically, they found that NMDA receptor antagonism significantly impaired trace fear conditioning with 15 s or 30 s trace intervals, but not with shorter trace intervals. With this in mind, the conclusions derived from the present data cannot be generalized to other trace intervals.

In conclusion, the present studies indicate that both pre- and post-training lesions of ventral hippocampus impair the acquisition and expression, respectively, of auditory trace fear conditioning. In addition, while pre-training lesions of dorsal hippocampus have no effect on the acquisition of trace fear conditioning, post-training lesions of dorsal hippocampus dramatically impair its expression. While animals with lesions of ventral hippocampus were found to be somewhat hyperactive, it is unlikely that the pattern of observed deficits can be attributed to this effect. Collectively these data suggest that the dorsal and ventral hippocampus may contribute differentially to the mnemonic processes underlying fear trace conditioning.

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