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Dorsal Versus Ventral Hippocampal Contributions to Trace and Contextual Conditioning: Differential Effects of Regionally Selective NMDA Receptor Antagonism on Acquisition and Expression

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ABSTRACT: The dorsal and ventral subregions of the hippocampus likely play dissociable roles in some forms of learning. For example, we have previously demonstrated that temporary inactivation of ventral, but not dorsal, hippocampus dramatically impaired the acquisition of trace fear conditioning, while temporary inactivation of dorsal, but not ventral, hippocampus impaired spatially guided reinforced alternation (Czerniawski et al. (2009) Hippocampus 19:20–32). Importantly, emerging data suggest that lesions, temporary inactivation, and NMDA receptor antagonism within these subregions can produce quite different patterns of behavioral effects when administered into the same region. Specifically, while neither lesions nor temporary inactivation of dorsal hippocampus impair the acquisition of trace fear conditioning, learning in this paradigm is severely impaired by pre-training administration of the NMDA receptor antagonist *dl*-2-phosphonovaleric acid (APV) in dorsal hippocampus; the effect of NMDA receptor antagonism within ventral hippocampus on the acquisition and expression of trace conditioning, or on learning in general, has not yet been systematically explored. The present study extends our previous work examining the differential effect of lesions or inactivation of the dorsal and ventral hippocampal subregions by systematically examining the effect of regionally selective pre-training or pre-testing administration of APV on the acquisition and expression of trace and contextual fear conditioning. The results of these studies demonstrate that while pre-training NMDA receptor antagonism within either the dorsal or ventral subregion of the hippocampus impaired the acquisition of both trace and contextual conditioning, pre-testing NMDA receptor antagonism within ventral, but not dorsal, hippocampus impaired the expression of previously-acquired trace and contextual fear conditioning. These data suggest that selectively manipulating the integrity of individual subregions may result in compensatory mechanisms that can support learning, and that NMDA-dependent plasticity within both dorsal and ventral hippocampus is normally required for the acquisition and maintenance of memory in trace and contextual fear conditioning. © 2011 Wiley Periodicals, Inc.

KEY WORDS: APV; fear conditioning; associative learning; neuronal plasticity; memory

INTRODUCTION

Converging evidence suggests that the dorsal hippocampal (DH) and ventral hippocampal (VH) subregions are anatomically dissociable, and that these anatomical distinctions are reflected in their differential participation in a variety of forms of learning (Canteras and Swanson, 1992; Moser and Moser, 1998; Bannerman et al., 1999; Pitkanen et al., 2000; Czerniawski et al., 2009; Yoon and Otto, 2007; Fanselow and Dong, 2010). Specifically, DH appears to play a particularly prominent role in spatial learning (O'Keefe and Nadel, 1978; Moser et al., 1993; Jung et al., 1994; Mao and Robinson, 1998; Ferbinteanu and McDonald, 2001; Czerniawski et al., 2009), while VH has been strongly implicated in anxiety and defensive behaviors (Kjelstrup et al., 2002; Bannerman et al., 2004; McHugh et al., 2004; Trivedi and Coover, 2004; Bertoglio et al., 2006; Pentkowski et al., 2006). Consistent with this emerging pattern, we have recently observed a double dissociation wherein inactivation of DH, but not VH, dramatically impaired performance in spatially guided delayed reinforced alternation, while inactivation of VH, but not DH, attenuated the acquisition and expression of trace fear conditioning (Czerniawski et al., 2009). We have also observed that lesions of VH, but not DH, dramatically impair the acquisition of trace fear conditioning (Yoon and Otto, 2007). Thus these data indicate that the integrity of VH, but not DH, is critical to the acquisition trace fear conditioning, a form of associative learning generally considered to be "hippocampus-dependent." Interestingly, however, lesions of the DH after training result in robust deficits in conditioned fear during subsequent testing (Yoon and Otto, 2007), suggesting that although DH appears to be unnecessary for the acquisition of trace fear conditioning, it may play a prominent role in the retention or expression of trace fear memories if it was intact during acquisition. Moreover, while neither inactivation nor lesions of DH impair the acquisition of trace fear conditioning, several recent reports suggest that that NMDA receptor antagonism in DH impairs the acquisition of this form of learning (Misane et al., 2005; Quinn et al., 2005; Wanisch et al., 2005). A similar pattern of effects has been observed with respect to the involvement of DH in contextual fear conditioning: pre-training excitotoxic lesions or temporary inactivation of DH typically have no effect on acquisition, while pre-training NMDA receptor antagonism dramatically attenuates freezing during subsequent testing (Young et al., 1994; Maren et al., 1997;

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Richmond et al., 1999; Bast et al., 2003; Wiltgen and Fanselow, 2003). Collectively, these data suggest that NMDA receptor-mediated plasticity within DH may normally participate in some forms of learning for which DH integrity may not be required.

Given the fact that VH has direct and reciprocal connections with the amygdala, whereas DH gains access to amygdalar processing only through its connections with VH (Pitkanen et al., 2000), it is likely that the integrity of VH is necessary for any behavior that may depend on communication between DH and the amygdala. However, the interactions between these areas and the extent to which regionally specific NMDA receptor-dependent plasticity contribute to learning in general, and various forms of fear conditioning in particular, is currently not well characterized. Thus the goal of the present studies was to provide a direct and systematic comparison of pre-training vs. pre-testing, regionally-specific NMDA receptor antagonism within DH and VH on the acquisition and expression of both trace and contextual conditioning. The data reported here are the first to demonstrate a regionally selective and behaviorally dissociable effect of pre-training and pre-testing NMDA receptor antagonism in DH and VH.

MATERIALS AND METHODS

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

General Methods

The present experiment examined the effect of infusion of the NMDA receptor antagonist *dl*-2-phosphonovaleric acid (APV) into DH or VH on the acquisition and expression of trace and contextual fear conditioning. Subjects received surgery prior to all behavioral experiments and were randomly assigned to one of four experimental groups (see Fig. 1): artifical cerebral spinal fluid (aCSF) before both training and testing (aCSF-aCSF), APV before training and aCSF before testing (APV-aCSF), aCSF before training and APV before testing (aCSF-APV), and APV before both training and testing (APV-APV). Data were analyzed using separate one- or two-way analyses of variance (ANOVAs) as appropriate. An α level of 0.05 was used for all statistical analyses. *Post hoc* comparisons, where necessary, were conducted using Student–Newman–Keul's (SNK) *post hoc* test.

Subjects

One hundred naive male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250–300 g at the time of surgery served as subjects. All subjects were housed individually in plastic cages in a colony room with a 12 h light/dark cycle with lights on at 7 am. All behavioral testing occurred during the light cycle. Subjects had access to food and water *ad libitum* and were handled for 2 min daily for 5 days prior to surgical procedures and behavioral training.

Apparatus

Fear conditioning and testing chambers. Auditory trace fear conditioning and context testing were conducted in a behavioral chamber (30 cm × 24 cm × 27 cm) enclosed in a sound-attenuating enclosure (56 cm × 41 cm × 42 cm). The floor of the chamber was composed of 16 stainless steel rods equally spaced by 1.9 cm which were connected to a shock generator (model H13-15, Coulbourn Instruments, Allentown, PA) designed to administer footshock unconditioned stimulus (US) (0.6 mA). Two of the opposing walls were composed of transparent Plexiglas and the other two were aluminum. When appropriate, a computer-generated tone (3.9 kHz, 80 dB) was presented through a speaker mounted 14 cm above the floor on the outside one of the aluminum chamber walls. A single light bulb (29 V, 0.04 A) was located 24.5 cm above the floor. A one-way glass window on the front door of the sound attenuating enclosure allowed an experimenter to observe and score the behavioral measure of freezing using a hand switch that was connected to the computer controlling all paradigmatic events. The training chamber was cleaned with a commercially available cage cleaner (Research Laboratories Inc.) between sessions.

The testing session for trace fear conditioning took place in a novel chamber located in a different experimental room. The testing chamber had the same measurements and configuration as the training chamber but was differentiated from the training chamber in that the entire floor was covered with black Plexiglas and a black and white striped panel was attached to one of the opposing walls. A one-way glass window on the front door of the sound attenuating enclosure allowed for an

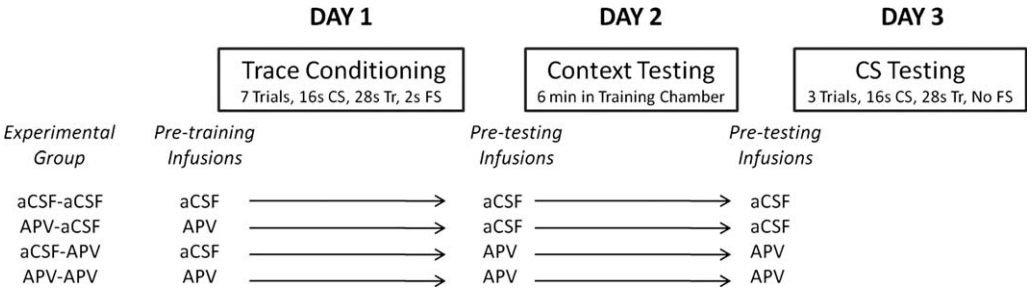

FIGURE 1. Experimental design.

observer to manually score freezing behavior with a hand switch. The testing chamber was cleaned with alcohol between sessions.

Open field chamber. Locomotor activity was assessed one week after trace fear conditioning in an open-field chamber (85 cm × 85 cm × 30 cm) made of black Plexiglas. The floor of the chamber was divided into 36 squares (14 cm). The chamber was located in a room lit with a single fixture (65 W), and a video camera placed approximately 1.5 m above the center of the chamber was used to record each session. An experimenter unaware of the experimental condition of each subject watched the video on a TV screen in a different room and manually recorded locomotor activity.

Procedure

Surgery. All subjects were first anesthetized with an i.p. administration of a ketamine (80 mg/kg; Butler Schein)–xylazine (12 mg/kg; Butler Shein) mixture before cannula implantation surgery. Each subject's head was shaved, mounted in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA), and cleaned with alcohol and betadine. Subcutaneous injections of marcaine (0.1 ml, 25%; Butler Schein) in several locations below the scalp served as a local anesthetic and vasoconstrictor. The scalp was then incised and retracted. Six small burr holes were drilled into the skull. For subjects receiving infusions into DH, double guide cannulae (22-gauge, 11 mm, Plastics1, Roanake, VA) were implanted bilaterally into the DH (anterior/posterior: −3.8 mm, medial/lateral: ± 2.5 mm from bregma; dorsal/ventral: −2.2 mm from dura). For subjects receiving infusions into VH, single guide cannulae (22-gauge, 11 mm, Plastics1, Roankae, VA) were implanted bilaterally into VH (anterior/posterior: −5.2 mm, medial/lateral: ±5 mm from bregma; dorsal/ventral: −5.5 mm from dura). The cannulae were affixed with acrylic cement and anchored to the skull via four stainless steel screws. The incision was then closed with stainless steel surgical staples and obdurators were placed into the guide cannula. All animals were closely monitored during the 7–10 day post surgical recovery period. Before behavioral testing subjects were randomly assigned to the different experimental groups for each of the experiments.

Auditory trace fear conditioning and testing. Trace fear conditioning was conducted 7–10 days after surgery. Auditory trace fear conditioning took place in a single session consisting of seven pairings of a tone (16 sec, 3.9 kHz, 80 dB) and footshock (2 sec, 0.6 mA), with a trace interval of 28 sec between the offset of the tone and onset of the shock. The first tone was presented after a 2 min acclimation period and subsequent trials were separated by a 2 min intertrial interval (ITI). The behavioral response of freezing, defined as a rigid posture and lack of movement except that required for respiration, was recorded throughout the entire conditioning session by an observer blind to the subjects' condition. These raw data were subsequently transformed into the percentage of time spent freezing during the ITI, conditioned stimulus (CS), and trace interval of the training session.

Conditioned fear to the training context was assessed 24 h after conditioning by placing each subject into the chamber in which conditioning occurred for 6 min. Freezing was recorded during the entire session by an observer blind to the subjects' condition. No stimuli (i.e., tone, shock) were presented during this session.

The testing session for trace fear conditioning was conducted in a novel chamber 48 h after conditioning (24 h after context testing) in one session consisting of three trials. The timing of stimulus delivery and duration of both the CS and ITI was identical to that used during training except that footshock was not presented and the number of trials was reduced to 3 to reduce the potential effect of extinction during testing. As during conditioning, the behavioral measure of freezing was recorded throughout the entire testing session.

Locomotor activity. In order to examine whether our manipulations affected basal levels of activity, locomotion was assessed in an open field in three sessions over three consecutive days one week after trace conditioning. At the start of each session subjects were placed in the center of the open chamber, and they were then allowed to explore freely for 10 min. Each session was recorded by a video camera placed approximately 1.5 m above the open field and scored by an observer who watched the video on a TV screen in another room. The experimenter, who was unaware of the experimental condition of the subjects, recorded both ambulation, defined as the crossing of all four legs from one square to another, and rearing, defined as lifting the two front legs off the floor. On the second day of locomotor activity testing, subjects received microinfusions of the same solution that was administered before behavioral testing; no infusions occurred on the first or third day of locomotor activity testing.

Infusions of APV or aCSF. Subjects received microinfusions of either aCSF (Harvard Apparatus) or APV (10 μg/μl; Sigma, St Louis, MO, pH 7.4). The infusions were administered via insertion of an infusion cannula into the guide cannula targeted at the DH or VH. The infusion cannula protruded 1 mm beyond the tip of the guide cannula, and was connected via polyethylene tubing to a 10 μl Hamilton syringe mounted in an infusion pump (Harvard Apparatus). A volume of 0.5 μl (0.25 μl/min) was infused bilaterally for a total volume of 1 μl for all subjects. The infusion cannula was left in position for 4 min following completion of infusion to allow for diffusion of the APV or aCSF. Subjects then had the infusion cannula replaced with a dummy cannula and were subsequently transferred to an experimental room to undergo behavioral training or testing as described in Figure 1. Initial sample sizes for subjects with cannula in DH were: aCSF-aCSF (n = 11), aCSF-APV (n = 11), APV-aCSF (n = 12), APV-APV (n = 11). Initial sample sizes for subjects with cannula in DH were: aCSF-aCSF (n = 14), aCSF-APV (n = 14), APV-aCSF (n = 14), APV-APV (n = 13). Final sample sizes for each group were

determined following histological assessment of cannula placement (see Cannula Placement, below).

Histology. Following completion of all behavioral testing, animals were administered a sub-lethal dose of sodium pentobarbital (100 mg/kg, i.p.) and perfused transcardially with 0.9% saline followed by buffered 10% formalin. The brain was removed and placed in a 10% formalin-30% sucrose solution for at least 3 days. The brain was then frozen and sliced into coronal sections with a thickness of 50 μm using a cryostat. Every other slice throughout the DH or VH was mounted on gelled glass microscope slides and subsequently stained with cresyl violet and coverslipped. An observer blind to the subject's condition verified cannula placement throughout the DH or VH. Subjects with inaccurate cannula placement or extensive damage were excluded from data analysis.

RESULTS

Cannula Placement

Following histological examination, nine subjects with cannula in DH and ten subjects with cannula in VH were excluded due to incorrect cannula placement. Thus, the final group numbers for subjects with cannula in DH were: aCSF-aCSF ($n = 9$), aCSF-APV ($n = 10$), APV-aCSF ($n = 9$), and APV-APV ($n = 8$). The final group numbers for subjects with cannula in VH were: aCSF-aCSF ($n = 14$), aCSF-APV ($n = 9$), APV-aCSF ($n = 12$), and APV-APV ($n = 10$). Figure 2 depicts a representative photograph of accurate cannula placement for subjects with cannula in DH (Fig. 2a) or VH (Fig. 2b).

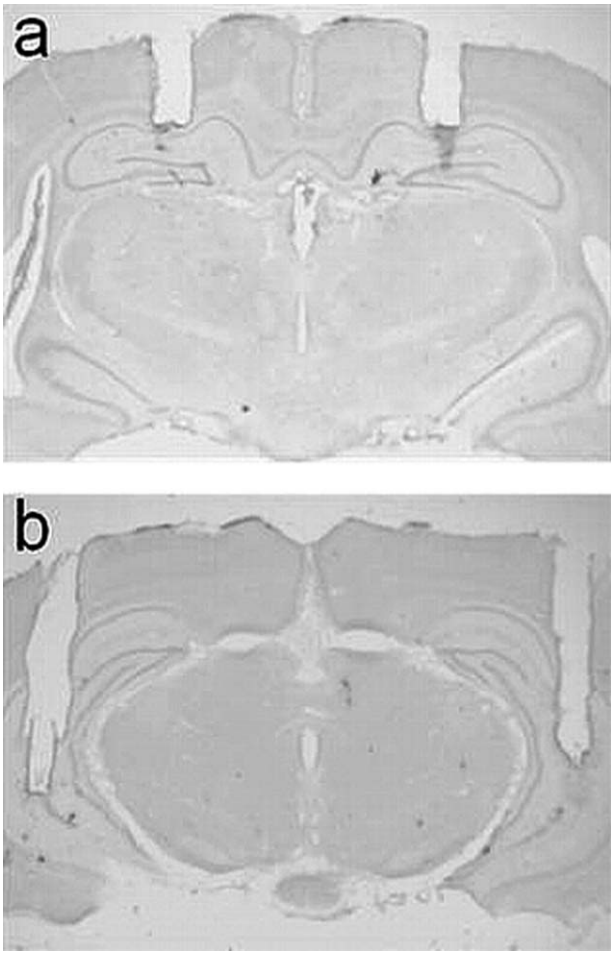

FIGURE 2. Representative photograph of cannula implantation in dorsal (a) or ventral (b) hippocampus.

Trace Fear Conditioning: Effect of APV or aCSF Infusions into Dorsal Hippocampus on Freezing During Training

The mean (±SEM) percentage of freezing exhibited by different infusion groups during conditioning is illustrated in Figures 3a–c. For analysis of data during the conditioning session, subjects were collapsed into two groups for training: those that received pre-training infusions of APV into DH (APV-APV and APV-aCSF, $n = 17$) and those that received pre-training infusions of aCSF (aCSF-aCSF and aCSF-APV, $n = 19$). The data for Trial 1, prior to the delivery of the first US, are interpreted as a "baseline" measure of freezing, and are separated from those for Trials 2–7. Because freezing for each subject was stable across Trials 2–7 during conditioning, the data for each subject after the first US presentation were averaged into a single value (Trials 2–7). For the ITI, a two way ANOVA with infusion condition as the between subjects factor and time point (Trial 1 (baseline) vs. Trials 2–7) as the within subjects factor revealed there was a main effect for infusion condition, $F(1,34) = 6.91$, $P = 0.0128$, a main effect for time point, $F(1,71) = 262.92$, $P < 0.0001$, and a significant interaction between infusion condition and time point, $F(1,71) = 8.69$, $P = 0.005$ during conditioning (Fig. 3a). Subsequent SNK *post hoc* analyses revealed that group APV exhibited significantly less freezing than group aCSF during Trials 2–7 but not during Trial 1 ($P < 0.05$). In addition, both groups exhibited significantly higher levels of freezing during Trials 2–7 than they did during baseline ($P < 0.05$).

For the CS, a two way ANOVA with infusion condition as the between subjects factor and time point (Trial 1 (baseline) vs. Trials 2–7) as the within subjects factor ANOVA revealed there was a main effect for time point, $F(1,71) = 104.02$, $P < 0.001$, but no main effect for infusion condition, $F(1,34) = 0.516$, $P = 0.477$, and no significant interaction between infusion condition and time point, $F(1,71) = 0.683$, $P = 0.414$ (Fig. 3b). Subsequent SNK *post hoc* analyses revealed that both groups exhibited significantly higher levels of freezing during Trials 2–7 of the CS than they did during Trial 1 (baseline), before the first shock was presented ($P < 0.05$).

For the trace interval, a two way ANOVA with infusion condition as the between subjects factor and time point (Trial 1 (baseline) vs. Trials 2–7) as the within subjects factor ANOVA

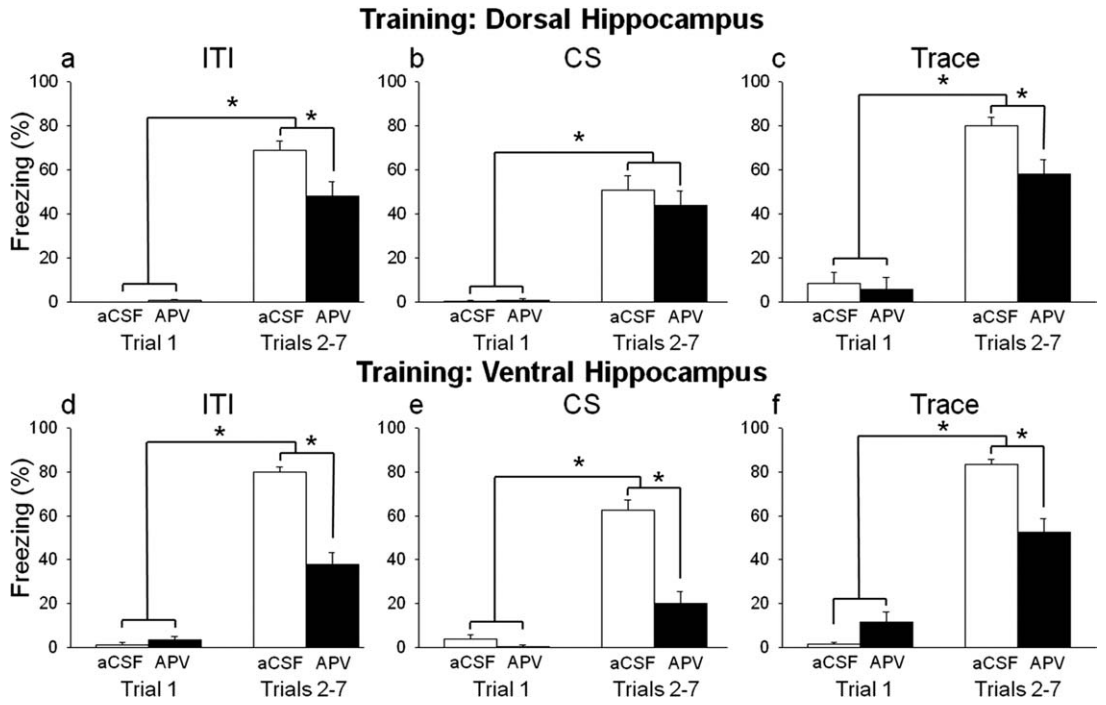

FIGURE 3. Mean (±SEM) percentage freezing during the ITI, CS, and trace interval of the conditioning session exhibited by different groups that received bilateral pre-training infusions of aCSF or APV into dorsal (a–c) or ventral (d–f) hippocampus. *$P < 0.05$.

revealed there was a main effect for infusion condition, $F(1,34) = 10.46$, $P = 0.002$ and a main effect for time point, $F(1,71) = 0.228.59$, $P < 0.0001$, but no significant interaction between infusion condition and time point, $F(1,71) = 2.58$, $P = 0.117$ (Fig. 3c). Subjects that received aCSF infusions exhibited significantly higher levels of freezing during the trace interval than those that received APV infusions into DH before conditioning. However, subsequent SNK *post hoc* analyses revealed that both groups exhibited significantly higher levels of freezing during Trials 2–7 of the trace interval than they did during the first trial (baseline), before the first shock was presented ($P < 0.05$).

Trace Fear Conditioning: Effect of APV or aCSF Infusions into Ventral Hippocampus on Freezing During Training

The mean (±SEM) percentage of freezing exhibited by different infusion groups during conditioning is illustrated in Figures 3d–f. For analysis of data during the conditioning session, subjects were collapsed into two groups for training: those that received pre-training infusions of APV into VH (APV-APV and APV-aCSF, $n = 22$) and those that received infusions of aCSF (aCSF-aCSF and aCSF-APV, $n = 23$). As with the animals receiving infusions into DH described above, performance during the first ITI (Trial 1), prior to the delivery of the first CS or footshock, is interpreted as a "baseline" measure of freezing, and is separated from those for Trials 2–7. Similarly, the data for each subject after the first US presentation were averaged into a single value (Trials 2–7). For the ITI, a two way ANOVA with infusion condition as the between subjects factor and time point (Trial 1 (baseline) vs. Trials 2–7) as the within subjects factor ANOVA revealed that there was a main effect for infusion condition, $F(1,43) = 34.0$, $P < 0.0001$, a main effect for time point, $F(1,89) = 419.8$, $P < 0.0001$, and a significant interaction between infusion condition and time point, $F(1,89) = 64.7$, $P < 0.0001$ for the ITI during conditioning (Fig. 3d). Subsequent SNK *post hoc* analyses revealed that group APV exhibited significantly less freezing than group aCSF during Trials 2–7 but not during Trial 1 ($P < 0.05$). In addition, both groups exhibited significantly higher levels of freezing during Trials 2–7 than they did during baseline ($P < 0.05$).

For the CS, a two way ANOVA with infusion condition as the between subjects factor and time point (Trial 1 (baseline) vs. Trials 2–7) as the within subjects factor ANOVA revealed that there was a main effect for infusion condition, $F(1,43) = 33.3$, $P < 0.0001$, a main effect for time point, $F(1,89) = 151.3$, $P < 0.0001$, and a significant interaction between infusion condition and time point, $F(1,89) = 37.7$, $P < 0.0001$ for the ITI during conditioning (Fig. 3e). Subsequent SNK *post hoc* analyses revealed that group APV exhibited significantly less freezing than group aCSF during Trials 2–7 but not during baseline ($P < 0.05$). In addition, both groups exhibited significantly higher levels of freezing during the CS presentations during Trials 2–7 than they did during baseline ($P < 0.05$).

For the trace interval, a two way ANOVA with infusion condition as the between subjects factor and time point (Trial 1

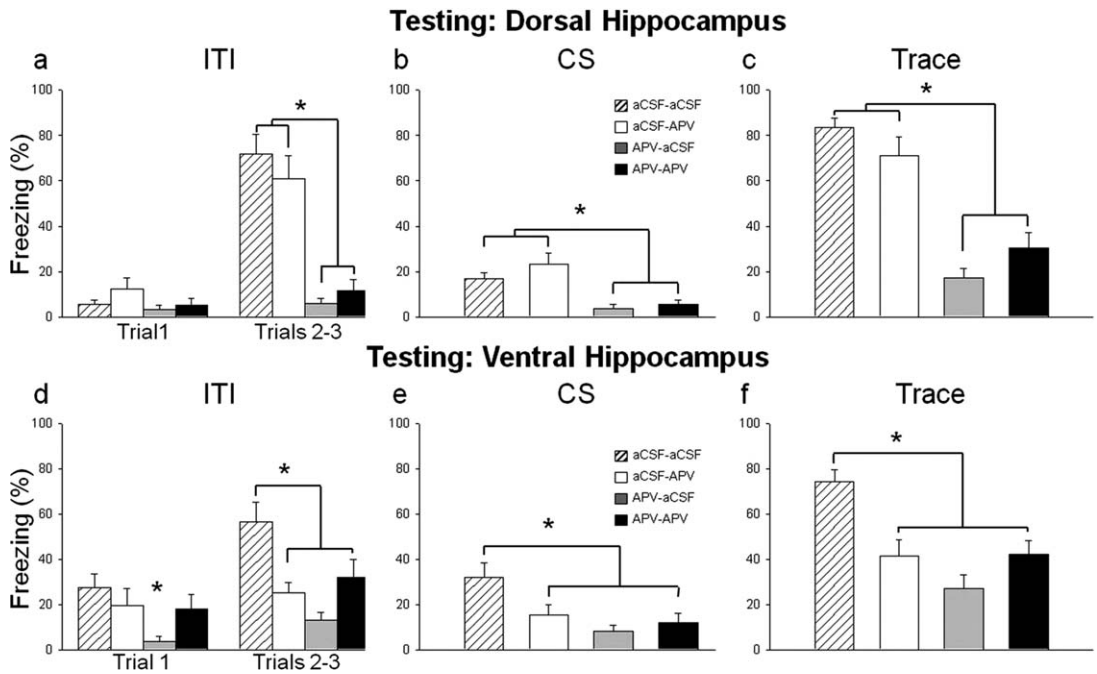

FIGURE 4. Mean (±SEM) percentage freezing during the ITI, CS, and trace interval of the testing session exhibited by different groups that received bilateral infusions of APV or aCSF into dorsal (a–c) or ventral (d–f) hippocampus. For dorsal hippocampus, infusions of APV prior to training or prior to both training and testing impaired conditioned freezing during the test session. For ventral hippocampus, infusions of APV prior to training, testing, or prior to both training and testing impaired conditioned freezing during the test session. *($P < 0.05$).

(baseline) vs. Trials 2–7) as the within subjects factor ANOVA revealed that there was a main effect for infusion condition, $F(1,43) = 4.82$, $P = 0.033$, a main effect for time point, $F(1,89) = 439.15$, $P < 0.0001$, and a significant interaction between infusion condition and time point, $F(1,89) = 48.56$, $P < 0.0001$ for the ITI during conditioning (Fig. 3f). Subsequent SNK *post hoc* analyses revealed that group APV exhibited significantly less freezing than group aCSF during Trials 2–7 but not during baseline ($P < 0.05$). In addition, both groups exhibited significantly higher levels of freezing during the trace interval of Trials 2–7 than they did during baseline ($P < 0.05$).

Trace Fear Conditioning: Effect of APV or aCSF Infusions into Dorsal Hippocampus on Freezing During Testing

The expression of conditioned fear exhibited during testing for subjects with cannula implanted in DH is depicted in Figures 4a–c. The mean (±SEM) percentage of freezing exhibited during the 2 min ITI is shown in Figure 4a. The first trial of the ITI consisted of the first 2 minu in the novel chamber before any CS presentations and thus served as a period during which baseline levels of freezing were measured; freezing behavior for each subject during the ITI for Trials 2 and 3 were averaged into a single value. A one-way ANOVA revealed there was no statistically significant difference between groups during the first ITI (baseline), $F(3,35) = 2.34$, $P = 0.0923$, but there was a significant difference in the percentage of time spent freezing during Trials 2–3 of the ITI, $F(3,35) = 19.8$, $P < 0.0001$. Subsequent *post hoc* analyses (SNK) revealed that there was no significant difference between groups aCSF-aCSF and aCSF-APV, nor was there a difference between groups APV-aCSF and APV-APV. However groups aCSF-aCSF and aCSF-APV were significantly different from groups APV-aCSF and APV-APV ($P < 0.05$), thus indicating that only subjects who received infusions of APV into DH before conditioning froze significantly less during the ITI presentations during testing 48 h later.

The mean (±SEM) percentage of freezing exhibited during the 16 sec CS presentation averaged across all three testing trials is shown in Figure 4b. A one-way ANOVA revealed that there was a significant difference between groups in the percentage of time spent freezing during the CS, $F(3,35) = 8.55$, $P = 0.003$. Subsequent *post hoc* analyses (SNK) revealed that there was no significant difference between groups aCSF-aCSF and aCSF-APV, nor was there a difference between groups APV-aCSF and APV-APV. However groups aCSF-aCSF and aCSF-APV were significantly different from groups APV-aCSF and APV-APV ($P < 0.05$), thus indicating that only subjects who received infusions of APV into DH before conditioning froze significantly less to the CS presentations during the testing session 48 h later.

The mean (±SEM) percentage of freezing exhibited during the 28 sec trace interval averaged across all three testing trials is shown in Figure 4c. A one-way ANOVA revealed that there was a significant difference between groups in the percentage of time spent freezing during the trace interval, $F(3,35) = 24.4$, $P < 0.0001$. Subsequent *post hoc* analyses (SNK) revealed

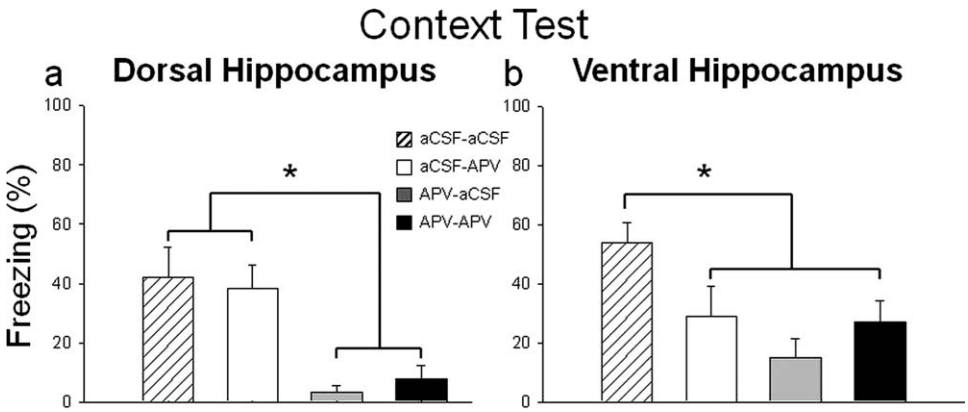

FIGURE 5. Mean (±SEM) percentage freezing during the context testing session exhibited by different groups that received bilateral infusions of APV or aCSF into (a) dorsal or (b) ventral hippocampus. Infusions of APV into dorsal hippocampus prior to training or prior to both training and testing impaired conditioned freezing during the context testing session. Infusions of APV into ventral hippocampus prior to training, prior to testing, or prior to both training and testing impaired conditioned freezing during the context testing session. *($P < 0.05$).

that there was no significant difference between groups aCSF-aCSF and aCSF-APV, nor was there a difference between groups APV-aCSF and APV-APV. However groups aCSF-aCSF and aCSF-APV were significantly different from groups APV-aCSF and APV-APV ($P < 0.05$), thus indicating that only subjects who received infusions of APV into DH before conditioning froze significantly less during the trace interval in the testing session 48 h later.

Trace Fear Conditioning: Effect of APV or aCSF Infusions into Ventral Hippocampus on Freezing During Testing

The expression of conditioned fear exhibited during the testing session for subjects with cannula implanted in VH is depicted in Figures 4d–f. The mean (±SEM) percentage of freezing exhibited during the 2 min ITI is shown in Figure 4d. A one-way ANOVA revealed there was a statistically significant difference between groups during the first ITI (baseline), $F(3,44) = 3.34$, $P = 0.0284$. Subsequent *post hoc* analyses revealed that group APV-aCSF froze significantly less than group aCSF-aCSF ($P < 0.05$) but that there were no other group differences. A one-way ANOVA revealed there was a significant difference in the percentage of time spent freezing during Trials 2–3 of the ITI, $F(3,44) = 7.83$, $P = 0.003$. Subsequent *post hoc* analyses (SNK) revealed that there was a significant difference between group aCSF-aCSF and all other groups ($P < 0.05$), thus indicating that subjects who received infusions of APV into VH before conditioning or before testing froze significantly less than control subjects during the ITI of the testing session.

The mean (±SEM) percentage of freezing exhibited during the 16 s CS presentation is shown in Figure 4e. A one-way ANOVA revealed there was a significant difference between groups in the percentage of time spent freezing during the CS, $F(3,44) = 5.41$, $P = 0.003$. Subsequent *post hoc* analyses (SNK) revealed that there was a significant difference between group aCSF-aCSF and all other groups ($P < 0.05$), thus indicating that subjects who received infusions of APV into VH before conditioning or before testing froze significantly less than control subjects during the CS presentations during the testing session 48 h later.

The mean (±SEM) percentage of freezing exhibited during the 28 sec trace interval is shown in Figure 4f. A one-way ANOVA revealed that there was a significant difference between groups in the percentage of time spent freezing during the trace interval, $F(3,44) = 12.6$, $P < 0.0001$. Subsequent *post hoc* analyses (SNK) revealed that there was a significant difference between group aCSF-aCSF and all other groups ($P < 0.05$), thus indicating that subjects who received infusions of APV into VH before conditioning or before testing froze significantly less than control subjects during the trace interval in the testing session 48 h later.

Contextual Fear Conditioning

For statistical analysis during testing, data from only the first three minutes of context testing were used because the behavior at the beginning of the session was least likely to be affected by extinction. The mean (±SEM) percentage freezing for each of the experimental groups is shown in Figure 5. For subjects receiving infusions in DH, a one-way ANOVA revealed a significant difference in freezing between groups, $F(3,35) = 7.81$, $P = 0.0005$ (Fig. 5a). Subsequent *post hoc* analyses (SNK) revealed that there was no significant difference between groups aCSF-aCSF and aCSF-APV, nor was there a difference between groups APV-aCSF and APV-APV. However groups aCSF-aCSF and aCSF-APV were significantly different than groups APV-aCSF and APV-APV ($P < 0.05$), thus indicating that only subjects who received infusions of APV into DH before conditioning froze significantly less during context testing 24 h later.

For subjects receiving infusions into VH, a one-way ANOVA revealed a significant difference in freezing between groups, $F(3,44) = 5.44$, $P = 0.003$ (Fig. 5b). Subsequent *post hoc*

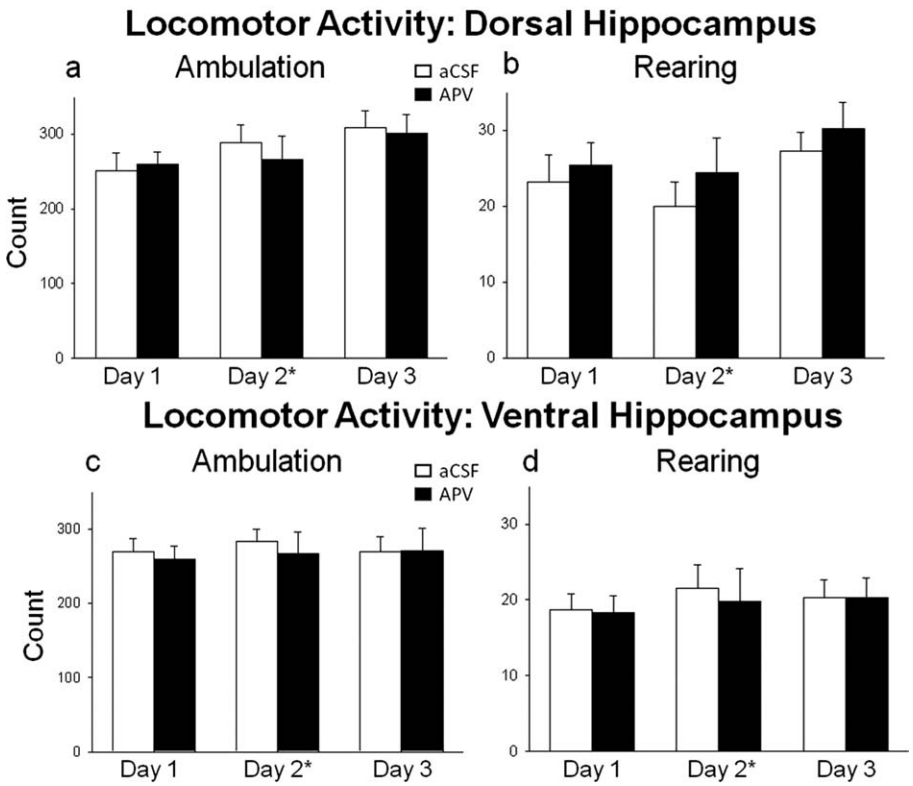

FIGURE 6. Mean (±SEM) number of ambulation and rearing counts during locomotor activity assessment for subjects that received APV or aCSF infusions into dorsal (a,b) or ventral (c,d) hippocampus. *Denotes that infusions were administered prior to placement in the open field on Day 2.

analyses (SNK) revealed that there was a significant difference between group aCSF-aCSF and all other groups ($P < 0.05$), thus indicating that subjects who received infusions of APV into VH before conditioning or before testing froze significantly less than control subjects during the context testing session.

Locomotor Activity

Subjects were placed into the open field on Days 1 and 3 of locomotor testing without receiving any infusions beforehand. Subjects were infused with whatever substance they received before testing on Day 2 of the locomotor activity test. Therefore, prior to locomotor testing on Day 2, subjects from group aCSF-aCSF or APV-aCSF received infusions of aCSF and were combined into one group (aCSF), and subjects from group aCSF-APV or APV-APV received infusions of APV and were combined into a separate group (APV). The final group numbers for subjects who received infusions of aCSF or APV into DH were 15 and 14, respectively. The final group numbers for subjects who received infusions of aCSF or APV into VH were 24 and 17, respectively.

The mean (±SEM) ambulation counts for subjects with infusions of APV or aCSF into DH are depicted in Figure 6a. A two-way ANOVA with day as the within-subjects factor and infusion condition as the between-subjects factor failed to reveal a significant main effect for condition, $F(1,27) = 0.083$, $P = 0.775$, or for day, $F(2, 86) = 2.91$, $P = 0.063$. There was no interaction between condition and day, $F(2,86) = 0.278$, $P = 0.757$. All subjects with cannula in DH exhibited similar levels of ambulation throughout all sessions of locomotor activity assessment, regardless of infusion condition.

The mean (±SEM) rearing counts for subjects with infusions of APV or aCSF into DH are depicted in Figure 6b. A two-way ANOVA with day as the within-subjects factor and infusion condition as the between-subjects factor failed to reveal a significant main effect for condition, $F(1,27) = 0.619$, $P = 0.438$, but did reveal a significant main effect for day, $F(2, 86) = 4.622$, $P = 0.014$. There was no interaction between condition and day, $F(2,84) = 0.131$, $P = 0.877$, indicating that the difference in rearing across days did not depend on the infusion condition.

The mean (±SEM) ambulation counts for subjects with infusions of APV or aCSF into VH are depicted in Figure 6c. A two-way ANOVA with day as the within-subjects factor and infusion condition as the between-subjects factor failed to reveal a significant main effect for condition, $F(1,39) = 0.158$, $P = 0.693$, or for day, $F(2, 122) = 0.127$, $P = 0.880$. There was no interaction between condition and day, $F(2,122) = 0.093$, $P = 0.911$. All subjects with cannula in VH exhibited similar levels of ambulation throughout all sessions of locomotor activity assessment, regardless of infusion condition.

The mean (±SEM) rearing counts for subjects with infusions of APV or aCSF into DH are depicted in Figure 6d. A two-way ANOVA with day as the within-subjects factor and infusion condition as the between-subjects factor failed to reveal a significant main effect for condition, $F(1,39) = 0.061$, $P = 0.805$, or for day, $F(2, 122) = 0.405$, $P = 0.667$. There was no interaction between condition and day, $F(2,122) = 0.055$, $P = 0.946$. All subjects with cannula in VH exhibited similar levels of rearing throughout all sessions of locomotor activity assessment, regardless of infusion condition.

DISCUSSION

In the present study, we examined the effect of NMDA receptor antagonism in DH and VH on trace and contextual fear conditioning. We observed that pre-training infusions of APV into either DH or VH dramatically impair acquisition, while pre-testing infusions of APV into VH but not DH impair the expression of trace and contextual fear conditioning. The implications of these data are described below.

Role of DH in Trace and Contextual Fear Conditioning: Dissociation of Integrity and Plasticity?

In the present study, pre-training but not pre-testing infusions of APV into DH resulted in a robust decrease in conditioned fear (freezing) during testing sessions assessing both trace and contextual conditioning. These data are generally consistent with other reports demonstrating that NMDA receptor antagonism in DH disrupts the acquisition but not expression of contextual or trace fear conditioning (Bast et al., 2003; Misane et al., 2005; Quinn et al., 2005; Wanisch et al., 2005; Matus-Amat et al., 2007; Schenberg et al., 2008), yet are in stark contrast to previous research from our laboratory and others demonstrating that neither lesions nor temporary inactivation of DH prior to training affect trace or contextual conditioning (Rogers et al., 2006; Yoon and Otto, 2007; Czerniawski et al., 2009).

The selective impairment in the acquisition vs. the expression of these associations after NMDA receptor antagonism strongly suggests that NMDA receptor activity is not required for the retrieval or expression of these memories. It is also important to stress that a transient blockade of NMDA receptors in DH dramatically impairs learning while more extensive temporary inactivation with muscimol or permanent lesions do not typically result in an acquisition deficit in these paradigms (Yoon and Otto, 2007; Czerniawski et al., 2009). Collectively, these data suggest that in the absence of DH functioning following lesion or temporary inactivation, compensatory mechanisms in other brain regions may subserve the acquisition of trace and contextual conditioning, but if the DH is intact, then NMDA receptor-mediated plasticity within this region normally occurs and is critical to either the maintenance or later recall of learned information. Consistent with this notion, we have recently reported a pronounced increase in *Arc* gene and protein expression after trace and contextual, but not delay, fear conditioning (Czerniawski et al., 2011). Moreover, in the same study, specific knockdown of *Arc* in DH using antisense oligodeoxynucleotides (ODNs) attenuated this learning-related increase in *Arc* protein expression and resulted in a dramatic impairment in both trace and contextual fear conditioning. Importantly, this learning-induced increase in *Arc* expression was also abolished by administration of APV, suggesting that when DH is intact, NMDA receptor-mediated *Arc* expression plays a prominent role in the consolidation of some forms of hippocampus-dependent learning. Collectively, our present data further support the notion that NMDA receptor-dependent synthesis of *Arc* protein may play a prominent role in the plasticity processes within DH that underlie several forms of learning, but that these processes can be mediated by other brain structures when the integrity of DH is compromised.

Role of VH in Trace and Contextual Fear Conditioning: Both Integrity of and Plasticity Within VH are Critical to the Acquisition and Expression of Trace and Contextual Conditioning

To date, this is the first study that has systematically examined the effect of APV administration in VH on the acquisition and subsequent expression of trace and contextual fear conditioning. It has been reported previously that administration of the noncompetitive NMDA receptor antagonist MK-801 into VH disrupted the acquisition of contextual fear conditioning (Zhang et al., 2001). In addition, we have previously demonstrated that pre-training or pre-testing lesions or inactivation of VH dramatically impair the acquisition and expression of trace fear conditioning (Yoon and Otto, 2007; Czerniawski et al., 2009). We now report that pre-training or pre-testing administration of APV also robustly attenuates the acquisition and expression of fear in these paradigms.

The present data suggest that, as in DH, NMDA receptor-dependent plasticity within VH may play a critical role in the acquisition of trace and contextual conditioning. Because there are direct reciprocal connections between VH, but not DH, and the amygdala (Pitkanen et al., 2000), it is likely that the integrity of VH is necessary for any behavior that may depend on communication between DH and the amygdala. However, few studies have examined whether plasticity within VH is required to form associations between contextual stimuli and an aversive US. The present data suggest that NMDA receptor-mediated plasticity in VH may mediate both contextual and trace fear conditioning, indicating it is likely more than just a "relay" between DH and the amygdala.

The fact that pre-testing infusions of APV within VH also impaired the expression of previously-acquired trace and contextual conditioning suggests that there may be an important role for NMDA receptors in the retrieval or expression of memory in these paradigms. Given that NMDA receptors are typically thought to be particularly important for the induction,

but likely not maintenance, of some forms of neuronal plasticity (Morris et al., 1990), it is generally thought that NMDA receptor antagonism should affect memory acquisition only, and that pre-testing infusions of APV should be without effect. At least two lines of evidence provide possible explanations for our observation. First, the results of several reports suggest that both pre-training and pre-testing NMDA receptor antagonism in the amygdala attenuates the expression of fear conditioning without affecting locomotion or sensory processing of the US (Maren et al., 1996; Lee and Kim, 1998; Lee et al., 2001), suggesting that APV may disrupt normal synaptic transmission necessary to process the CS (Lee et al., 2001). These data are consistent with our findings suggesting that the role of NMDA receptors within the VH may not be limited to NMDA receptor-dependent plasticity processes involved in memory acquisition in these paradigms. Rather, these data suggest that the expression and/or retrieval of trace and contextual fear conditioning likely depend upon NMDA receptor-mediated synaptic transmission in VH and amygdala. Notably, there is a significantly lower density of NMDA and AMPA receptors in VH compared to DH, as well as lower levels of mRNA and protein expression for the NMDA receptor units NR2A and NR2B in VH (Pandis et al., 2006). As the NR2A subunits are particularly important to the induction of long-term potentiation (LTP) in hippocampal neurons (Liu et al., 2004), this may explain why there is an increased threshold for LTP induction in VH compared to DH (Papatheodoropoulos and Kostopoulos, 2000). Furthermore, a higher ratio of AMPA/NMDA receptor sites in VH compared to DH suggests there may be a functional difference in excitatory glutamatergic transmission in these subregions (Pandis et al., 2006). Collectively, the differences in glutamate receptor density and subunit composition in DH and VH suggest differences in glutamatergic function, with NMDA receptor-mediated plasticity more prominent in DH and fast excitatory transmission more prominent in VH. A second, but not mutually exclusive, possibility is that NMDA receptor activation is an important substrate underlying the new learning that occurs during extinction. However, in the current paradigm we cannot distinguish between a possible role for NMDA receptors in expression vs. extinction because the testing session consisted of three trials and we did not retest the animals to the CS or context. Future studies will be required in order to further explore potential contributions of VH to the expression vs. extinction of these forms of aversive associative learning.

The striking similarity in the effect of pre-training and pre-testing NMDA receptor antagonism in VH and the amygdala, coupled with their direct reciprocal connections, strongly point toward a highly important interaction of these structures for mediating various aspects of aversive learning. Consistent with this notion, several laboratories have reported that amygdala and VH play critical but dissociable roles in fear and anxiety, respectively (Phillips and LeDoux, 1992; Bannerman et al., 2004; McHugh et al., 2004). Therefore, some manipulations of VH, such as temporary inactivation or permanent lesion, and perhaps NMDA receptor antagonism, may attenuate the expression of conditioned fear as a consequence of anxiety reduction during training and/or testing. Because of its strong and reciprocal connections with the amygdala, nucleus accumbens, and hypothalamus, an alternate possibility is that VH is generally important for attaching emotional value to specific learning events.

Effect of NMDA Receptor Antagonism in DH or VH on Freezing During Trace Fear Conditioning

During the trace fear conditioning session, subjects that received pre-training infusions of the NMDA receptor antagonist APV exhibited generally lower levels of freezing during the ITI, CS, and trace interval compared to subjects who received infusions of aCSF. This reduction in freezing was observed regardless of whether APV was administered into DH or VH, although the effect was notably milder for subjects receiving infusions into DH. The more pronounced effect of NMDA receptor antagonism in VH may be due to altered extrinsic connections between VH and amygdala as well as the aforementioned intrinsic differences in NMDA receptor functioning between VH and DH. It is also possible that blocking NMDA receptors affected short-term memory processes during conditioning. These are the first data exploring the effect of APV administration within VH on behavior during trace conditioning; however with respect to DH, this observation is similar to that reported by others (Quinn et al., 2005). In light of the fact that APV administration had no observable effect on locomotor behavior, this reduction in freezing during training is unlikely to be due to the potentially confounding effects of manipulation-induced hyperactivity. Moreover, it is important to point out that all subjects, regardless of infusion condition or brain region, froze significantly more during Trials 2–7 than they did during Trial 1 (before the first US presentation). Furthermore, Misane et al. (2005) have demonstrated that intrahippocampal pre-training APV administration impairs freezing to an auditory CS in trace but not delay fear conditioning. Therefore, it is unlikely that APV administration into DH affected sensory processing of the auditory CS or footshock during training. However, as discussed above, it is possible that APV infusions differentially disrupt ongoing synaptic transmission in VH relative to DH, which in turn could potentially account for the differential attenuation in freezing in VH compared to DH during conditioning.

Infusions of APV into DH or VH Do Not Affect Locomotor Activity

Several studies have reported that some manipulations of the hippocampus can alter locomotor processes that may, in turn, affect performance of freezing behavior used to infer learning (Good and Honey, 1997; Richmond et al., 1999). In the present study, infusions of APV into either DH or VH had no effect on locomotor behavior as measured by ambulation and rearing counts in the open field. In addition, subjects receiving infusions of APV before both training and testing exhibited significantly less freezing during testing compared to control subjects. Because all animals with pre-training APV infusions

exhibited attenuated freezing responses during testing regardless of their pre-testing infusion condition, our results cannot be interpreted as a state-dependent effect of drug infusions. Thus, any behavioral observations of reduced freezing in the experimental groups likely reflect a learning and not performance deficit.

NMDA Receptor Antagonism in Both DH and VH Impair the Acquisition of Contextual and Trace Fear Conditioning: An Argument Against a Functional Dissociation?

Pre-training NMDA receptor antagonism in DH or VH resulted in similarly robust performance deficits during subsequent testing sessions assessing acquisition of both trace and contextual conditioning, suggesting that NMDA receptor-mediated plasticity within both DH and VH may normally play an important role in this form of learning. This notion may appear, at least initially, to conflict with recent reports of a functional dissociation between DH and VH based on lesions or inactivation (Moser and Moser, 1998; Bannerman et al., 1999; Richmond et al., 1999; Pitkanen et al., 2000; Yoon and Otto, 2007; Czerniawski et al., 2009). However, when considered together, these emerging data suggest that while acquisition in some behavioral paradigms may not require the integrity of DH or VH, NMDA receptor-dependent plasticity within those same brain areas may normally underlie the acquisition and subsequent retention of memory if they are intact during learning. Moreover, it is possible that both subregions are typically engaged in the same types of learning to serve a "redundancy" function. For instance, we suggest that DH normally participates in the acquisition of trace and contextual fear conditioning, but that in its absence VH can serve a compensatory function that may involve an upregulation of activity in VH. Future studies will investigate the potentially dynamic interaction between these subregions involved in memory formation.

Do Contextual and Trace Fear Conditioning Rely on a Common Underlying Function of the Hippocampus?

The fact that NMDA receptor antagonism impairs the acquisition of both contextual and trace fear conditioning suggests that learning and memory in these paradigms may engage the hippocampus in a similar fashion and depend upon a common underlying chemistry. Why is the hippocampus important for contextual and trace conditioning but not delay conditioning? One possibility is that learning associations in trace and contextual conditioning is more difficult than in delay conditioning. However this is unlikely because all three of these forms of associative learning can be learned in a single trial (Misane et al., 2005). In the present study, the robust level of freezing expressed by control subjects during the trace interval compared to the CS suggests that they learned the temporal association between the CS and US. In fact, we consistently observe relatively low levels of freezing to the CS during trace (Otto and Yoon, 2007; Czerniawski et al., 2009) compared to delay fear conditioning (Czerniawski et al., 2011). Furthermore, control subjects freeze significantly more to the context in which they were trained and during the trace interval than during the baseline period in a novel context, supporting the notion that the observed levels of freezing reflect learned associations and not non-associative responses. Thus, because of these robust and discriminative levels of conditioned responding, it is unlikely that differences in task difficulty can account for the engagement of the hippocampus in trace and contextual, but not delay fear conditioning. Another possible explanation involves an important distinction between contextual or trace conditioning and delay conditioning with respect to the temporal specificity among stimuli in these paradigms. Unlike delay conditioning, in trace conditioning the CS and US are discontiguous, and in typical contextual conditioning the "context" itself is composed of a set of multimodal, temporally diffuse, and relatively imprecise stimuli that are not temporally phasic and do not co-terminate with the US. Therefore, in both contextual and trace conditioning, the hippocampus may serve to integrate temporally and/or spatially diffuse multi-modal information (Wallenstein et al., 1998).

Although traditional context learning may depend more on spatial processing and trace conditioning more on temporal processing, the hippocampus may play a general role in putting together the "what," "where," and "when" of a stimulus or event (Ergorul and Eichenbaum, 2004). Thus, while the DH and VH may be differentially involved in some learning paradigms, both are likely involved in forming relationships between temporally or spatially diffuse stimuli that can be used to influence behavior. For example, the spatial learning paradigms that depend on DH typically require the formation of a unified representation of the various stimuli that comprise the place (O'Keefe and Nadel, 1978; Moser and Moser, 1998). In addition, anxiety-related behaviors that tend to depend on the integrity of VH also include forming relationships between temporally diffuse stimuli, including a variety of motivational or emotional states (Davidson and Jarrad, 1993; Hock and Bunsey, 1998). Thus, it is likely that while DH and VH are sometimes recruited differentially for some types of learning, both subregions are important for forming configural, multimodal representations of spatially or temporally diffuse stimuli that enable animals to better learn about, and discriminate between, places and events.

REFERENCES

Bannerman DM, Yee BK, Good MA, Heupe MJ, Iversan SD, Rawlins JNP. 1999. Double dissociation of function within the hippocampus: A comparison of dorsal, ventral, and complete hippocampal cytotoxic lesions. Behav Neurosci 113:1170–1188.

Bannerman DM, Rawlins ,JNP, McHugh SB, Deacon RMJ, Yee BK, Bast T, Zhang WN, Pothuizen HHJ, Feldon J. 2004. Regional dissociations within the hippocampus—Memory and anxiety. Neurosci Behav Rev 28:273–283.

Bast T, Zhang WN, Feldon J. 2003. Dorsal hippocampus and classical fear conditioning to tone and context in rats: Effects of local NMDA-receptor blockade and stimulation. Hippocampus 13:657–675.

Bertoglio LJ, Lourenco Joca SR, Guimaraes FS. 2006. Further evidence that anxiety and memory are regionally dissociated within in the hippocampus. Behav Brain Res 175:183–188.

Canteras NS, Swanson LW. 1992. Projections of ventral subiculum to the amygdala, septum and hypothalamus: A PHAL anterograde tract-tracing study in the rat. J Comp Neurol 324:180–194.

Czerniawski J, Yoon T, Otto T. 2009. Dissociating space and trace in dorsal and ventral hippocampus. Hippocampus 19:20–32.

Czerniawski J, Ree F, Chia C, Ramamoorthi K, Kumata Y, Otto TA. 2011. The importance of having *Arc*: Expression of the immediate-early gene *Arc* is required for hippocampus-dependent fear conditioning and blocked by NMDA receptor antagonism. J Neurosci 31:11200–11207.

Davidson TL, Jarrad LE. 1993. A role for hippocampus in the utilization of hunger signals. Behav Neural Biol 59:167–171.

Ergorul C, Eichenbaum H. 2004. The hippocampus and memory for "what", "where", and "when". Learn Mem 11:397–405.

Fanselow MS, Dong H. 2010. Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 65:7–19.

Ferbinteanu J, McDonald RJ. 2001. Dorsal/ventral hippocampus, fornix, and conditioned place preference. Hippocampus 11:187–200.

Good M, Honey RC. 1997. Dissociable effects of selective lesions to hippocampal subsystems on exploratory behavior, context learning, and spatial learning. Behav Neurosci 111:487–493.

Hock BJ, Bunsey MD. 1998. Differential effects of dorsal and ventral hippocampal lesions. J Neurosci 18:7027–7032.

Jung MW, Wiener SI, McNaughton BL. 1994. Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. J Neurosci 14:7347–7356.

Kjelsrup KG, Tuvnes FA, Steffenach H, Murison R, Moser E, Moser M. 2002. Reduced fear expression after lesions of the ventral hippocampus. Proc Natl Acad Arts Sci 99:10825–10830.

Lee H, Kim JJ. 1998. Amygdalar NMDA receptors are critical for new fear learning in previously fear-conditioned rats. J Neurosci 18:8444–8454.

Lee HJ, Choi JS, Brown TJ, Kim, JJ. 2001. Amygdalar NMDA receptors are critical for the expression of multiple conditioned fear responses. J Neurosci 21:4116–4124.

Liu L, Wong TP, Pozza MF, Lingenhoehl K, Wang Y, Sheng M, Auberson YP, Wang YT. 2004. Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. Science 304:1021–1024.

Mao J, Robinson JK. 1998. Microinjection of GABA-A agonist muscimol into the dorsal but not the ventral hippocampus impairs nonmnemonic measures of delayed non-matching-to-position performance in rats. Brain Res 784:139–147.

Maren S, Aharonov G, Fanselow MS. 1996. Retrograde abolition of conditioned fear after excitotoxic lesions in the basolateral amygdala of rats: Absence of a temporal gradient. Behav Neurosci 15:7548–7564.

Maren S, Aharonov G, Fanselow MS. 1997. Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. Behav Brain Res 88:261–274.

Matus-Amat P, Higgins EA, Sprunger D, Wright-Hardesty K, Rudy JW. 2007. The role of dorsal hippocampus and basolateral amygdala NMDA receptors in the acquisition and retrieval of context and contextual fear memories. Behav Neurosci 121:721–731.

McHugh SB, Deacon RMJ, Rawlins JNP, Bannerman DM. 2004. Amygdala and ventral hippocampus contribute differentially to mechanisms of fear and anxiety. Behav Neurosci 118:63–78.

Misane I, Tovote P, Meyer M, Spiess J, Ogren SO, Steidl O. 2005. Time-dependent involvement of the dorsal hippocampus in trace fear conditioning in mice. Hippocampus 15:418–426.

Morris RG, Davis S, Butcher SP. 1990. Hippocampal synaptic plasticity and NMDA receptors: A role in information storage? Philos Trans R Soc Lond B Sci 329:187–2004.

Moser M, Moser EI. 1998. Functional differentiation in the hippocampus. Hippocampus 8:608–619.

Moser E, Moser M, Anderson P. 1993. Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. J Neurosci 13:3916–3925.

O'Keefe J, Nadel L. 1978. The Hippocampus as a Cognitive Map. New York: Oxford University Press.

Pandis C, Sotiriou E, Kouvaras E, Asprodini E, Papatheodoropoulos C, Angelatou F. 2006. Differential expression of NMDA and AMPA receptor subunits in rat dorsal and ventral hippocampus. Neuroscience 140:163–175.

Papatheodoropoulos C, Kostopoulos G. 2000. Decreased ability of rat temporal hippocampal CA1 region to produce long-term potentiation. Neurosci Lett 279:177–180.

Pentkowski NS, Blanchard DC, Lever C, Litvin Y, Blanchard RJ. 2006. Effects of lesions to the dorsal and ventral hippocampus on defensive behaviors in rats. Eur J Neurosci 23:2185–2196.

Phillips RG, LeDoux JE. 1992. Differential contributions of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106:274–285.

Pitkanen A, Pikkarainen M, Nurminen N, Ylinen A. 2000. Reciprocal connections between the amygdala and hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. Ann NY Acad Sci 911:369–391.

Quinn JJ, Loya F, Ma QD, Fanselow MS. 2005. Dorsal hippocampus NMDA receptors differentially mediate trace and contextual fear conditioning. Hippocampus 5:665–674.

Richmond MA, Yee BK, Pouzet B, Veenman L, Rawlins JN, Feldon J, Bannerman DM. 1999. Dissociating context and space within the hippocampus. Effects of complete, dorsal, and ventral excitotoxic lesions on conditioned freezing and spatial learning. Behav Neurosci 113:1189–1203.

Rogers JL, Hunsaker MR, Kesner RP. 2006. Effects of dorsal and ventral CA1 subregional lesions on trace fear conditioning. Neurobiol Learn Mem 8:72–81.

Schenberg EE, Oliveira MGM. 2008. Effects of pre or posttraining dorsal hippocampal D-AP5 injection on fear conditioning to tone, background, foreground context. Hippocampus 18:1089–1093.

Trivedi MA, Coover GD. 2004. Lesions of the ventral hippocampus, but not dorsal hippocampus, impair conditioned fear expression and inhibitory avoidance on the elevated T-maze. Neurobiol Learn Mem 81:172–184.

Wallenstein GV, Eichenbaum H, Hasselmo ME. 1998. The hippocampus as an associator of discontiguous events. Trends Neurosci 8:317–323.

Wanisch K, Tang J, Mederer A, Wotjak C. 2005. Trace fear conditioning depends on NMDA receptor activation and protein synthesis within the dorsal hippocampus of mice. Behav Brain Res 157:63–69.

Wiltgen BJ, Fanselow MS. 2003. A model of hippocampal-cortical-amygdala interactions based on contextual fear conditioning. In: Jeffery KJ, editor. The Neurobiology of Spatial Behaviour. New York: Oxford UP. pp.83–103.

Yoon T, Otto T. 2007. Differential contributions of the dorsal and ventral hippocampus in rats to trace fear conditioning. Neurobiol Learn Mem 87:464–475.

Young SL, Bohenek DL, Fanselow MS. 1994. NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: immunization against amnesia by context preexposure. Behav Neurosci 108:19–29.

Zhang WN, Bast T, Feldon J. 2001. The ventral hippocampus and fear conditioning in rats: Different anterograde amnesias of fear after infusion of *N*-methyl-D-aspartate or its noncompetitive antagonist MK-801 into the ventral hippocampus. Behav Brain Res 126:159–174.