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Effects of ventral and dorsal CA1 subregional lesions on trace fear conditioning

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Abstract

Recent lines of research have focused on dissociating function between the dorsal and ventral hippocampus along space and anxiety dimensions. In the dorsal hippocampus, the CA1 subregion has been implicated in the acquisition of contextual fear as well as in the trace interval in trace fear conditioning. The present study was designed to test the relative contributions of dorsal (dCA1) and ventral CA1 (vCA1) in trace fear conditioning. Long–Evans rats received ibotenate lesions of the ventral CA1 ($n = 7$), dorsal CA1 ($n = 9$), or vehicle control lesions ($n = 8$) prior to trace fear conditioning acquisition. Results suggest dCA1 and vCA1 groups show no significant deficits during acquisition when compared to control groups. dCA1 and vCA1 both show deficits in the retention of contextual fear when tested 24 h post-acquisition ($P < .05$ and $P < .01$, respectively), and vCA1 was impaired relative to dCA1 ($P < .05$). This is suggestive of a graded involvement in contextual retention between the dorsal and ventral aspects of CA1. dCA1 showed no deficit for retention of conditioned fear to the tone or the trace when tested 48 h post-acquisition, whereas vCA1 did show a significant deficit for the trace interval and a slight, non-significant reduction in freezing to the tone, when compared to the control group ($p < .05$). Overall the data are suggestive of a graded involvement in retention of fear conditioning between the dorsal and ventral aspects of CA1, but it is likely that vCA1 may be critically involved in retention of trace fear conditioning.

Keywords: Trace fear conditioning; Ventral hippocampus; Dorsal hippocampus; CA1

1. Introduction

In addition to its role in associating conditioned fear with contextual stimuli (Kim & Fanselow, 1992; Maren, Aharonov, & Fanselow, 1997), the hippocampus appears to be necessary to form associations between a conditioned stimulus (CS) and unconditional stimulus (US) when a temporal gap or 'trace' separates the CS from the US ("trace" conditioning). In both eye-blink conditioning in rabbits (Kim, Rison, & Fanselow, 1995; McEchron & Disterhoft, 1999; Moyer, Deyo, & Disterhoft, 1990) and fear conditioning in rats (McEchron, Bouwmeester, Tseng, Weiss, & Disterhoft, 1998; Quinn, Oommen, Morrison, & Fanselow, 2002; Tseng, Guan, Disterhoft, & Weiss, 2004; Weiss, Bouwmeester, Power, & Disterhoft, 1999), lesions of the hippocampus retard or attenuate trace conditioning; however, both Weiss et al. (1999) and Moyer et al. (1990) reported that when the CS and US coincide (delay conditioning) animals with lesions of the hippocampus perform as well as controls. These data suggest the hippocampus plays a role in the acquisition of trace fear conditioning but not classical conditioning of fear. In all of the above studies, except the Quinn et al. (2002), lesions were made to the hippocampus prior to conditioning, suggesting that the hippocampus is necessary for the acquisition of trace conditioning. Plasticity in the hippocampus plays an important role given that in mice and rats the acquisition of trace fear or trace eye-lid conditioning is impaired with NMDA receptor antagonist (APV) injections into the dorsal

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hippocampus prior to learning (Misane et al., 2005; Quinn, Loya, Quang, & Fanselow, 2005; Sakamoto et al., 2005). In humans, Knight, Cheng, Smith, Stein, and Helmstetter (2004) and Buchel, Dolan, Armony, and Friston (1999) found an increase in hippocampal activity during trace, but not delay conditioning using functional magnetic resonance imaging (fMRI); supporting the findings in both rabbits and rats. Furthermore, post-conditioning lesions of the hippocampus appear to cause a temporally graded retrograde amnesia (Anagnostaras, Maren, & Fanselow, 1999; Takehara, Kawahara, & Kirino, 2003), suggesting that the hippocampus may play a role in the consolidation and/or retrieval of previously conditioned contextual fear (for review see Maren & Holt, 2000). It appears that the hippocampus is necessary for the acquisition, consolidation, and retrieval of temporal information in trace fear conditioning; an idea supported by both computational and cognitive models (Kesner & Rogers, 2004; Kesner, 1998; Rolls, 1996).

The hippocampus primarily receives cortical information from the entorhinal cortex (EC) (Siegal & Tassoni, 1971; Swanson & Cowan, 1977). Steffenach, Witter, Moser, and Moser (2005) found that fiber-sparing lesions of the dorsolateral band of the EC, which projects mainly to the dorsal hippocampus, retarded rats' retention as well as inhibited new learning on the water maze while showing no discernible effect on anxiety-related behaviors, measured as activity on the elevated plus-maze. In contrast, fiber-sparing lesions of the ventromedial band of the EC, which projects mainly to the ventral hippocampus, reduced anxiety-related behaviors on the elevated plus-maze, but had no discernible effect on water maze training. Some investigators suggest that the contribution of the EC could vary as a function of the targets within the hippocampus. For example, axons terminating in the dorsal hippocampus, defined as the 50% of hippocampal volume starting at the septal pole (homologous to the posterior hippocampus in primates and humans), are thought to mediate behaviors related to spatial learning and memory; whereas axons terminating in the ventral hippocampus, 50% of hippocampal volume starting at the temporal pole (homologous to the anterior hippocampus), are thought to mediate behaviors related to anxiety, fear, and/or stress (Bannerman et al., 1999, 2004; Moser & Moser, 1998). Furthermore, the ventral hippocampus appears to have more connections to other limbic structures implicated in anxiety, fear, and/or stress related behaviors: namely the prefrontal cortex (Barbas & Blatt, 1995; Goldman-Rakic, Selemon, & Schwartz, 1984), bed nucleus of the stria terminalis, and the amygdala (Henke, 1990) which has reciprocal connections with the ventral hippocampus (Pitkanen, Pikkarainen, Nurminen, & Ylinen, 2000). Bast and colleagues (2001) reported that inactivation of the ventral hippocampus attenuated freezing to discrete and contextual cues during delay conditioning, offering behavioral support to the anatomical data; however, Kjelstrup et al. (2002) have reported that ventral hippocampal lesions fail to impair contextual conditioning. These authors also reported that ventral hippocampal lesions show reduced anxiety-related behaviors on the elevated plus-maze, a finding supported by Trivedi and Coover (2004). Recent findings from Burman, Starr, and Gewirtz (2006) found that lesions of the dorsal hippocampus attenuated acquisition of trace conditioning, whereas, larger lesions, encompassing both dorsal and ventral hippocampus, impaired retention of trace conditioning. Despite these apparent contradictions, the anatomical data suggest a potential dissociation for hippocampal function along the dorsal–ventral axis with the dorsal hippocampus implicated in spatial learning and memory and the ventral hippocampus implicated in more anxiety-related behaviors.

In their review of the roles of the different subregions of the hippocampus in learning and memory, Kesner, Lee, and Gilbert (2004) suggest that the CA1 subregion mediates temporal information processing by means of temporally "chunking" information and temporal pattern separation. In support of this view, Gilbert, Kesner, and Lee (2001) found that lesions of the CA1 subregion disrupted memory for temporal order using an eight-arm radial maze. Huerta, Sun, Wilson, and Tonegawa (2000) reported that mice lacking NMDA receptors in the CA1 subregion (dorsal plus ventral) failed to learn a 30-s trace fear conditioning paradigm, but were unimpaired during delay conditioning. Furthermore, McEchron, Tseng, and Disterhoft (2003) found that a significant portion of CA1 neurons fired maximally following CS offset (trace interval) during a 10- and 20-s trace conditioning paradigm in rabbits. These authors also reported that neuronal firing was largest when the fear responses were greatest, suggesting a relationship between CA1 activity and behavior. These data suggest that the CA1 subregion of the hippocampus is critically involved in information processing involving CS–US associations if a temporal component (trace) is interposed.

Given that the CA1 subregion of the hippocampus seems to be important for temporal processing, the present study sought to investigate potential differences between the respective roles of the dorsal and ventral components of CA1 in trace fear conditioning. Based on the findings above, it was hypothesized that lesions of dorsal CA1 would produce a deficit in contextual conditioning; however, the inconsistent findings with the ventral hippocampus made it unclear whether or not ventral CA1 lesions would attenuate contextual conditioning. In addition, it was unclear whether either lesion would selectively impair acquisition, retention, or would impair both. Furthermore, it was hypothesized that ventral CA1 lesions would attenuate tone and trace conditioning, but the extent to which ventral CA1 lesions would impair freezing to the tone was unknown. Finally, if the ventral hippocampus is involved in anxiety-related behavior, an overall increase in activity may be found, whereas if the dorsal hippocampus processes spatial learning and memory a deficit in context and/or trace, but not the tone, would be seen. At present, no study has examined the role of the ventral hippocampal subregions (i.e., CA1, CA3) in any behavioral task. Understanding the

role of hippocampal subregions along the dorsal–ventral axis is critical in order to create a holistic theory/model of hippocampal function. In the present paradigm, a 10-s temporal gap, or trace interval, separated the tone stimulus (CS) from the foot-shock (US). In addition, rats were given a 2-min pre-exposure period, critical for encoding contextual information (Fanselow, 1990). This paradigm provides a measure of CA1 function in the dorsal and ventral components of the hippocampus as it (a) allows for the investigation of the encoding and retrieval of fear conditioning during the trace interval separate from the shock, tone, or context and (b) separates encoding and retention of fear conditioning for the context from the shock, tone, or trace.

2. Materials and methods

2.1. Animals

Twenty-four Long–Evans rats (Simonsen Laboratories, Inc., Gilroy, CA), approximately 4 months of age and weighing 300–400 g at the start of experimentation served as subjects. The rats were housed individually in plastic tubs located in a colony with a 12-h light–dark cycle. All testing was conducted during the light portion the light–dark cycle. All rats were free fed and had ad libitum access to water. All experiments were conducted according to the NIH Guide for the Care and Use of Laboratory Animals and the University of Utah Institutional Animal Care and Use Committee.

2.2. Surgery

Rats were randomly assigned to a surgery group. Rats were anesthetized and maintained with sodium pentobarbitol (60 mg/kg i.p.) and given atropine sulfate (0.2 mg/kg i.m.) as a prophylactic. Rats that received a lesion of the ventral CA1 subregion were given 0.75 mL diazepam (2 mg/mL i.p.) 10 min prior to surgery to prevent any seizure activity that may result from the excitotoxic lesion. Each rat was placed in a stereotaxic apparatus (David Kopf Instruments, Tunjana, CA) with its head level. The scalp was incised and retracted to expose bregma and lambda which were adjusted into the same horizontal plane by moving the incisor bar dorsoventrally. Dorsal CA1 lesions ($n = 9$) were made using ibotenic acid (Biosearch Technologies, Inc., San Rafael, CA, 8 mg/mL), infused using a micro infusion pump (Cole-Parmer; Vernon Hills, IL) and a 10-μL Hamilton syringe (Hamilton; Reno, NV) at a rate of 6 μL/hr and a volume of 0.10 or 0.15 μL depending on the site, bilaterally into three sites. After infusion, the cannula remained in each site for 1 min to allow diffusion of the injected excitotoxin. The coordinates for dorsal CA1 lesions based on the Paxinos and Watson atlas (1997) were 3.6 mm posterior to bregma, 1.0 mm lateral to the midline, 2.4 mm ventral to dura (0.1 μL volume ibotenic acid injected); 3.6 mm posterior to bregma, 2.0 mm lateral to the midline, 2.1 mm ventral to dura (0.1 μL); 3.6 mm posterior to bregma, 3.0 mm lateral to the midline, 2.3 mm ventral to dura (0.15 μL). Ventral CA1 lesions ($n = 7$) were also made using ibotenic acid, infused following the same procedures as dorsal CA1 lesions, into three sites within the ventral hippocampus located 5.3 mm posterior to bregma, 3.0 mm lateral to the midline, 2.8 mm ventral to dura (0.1 μL); 5.3 mm posterior to bregma, 5.2 mm lateral to the midline, 4.0 mm ventral to dura (0.1 μL); 5.3 mm posterior to bregma, 5.8 mm lateral to the midline, 6.2 mm ventral to dura (0.15 μL). Vehicle control lesions (vCA1 vehicle $n = 4$ and dCA1 vehicle $n = 4$) were made using the same coordinates and procedures as the dCA1 group; however, physiological saline was infused. Following surgery, the incision was sutured, 1.5 mL saline was injected into each hip subcutaneously to expel the anesthetic, and the rats were allowed to recover on a heating pad before returning to their home cage. In addition, rats received acetaminophen (Children's Tylenol; 200 mg/100 mL water) as an analgesic and mashed food for three days following surgery. The behavior of all animals was monitored for epileptiform activity for 7 days post-surgery. No epileptic behavior was observed.

2.3. Histology

After all behavioral testing was completed, rats were euthanized with a lethal dose of sodium pentobarbital (70 mg/mL i.p.) and perfused intracardially with 0.9% phosphate buffered saline (pH 6.0) for 2 min followed by 10% buffered formalin (pH 7.0) for another 5 min. The brains were then extracted and stored in 30% sucrose formalin for 72 h before being frozen and sliced into 40 μm sections with a freezing-stage microtome. Control and dorsal CA1 lesions were cut along the coronal plane, whereas ventral CA1 lesions were cut along the transverse plane. In both cases, every third section from the tissue block containing the hippocampus was mounted on microscopic slides and stained with cresyl violet for microscopic verification of the lesions. The percent damage for each animal was averaged from the sections and then summed across animals. Sections were photographed and imported into Image J (National Institute of Health, Bethesda MD) for quantitative lesion analysis.

2.4. Experimental apparatus

Two observation chambers were used during the three consecutive days of testing. The first chamber was used for conditioning and the contextual retention test. This chamber (28 × 21 × 22 cm; Coulbourn Instruments, Allentown, PA) consisted of two clear Plexiglas walls (rear wall and front door) and two aluminum sidewalls. The chamber floor contained 18 steel rods connected to a precision-regulated shocker (Coulbourn Instruments, Allentown, PA) delivering an electric foot-shock stimulus. A speaker was inserted into one of the aluminum sidewalls of the conditioning chamber to deliver the tone. A computer program (Graphic State, Coulbourn Instruments, Allentown, PA) controlled the presentation of all stimuli. The chamber was located in an isolated room lit with fluorescent and halogen lamps. Numerous visual cues such as toys and posters were located around the conditioning chamber to provide contextual cues. A video camera recorded the animal's behavior. The chamber was cleaned using a weakened cleaning solution (HDQ cleaner). A second observation chamber tested the retention of the tone stimulus and trace interval in the absence of any contextual cues or shock. This chamber (32 × 32 × 32 cm) was constructed from clear Plexiglas on all sides of the chamber. A speaker was attached to a hole (2.5 cm diameter) made on one of the walls of the chamber to deliver auditory stimuli. The chamber was located in a different room surrounded by completely different visual cues. The chamber was cleaned between sessions to remove stress odors using water.

2.5. Procedure

2.5.1. Day 1—acquisition

Rats were placed in the fear conditioning chamber for 2 min without a tone stimulus as a baseline. After the 2 min baseline period, rats received 15 trials of tone-trace-shock pairings. A tone (32 s, 2 kHz, 85-dB), presented through a speaker, initiated each trial. A trace period (10-s) separated the tone and shock. An electric foot-shock (2-s, 0.5 mA) was delivered through the shock-floor after the trace period. A 72-s intertrial interval (ITI) separated each successive trial. After the 15th and final tone-trace-shock pairing, the rats remained in the chamber for an additional 2 min without tone or shock stimuli. A freezing response (e.g., absence of movement minus respiratory movement) was measured by a blind observer, who scored freezing behavior every 8-s for the baseline, tone, and ITI and every 5-s for the trace; resulting in 15 total baseline observations, as well as 4 tone observations, 2 trace observations, and 9 ITI observations for each trial.

2.5.2. Day 2—contextual retention test

Each rat was tested for retrieval of contextual conditioning 24 h after acquisition. The rat was placed in the same chamber used during the acquisition period for 8 min in the absence of the tone stimulus. Freezing

behavior was measured every 8 s by a blind observer. Due to extinction, only the first 6 min of testing was analyzed.

2.5.3. Day 3—tone and trace retention test

Each rat was placed in the clear Plexiglas chamber 48 h after acquisition. The rat received a 2-min pre-exposure period followed by 15 tone-trace combinations (the same tone presented in Day 1 acquisition). The tone was presented for 32 s, followed by a 12-s trace period. The 12 s trace reflected the 10-s trace period during acquisition plus 2 s for the duration of the shock which was absent in the test. Freezing behavior was measured every 8 s by a blind observer during the 2-min baseline and the 15 tone-trace periods. Due to extinction, only the first 9 tone-trace periods were analyzed.

2.6. Data analysis

Freezing scores were transformed to percent scores of total observations and blocked into groups of three trials for data analysis. Repeated measures analysis of variance (ANOVA) was employed for testing group differences during acquisition and retention of the context, tone stimulus, and trace interval. Furthermore, a post hoc comparison (Student–Newman–Keuls (SNK)) was made when necessary. All effects were considered statistically significant if $P < .05$. Acquisition data are presented in five blocks of trials with three trials per block, shown as means ± SEMs.

3. Results

3.1. Histology

Ibotenic acid was used to produce selective lesions of pyramidal cells in dorsal and ventral CA1 (Fig. 1). Jarrard (1989) demonstrated ibotenic acid's efficacy as an axon-sparing excitotoxin, making it a suitable toxin for the present lesions. As CA1-specific neurotoxins are not available, the present study used previously established methods for producing selective damage to CA1 (Gilbert et al., 2001; Lee & Kesner, 2004). As shown in Fig. 1, ibotenic acid produced almost complete degeneration of the pyramidal cells when infused into the dorsal CA1 (Fig. 1B) and ventral CA1 (Fig. 1C); however, the pyramidal cells in CA3 and the granule cells in the dentate gyrus (DG) remained intact. A quantitative analysis using Image J showed that for the dorsal CA1 group, the lesions resulted in 90% damage to the pyramidal cells in CA1 with approximately 20% spread into CA2, but in no cases was CA3 affected. In some sections, some damage spread ventrally to the upper blade of the dentate gyrus, but this caused less than 5% damage to the dentate gyrus granule cells in the upper blade. There was no damage to overlying cortices. The ventral CA1 lesions were as precise as the dorsal CA1 lesions. There was usually some sparing at the most temporal pole of CA1. Approximately 80% of the CA1 pyramidal cells were ablated in the ventral CA1 lesions with sparing in the most CA2 aspect of CA1. There was usually limited damage to CA2, but never CA3. In no cases was damage to surrounding cortices observed. The dorsal CA1 lesions in the present study are consistent with results found from previous studies conducted in the lab (Gilbert et al., 2001; Lee & Kesner, 2004) and the ventral lesions show the same level of subregional specificity.

3.2. Data analysis

3.2.1. Acquisition of the intertrial interval

The results, shown in Fig. 2A, indicate that during acquisition all groups readily displayed freezing behavior during the intertrial interval (ITI). A repeated measures ANOVA with groups as the between factor and blocks of trials as the within factor revealed no significant main effect for groups [$F(2,21) = 1.29$; $P = .30$]; however, there was a significant

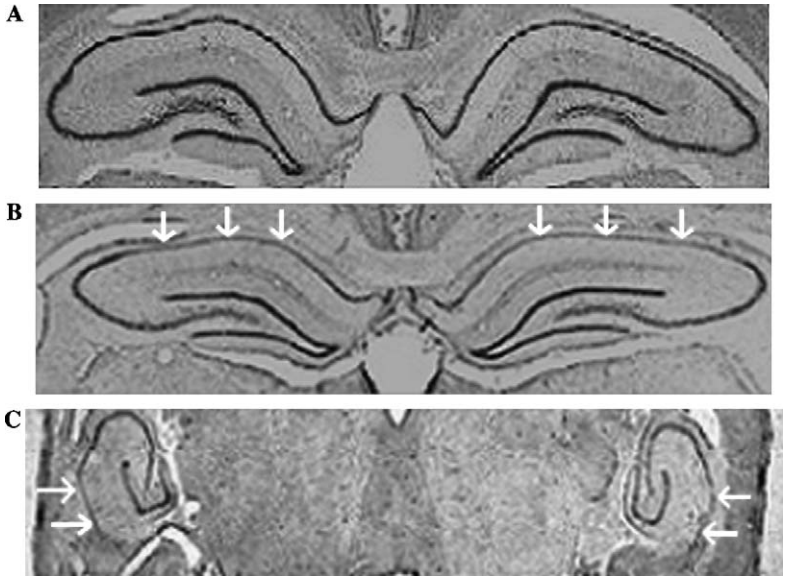

Fig. 1. Representative photomicrographs of subregion-specific lesions of the hippocampus. (A) Coronal section of a vehicle control. (B) Coronal section of a dorsal CA1 lesion. Note the degeneration of pyramidal cells in dorsal CA1 as compared to the control group and intact dorsal CA3 and dorsal DG. (C) Transverse section of a ventral CA1 lesion. Note the degeneration of pyramidal cells in ventral CA1 as compared to the control group and relatively spared ventral DG and ventral CA3.

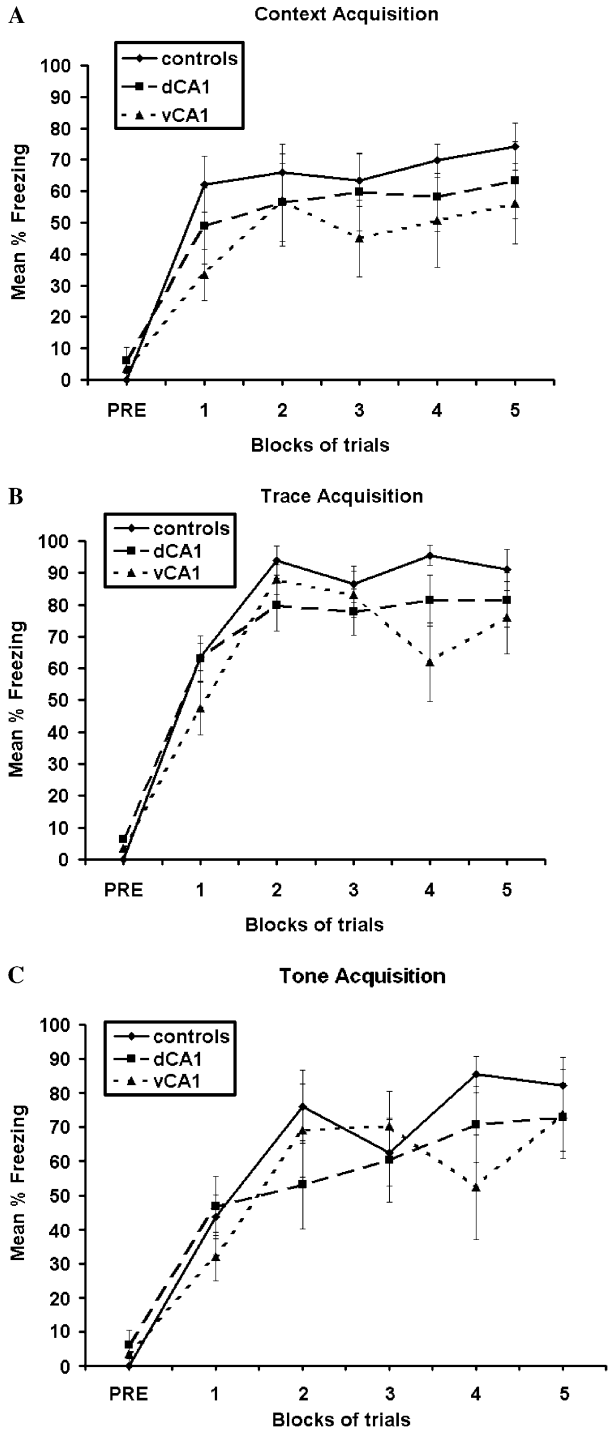

Fig. 2. Percent freezing during acquisition. (A) Context following the pre-acquisition baseline period (PRE) on Day 1. Data are presented as blocks of trials where each block represents three trials. Note that there are no significant differences among the groups. (B) Trace interval following the pre-acquisition baseline period (PRE) on Day 1. Data are presented as blocks of trials where each block represents three trials. Note that there are no significant differences among the groups. (C) Tone stimulus following the pre-acquisition baseline period (PRE) on Day 1. Data are presented as blocks of trials where each block represents three trials. Note that there are no significant differences among the groups.

main effect for blocks of trials [$F(4,84) = 3.06$; $P = .02$], suggesting that all rats learned the task. There were no significant interactions between the groups and trials during ITI acquisition [$F(8,84) = 0.47$; $P = .87$].

3.2.2. Acquisition of the trace interval

The results, shown in Fig. 2B, indicate that during acquisition all groups readily displayed freezing behavior during the trace interval. A repeated measures ANOVA with groups as the between factor and blocks of trials as the within factor revealed no significant main effect for groups [$F(2,21) = 3.11$; $P = 0.06$]. Since the P value approached significance and the trace acquisition graph suggested a possible dissociation between lesion groups that may have been missed by the ANOVA, a SNK post hoc was performed. Neither dCA1 nor vCA1 were significantly different from controls ($P > .05$). There was a significant main effect for blocks of trials [$F(4,84) = 9.31$; $P < .0001$], suggesting that all rats learned the task. There were no significant interactions between the groups and trials during acquisition of the trace interval [$F(8,84) = 1.18$; $P = .32$].

3.2.3. Acquisition of the tone stimulus

The results, shown in Fig. 2C, indicate that during acquisition all groups readily displayed freezing behavior during the tone stimulus. A repeated measures ANOVA with groups as the between factor and blocks of trials as the within factor revealed no significant main effect for groups [$F(2,21) = 1.38$; $P = .27$]; there was a significant main effect for blocks of trials [$F(4,84) = 11.59$; $P < .0001$], suggesting that all rats learned the task. There were no significant interactions between the groups and trials during acquisition of the tone stimulus [$F(8,84) = 1.75$; $P = .10$].

3.2.4. Contextual retention

Although the animals were tested on context retention for 8 min, only 6 min was analyzed and divided into three blocks of 2 min trials, as the control group began to extinguish to the context after 6 min. The results, shown in Fig. 3A, indicate that during retention of the context the ventral CA1 group froze significantly less than either the dorsal CA1 group or control group, whereas the dorsal CA1 group froze significantly less than the control group. A repeated measures ANOVA conducted on the context test administered 24 h post-training with groups as the between factor and blocks of trials as the within factor revealed a significant main effect for groups [$F(2,21) = 13.56$; $P < .0006$]. Post hoc analysis (SNK) indicated that the ventral CA1 lesion group froze significantly less than either the control ($P < .01$) or dorsal CA1 groups ($P < .05$), whereas the dorsal CA1 group froze significantly less than the control group ($P < .05$). This is suggestive of a graded involvement in contextual retention between the dorsal and ventral aspects of CA1. There was no main effect for blocks of trials [$F(2,42) = 2.08$; $P = .14$], nor was there an interaction between groups and blocks of trials [$F(4,42) = 0.60$; $P = .66$].

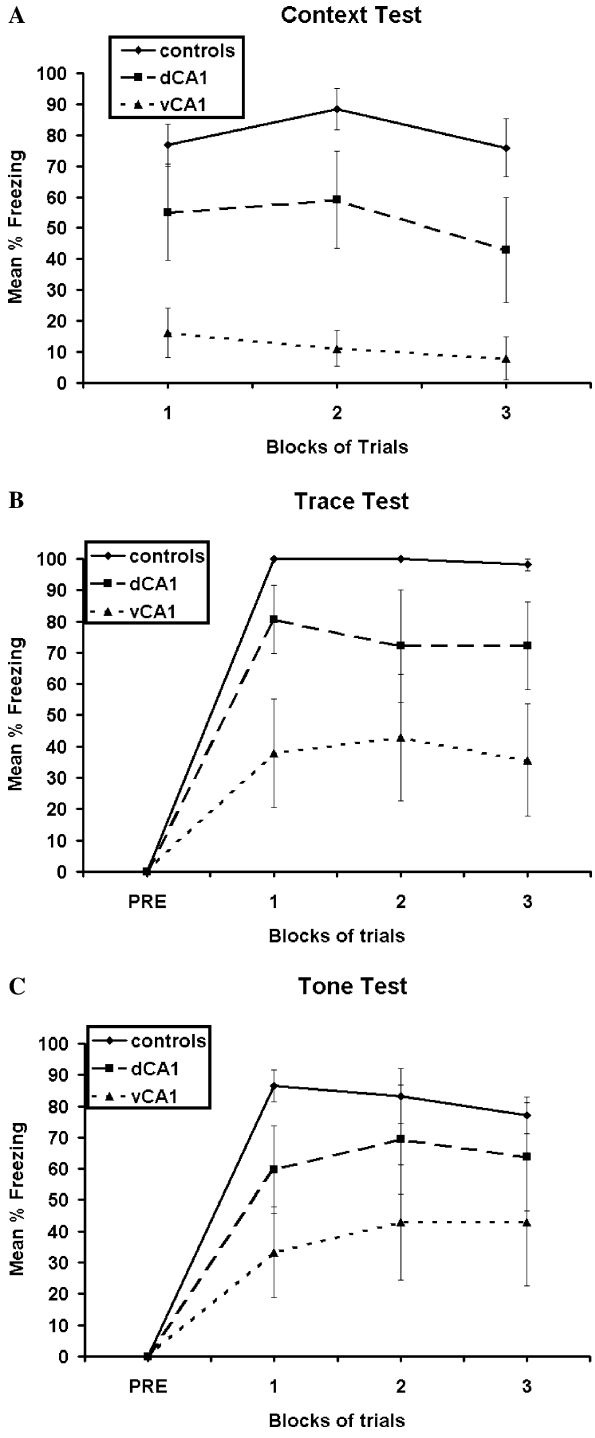

Fig. 3. Percent freezing during retention tests given post-conditioning. (A) Contextual retention tested 24 h post-conditioning. Data are presented as a function of blocks of trials where each block represent a 2-min time period. Note that the vCA1 group froze significantly less than either control or dCA1 groups, whereas the dCA1 group froze significantly less than the control group. (B) Trace retention test given following pre-retention baseline period (PRE) 48 h post-conditioning. Data are presented as blocks of trials where each block represents three trials. Note that the vCA1 group froze significantly less than either dCA1 group or control group, whereas the dCA1 group was not significantly different from the control group. (C) Tone retention test given following pre-retention baseline period (PRE) 48 h post-conditioning. Data are presented as blocks of trials where each block represents three trials. Note that there are no significant differences between groups.

3.2.5. Trace interval retention

The results, shown in Fig. 3B, indicate that during retention the ventral CA1 group froze significantly less than the controls, whereas the dorsal CA1 group showed no impairment during the trace test administered 48 h post-training. A repeated measures ANOVA with groups as the between factor and blocks of trials as the within factor revealed a significant main effect for groups during retention of the trace interval [$F(2,21) = 7.07$; $P = .005$]. Post hoc analysis (Student–Newman–Keuls) indicated that the ventral CA1 lesion group froze significantly less than the control group ($P < .01$) as well as significantly less compared to the dCA1 group ($P < .05$). Dorsal CA1 lesion animals did not differ from controls ($P > .05$). The vCA1 appears to be critically involved in subserving the trace component of fear conditioning, whereas dCA1 seems to play only a minor role. There was no main effect for blocks of trials during retention of the trace period [$F(2,42) = 0.81$; $P = .45$], nor was there an interaction between the groups and blocks of trials [$F(4, 42) = 0.62$; $P = .58$].

3.2.6. Tone stimulus retention

The results, shown in Fig. 3C, indicate that during retention all groups readily displayed freezing behavior during the tone test administered 48 h post-training. A repeated measures ANOVA with groups as the between factor and blocks of trials as the within factor revealed no significant main effect for groups during retention of the tone stimulus [$F(2,21) = 2.96$; $P = .08$]. Since the P value approached significance, an SNK post hoc analysis was performed on the data. No significant differences between groups was revealed ($P > .05$). Furthermore, there was no main effect for blocks of trials during retention of the tone period [$F(2, 42) = 0.56$; $P = .57$], nor was there an interaction between the groups and blocks of trials [$F(4,42) = 0.70$; $P = .59$].

4. Discussion

The present study sought to investigate potential dissociations between the roles of the dorsal and ventral components of CA1 during trace fear conditioning. It was hypothesized that lesions of the dorsal CA1 (dCA1) would produce a deficit in contextual conditioning; however, it was unclear whether or not ventral CA1 (vCA1) lesions would attenuate contextual conditioning. In addition, it was unclear whether either lesion would impair acquisition, retention, or both. Furthermore, it was hypothesized that ventral CA1 lesions would attenuate trace conditioning, but the extent to which ventral CA1 lesions would impair freezing to the tone was unknown. The present paradigm employed a 10-s trace interval to separate the tone stimulus (CS) from the foot-shock (US). In addition, rats were given a 2-min pre-exposure period, critical for encoding contextual information (Fanselow, 1990).

Surprisingly, acquisition of conditioned fear was uninterrupted by lesions of the dorsal or ventral CA1. Animals readily froze to the context during the intertrial interval

(ITI) (Fig. 2A), trace interval (Fig. 2B), or tone stimulus (Fig. 2C). Although it appears that there was some retardation of freezing during acquisition for the vCA1 lesion group, this effect was not statistically significant and was deemed unreliable. The data also indicate that both dCA1 and vCA1 lesions significantly reduced freezing during the context test administered 24 h post-conditioning (Fig. 3A); however, the vCA1 lesion group froze significantly less than either control or dCA1 lesion groups. Freezing behavior of the vCA1 lesion group was all but abolished when returned to the conditioning chamber for the context test. The pattern of the data is suggestive of a gradient in function along the dorso-ventral axis of the hippocampus. The dCA1 showed a deficit when compared to controls, but vCA1 showed a deficit when compared to dCA1 and a larger deficit when compared to controls. This means that dCA1 is in fact involved in contextual fear recollection, but vCA1 appears to be more important.

Similarly, the vCA1 lesion group froze significantly less than either control or dCA1 lesion group during the trace test administered 48 h post-conditioning (Fig. 3B). The dCA1 group showed a mild attenuation of freezing during the trace test; however, this was not reliable. The dCA1 group was not significantly different than controls, but the data indicate a marginal effect. Finally, no significant group differences were observed during the tone test also administered 48 h post-conditioning (Fig. 3C). The lesion groups showed attenuation of freezing, but this effect was not reliable. Thus, the findings indicate no differences among the groups during acquisition of trace fear conditioning, or retention of the tone (CS). The vCA1 lesion group displayed significantly reduced freezing during the context test as well as during the trace test, whereas the dCA1 lesioned group displayed significantly reduced freezing only during the context test and a small, insignificant attenuation of freezing to the trace interval. One caveat is that all animals received the context test before the tone-trace test. Although there were no overlapping stimuli, future studies may be needed to determine if any order effects exist.

It should be noted that in the present experiment the ventral CA1 lesion animals did not display hyperactivity. A close look at the acquisition data reveal the ventral CA1 animals were able to acquire the trace fear conditioning task as readily as the dCA1 and control groups. The vCA1 group was able to display freezing behavior during the acquisition phase. Also, the tone test data suggest the ventral CA1 animals, although slightly impaired, were able to freeze to the discrete CS 48 h post-acquisition; thus it is argued that hyperexcitability or hyperactivity were not factors in the deficits displayed by the ventral CA1 lesion animals. Also, during the 2-min baseline period prior to acquisition as well as prior to the trace and tone test the vCA1 animals did not show any excessive running, rearing, jumping, or other ambulatory behaviors compared to any other group.

The reduced freezing during the context test for the dCA1 group is consistent with previous findings with dorsal hippocampal lesions (Kim & Fanselow, 1992; Maren et al., 1997), as well as dCA1 lesions (Lee & Kesner, 2004), during delay conditioning. The reduced freezing during the context test for the vCA1 group is consistent with previous findings from Bast, Zhang, and Feldon (2001), who showed that temporary inactivation of the ventral hippocampus via tetrodotoxin (TTX) or the $GABA_A$ agonist muscimol attenuated freezing when animals were returned to the conditioned fear chamber. Rudy and Matus-Amat (2005) found that muscimol inactivation of the ventral hippocampus prior to conditioning and anisomycin infusions into the ventral hippocampus prior to a retention test after 48 h post-acquisition significantly reduced rats' freezing. Thus, the present findings are clearly consistent with these findings, suggesting that the hippocampus may be necessary for contextual associations during conditioned fear. The present data indicate that for conditioned contextual fear dCA1 is involved in retention but vCA1 appears to be even more important in the retention of conditioned fear.

Given that mice lacking NMDA receptors in the CA1 subregion (dorsal plus ventral) failed to learn a 30-s trace fear conditioning paradigm (Huerta et al., 2000) and CA1 neurons encoded trace duration during a 10- and 20-s trace conditioning paradigm in rabbits (McEchron et al., 2003), one might expect in the present study that acquisition would be retarded by the CA1 lesion groups. Although the lesions were present during acquisition, lesions of the CA1 subregion, either dorsal or ventral, seemed to have little effect on acquisition but did have an effect on the retention tests conducted 24 and 48 h post-conditioning. These data would suggest that the CA1 subregion of the hippocampus is not necessary for the acquisition of contextual or temporal information. One potential explanation might be that other subregions (i.e., DG or CA3, or some combination) of the hippocampus may play an important role in the acquisition of trace fear conditioning. For example, a recent study by Fendt, Koch, and Fanselow (2005) found that NMDA lesions of the dorsal hippocampus significantly impaired acquisition of trace, but not classical, fear conditioning when measured using fear potentiated startle. Quinn et al. (2005) found that infusions of the NMDA antagonist APV into the dorsal hippocampus prior to trace conditioning, but not the test, significantly reduced freezing during the context test. Furthermore, infusions of APV into the dorsal hippocampus either prior to trace conditioning or prior to the test significantly reduced freezing during the trace interval during the tone test. Both of these studies were aimed at the dorsal hippocampus, whereas the present study specifically investigated the CA1 subregion in both the dorsal and ventral components of the hippocampus. It is conceivable, therefore, that the entire hippocampus, or perhaps the CA3 subregion in conjunction with CA1, is necessary for the acquisition of trace fear conditioning (see Kesner, 2005).

Another hypothesis behind the present study was that lesions of the CA1 would impair trace conditioning. The present findings offer some support for this hypothesis;

however dCA1 lesions had no significant effect on trace acquisition or retention, although the graphed results are suggestive of a small effect. Only lesions of the vCA1 had a significant effect on trace retention. It must be noted, however, that the SNK post hoc indicated that vCA1 was significantly different relative to controls to $P > .01$, whereas vCA1 was different from dCA1 to $P > .05$. These data suggest that dCA1 is somehow involved, but vCA1 may be more critical for the retention of conditioned fear to the trace interval. Thus, even though the graphs appear to show a graded effect, based on the statistical analysis one cannot rule out the possibility that the vCA1, but not the dCA1, subserves the trace component of trace fear conditioning. Also, because a previous study showed that lesions or pharmacological blockade of the dorsal hippocampus disrupts trace fear conditioning with a 30-s trace interval (Quinn et al., 2005), it is possible that the dCA1 might be more important if a 30-s rather than a 10-s trace interval had been used. These data are consistent with Quinn et al. (2002) who suggest that the hippocampus is necessary for the consolidation and/or expression of conditioned fear to the trace interval. Additionally, Takehara and colleagues (2003) found that hippocampal lesions produced a temporally graded retrograde amnesia during trace eye-blink conditioning where the deficits were greatest one day after conditioning. In contrast, lesions of the mPFC produced the most profound deficit after four weeks, after which the hippocampal lesions no longer produced a deficit. These data in conjunction with the present data suggest that the hippocampus, specifically vCA1, is involved in the consolidation, expression, or retrieval of trace information. Long-term storage of this temporal information may reside in the prefrontal cortex. Runyan, Moore, and Dash (2004) reported that inhibition of extracellular signal-regulated kinase cascade (Erk) in the medial prefrontal cortex (mPFC) attenuated the retention of trace fear conditioning, but had no effect on trace encoding. Efferent connections from the ventral hippocampus CA1 synapse onto the prefrontal cortex (Barbas & Blatt, 1995; Goldman-Rakic et al., 1984). In part, this can be accomplished by the ventral CA1 and subiculum connection to the prelimbic cortex via the hippocampo-temporal pathway in rats (Jay & Witter, 1991) and monkeys (Goldman-Rakic et al., 1984). Furthermore, stimulation of the ventral hippocampus pathway can trigger LTP in the prelimbic cortex (Laroche, Jay, & Thierry, 1990) and it appears that many mPFC neurons are phase locked and entrained to hippocampal theta rhythm, suggesting that these neurons can gate information flow between the hippocampus and mPFC (Hyman, Zilli, Paley, & Hasselmo, 2005; Siapis, Lubenov, & Wilson, 2005). Clearly, these data implicate interactions between the hippocampus and prefrontal cortex. Lee and Kesner (2003) trained rats on a delayed non-matching-to-place task in an 8-arm radial maze requiring memory for a single spatial location following short-term (i.e., 10-s) delays or intermediate-term (i.e., 5-min) delays. These authors found that the dHIP and medial prefrontal cortex process short-term memory for spatial information in parallel; however, with 10-s and 5-min delays a combination of a lesion of the dHIP with inactivation of the mPFC or vice versa resulted in a severe impairment of short-term and intermediate-term memory for spatial information. Although the involvement of the hippocampus and mPFC depended on the duration of the temporal delay, interactions were necessary in order to solve the delayed non-matching-to-place task following short-term (i.e., 10-s) delays or intermediate-term (i.e., 5-min) delays.

Finally, research by Bannerman et al. (2004) implicates the ventral hippocampus in associating discrete cues during conditioned fear. In the present study, one might expect the vCA1 lesion group to have impairments during the tone test. Although vCA1 lesions produced a mild deficit, these data were not found to be statistically significant (defined as $P < .05$ in our analysis) and were deemed unreliable. It is possible, however, that full ventral hippocampal lesions could, in fact, disrupt discrete cue conditioning (i.e., tone), but this was not the goal of the present study. Clearly, more data are required to elucidate the role of the ventral hippocampus in associating discrete cues during trace conditioning.

In general, the present results demonstrate that the ventral hippocampus processes contextual information (i.e., spatial memory) as well as temporal (trace) information, suggesting a role for the ventral hippocampus in the retrieval of spatial and/or temporal information. These data are consistent with the notion that the hippocampus is necessary for the consolidation, expression, and/or retrieval of temporal (trace) information. Clearly, more research is needed to dissociate the roles of ventral CA1 from dorsal CA1. More research is needed to extend the present findings to the other hippocampal subregions, namely DG and CA3. The present study is consistent with many other reports in the literature, while offering some novel findings: namely a subregional analysis of hippocampal function across the dorsal–ventral axis. Further studies are needed to extend this finding to other memory experiments in which dorsal CA1 lesions produce a deficit, such as the radial eight-arm maze (Lee & Kesner, 2003), the object-trace-odor paired-association task (Kesner, Hunsaker, & Gilbert, 2005), and temporal pattern separation (Kesner et al., 2004). Finally, the interactions of the ventral hippocampus with extrahippocampal structures, namely the prefrontal cortex, need investigation to further elucidate the role of the ventral hippocampus in trace fear conditioning.

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