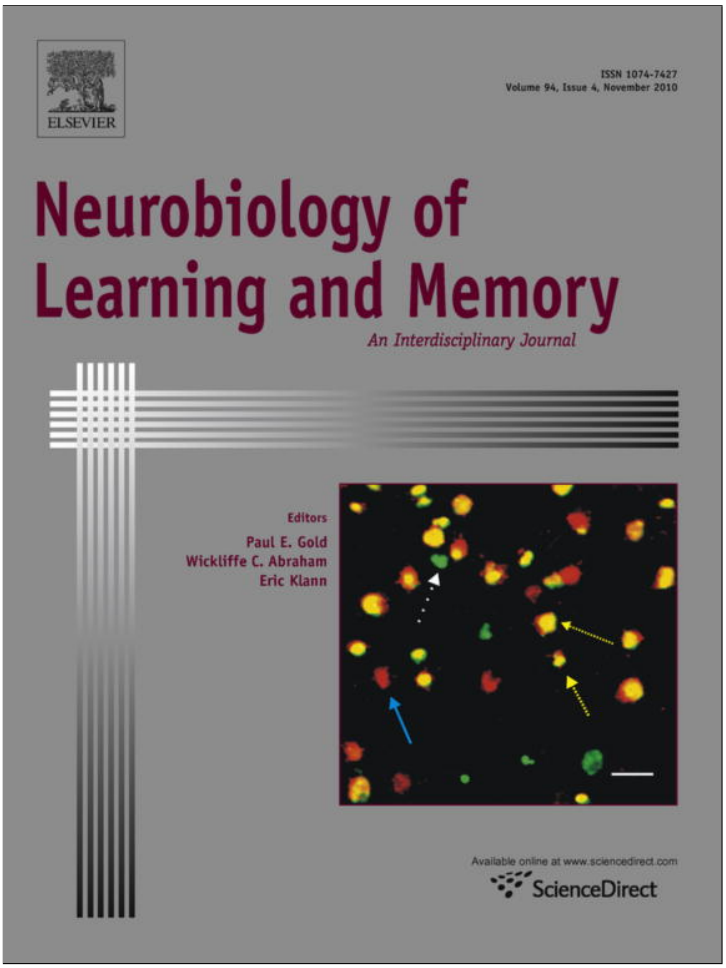

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Time-limited involvement of dorsal hippocampus in unimodal discriminative contextual conditioning

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ABSTRACT

Converging evidence examining the effects of post-training manipulations of the hippocampus suggests that the hippocampus may play a time-limited role in the maintenance of a variety of forms of memory. In particular, either lesions or inactivation of the dorsal hippocampus results in many cases in a time-limited retrograde impairment in nondiscriminative contextual conditioning paradigms. However, the extent to which hippocampal manipulations result in a time-limited retrograde amnesia for a variety of forms of learning has recently been called into question (reviewed in Sutherland, Sparks, & Lehmann (2010)). The present study examined the effect of inactivation of the dorsal hippocampus either 7, 28, or 42 days following training in an explicitly nonspatial, discriminative contextual conditioning paradigm (Otto & Poon, 2006; Parsons & Otto, 2008). Inactivation of the dorsal hippocampus resulted in a significant deficit in the expression of contextual conditioning at 7 and 28 days, but not 42 days, following training. Importantly, inactivation of the hippocampus did not affect either baseline freezing levels or conditioning to an explicit CS. Together with previous data exploring hippocampal contributions to discriminative unimodal contextual conditioning, these data suggest that the hippocampus may play a particularly prominent role in the temporary maintenance of memory in discriminative contextual paradigms.

1. Introduction

Converging evidence suggests that the hippocampus may play an important role in the acquisition and subsequent expression of a variety of forms of contextual conditioning (Anagnostaras, Maren, & Fanselow, 1999; Kim & Fanselow, 1992; Maren, Aharonov, & Fanselow, 1997; Otto & Poon, 2006; Parsons & Otto, 2008; Phillips & LeDoux, 1992; Rempel-Clower, Zola, Squire, & Amaral, 1996; Winocur, 1990; Winocur, Moscovitch, & Sekeres, 2007; Zola-Morgan & Squire, 1990). Many of these studies have focused on the role of the hippocampus in nondiscriminative contextual fear conditioning. Typically, training in these paradigms consists of one or more presentations of either an aversive unconditioned stimulus (US) alone, or pairings of an explicit conditioned stimulus (CS) with an aversive US, in a given spatial environment. Freezing behavior, typically defined as the cessation of movement except for that required for respiration and thought to be a reliable behavioral index of "fear", is assessed upon re-exposure to the conditioning chamber (e.g., Fanselow, 1980). Examinations of post-training manipulations of the hippocampus in nondiscriminative contextual fear conditioning often reveal a retrograde gradient, in which lesions of the hippocampus result in more severe impairment in recent (as opposed to remote) contextual conditioning (Anagnostaras et al., 1999; Kim & Fanselow, 1992; Maren et al., 1997). This general pattern of evidence is consistent with a temporally-graded mnemonic function of the hippocampus in both rodents and primates (Anagnostaras, Gale, & Fanselow, 2000; Rempel-Clower et al., 1996; Zola-Morgan & Squire, 1990; but see Sutherland, O'Brien, & Lehmann, 2008; Sutherland et al., 2010). While there is mounting evidence that post-training manipulations (typically lesions) of the hippocampus result in a temporally-limited retrograde amnesia for some forms of memory, other data suggest a significantly more limited role for the hippocampus in the temporary maintenance of memory (Sutherland, O'Brien, & Lehmann, 2008; reviewed in Sutherland et al. (2010)). Moreover, the specific post-training interval during which hippocampal processing is required varies considerably between studies in which a retrograde impairment has been found (reviewed in Sutherland et al. (2010)). The inconsistencies both within and between laboratories regarding the extent of retrograde amnesia following hippocampal manipulations may be due to a variety of factors, including but not limited to variable effects of lesions vs. temporary inactivation, variations among the stimulus modality required for learning, and the extent to which discriminative vs. nondiscriminative contextual conditioning paradigms are used. The present studies address a subset of these

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controversies, and expand on our recent finding that post-training inactivation of DH results in robust deficits in the expression of explicitly nonspatial, discriminative contextual conditioning 24 and 48 h after learning.

Like the previously described findings that post-training manipulations of the hippocampus often, but not always, result in a temporally-graded amnesia for contextual conditioning, the effects of pretraining hippocampal manipulations on contextual conditioning are relatively inconsistent (Bast, Zhang, & Feldon, 2001; Cho, Friedman, & Silva, 1999; Frankland, Cestari, Filipkowski, McDonald, & Silva, 1998; Kim, Rison, & Fanselow, 1993; Maren, Anagnostaras, & Fanselow, 1998; Maren & Fanselow, 1997; Maren et al., 1997; Phillips & LeDoux, 1992, 1994; Young, Bohenek, & Fanselow, 1994). In several cases, pretraining hippocampal manipulations impair the acquisition of contextual conditioning (Kim et al., 1993; Otto & Poon, 2006; Parsons & Otto, 2008; Phillips & LeDoux, 1992), while other studies report that such manipulations result in sparing, or only mild impairment, of contextual conditioning (Cho et al., 1999; Frankland et al., 1998; Maren et al., 1997; Phillips & LeDoux, 1994; Wiltgen, Sanders, Anagnostaras, Sage, & Fanselow, 2006). Emerging evidence suggests that these apparently disparate results may reflect a more pronounced role of the hippocampus in discriminative contextual conditioning (Antoniadis & McDonald, 2000; McDonald, King, Wasiak, Zelinski, & Hong, 2007; Otto & Poon, 2006; Parsons & Otto, 2008; Wang, Teixeira, Wheeler, & Frankland, 2009), relative to nondiscriminative, or simple, fear conditioning to context (e.g., Annau & Kamin, 1961; Estes & Skinner, 1941; McAllister & McAllister, 1971). For example, Frankland et al. (1998) reported that pretraining lesions of the hippocampus impaired the acquisition of discriminative contextual fear conditioning while leaving the acquisition of nondiscriminative contextual fear conditioning intact. These data suggest that contextual fear conditioning is more likely to depend on hippocampal integrity when the situation requires discrimination between multiple contextual stimuli; however, if an elemental solution is possible, hippocampal-lesioned animals may exhibit intact contextual fear conditioning (Anagnostaras et al., 2000; Frankland et al., 1998; Maren et al., 1997, 1998). Importantly, like nondiscriminative contextual conditioning, hippocampal participation in the maintenance of discriminative contextual conditioning appears to be temporally-graded (Wang et al., 2009).

Because of the known role of hippocampal processing in spatial memory, we have previously argued that an examination of explicitly nonspatial contextual conditioning may help dissociate the extent to which previously described effects of hippocampal manipulations on contextual conditioning are secondary to deficits in spatial processing. To this end, we have recently examined the role of the hippocampus in the acquisition and expression of explicitly nonspatial, olfactory contextual fear conditioning (Otto & Poon, 2006; Parsons & Otto, 2008). Specifically, we found that both excitotoxic lesions and temporary inactivation of dorsal hippocampus (DH) using the GABAa agonist muscimol disrupted the acquisition (Otto & Poon, 2006) and expression (Parsons & Otto, 2008) of a nonspatial, olfactory contextual discrimination while sparing conditioning to an explicit olfactory or auditory conditioned stimulus. Collectively these data suggest that the DH participates in the acquisition of and memory for discriminative contextual information, and are consistent with the view that the hippocampus plays an important role in the temporary maintenance of those memories prior to their ultimate consolidation in extra-hippocampal areas. However, our previous assessment of retrograde impairments in nonspatial contextual conditioning following post-training hippocampal inactivation examined 24 or 48 h training–testing intervals only; robust deficits were found at both of these post-training time points (Parsons & Otto, 2008). In order to address whether inactivation of the DH results in a temporally-graded retrograde amnesia, the current study examined the effects of DH inactivation on memory expression 7, 28, or 42 days after initial acquisition of our olfactory discriminative contextual paradigm.

2. General methods

All procedures were approved by the Institutional Animal Care and Use Committee of Rutgers University (Protocol #96-033).

2.1. Animals

Fifty-two naïve male Sprague–Dawley rats (Harlan, Indianapolis, IN), weighing between 249 and 275 g prior to surgical procedures, served as subjects. They were individually housed in plastic tubs in an environment-controlled vivarium on a 12-h light/dark cycle with access to food and water *ad libitum*. Animals were handled 2 min daily for 5 consecutive days prior to surgery. All behavioral procedures took place during the light phase of the cycle.

2.2. Experimental apparatus

Two identical behavioral chambers were used during training. Fear conditioning took place in a behavioral chamber ($30 \times 24 \times 42$ cm) housed in a sound-attenuating aluminum box ($56 \times 41 \times 42$). Aluminum composed one pair of opposing walls, and transparent Plexiglas constituted the other pair of walls and the ceiling. Scrambled footshock (0.5 mA) US was delivered through the chamber floor, which consisted of 16 stainless-steel rods (diameter 5 mm) equally spaced by 1.9 cm and connected to a shock generator (H13-15, Coulbourn Instruments, Allentown, PA). Illumination of the chamber was provided via a light bulb (28 V, 0.4 A) mounted 24.5 cm above the chamber floor. A tray containing animal bedding was placed under the floor of the chamber. When appropriate, a wall-mounted speaker presented a computer-generated tone CS (3.9 kHz, 80 dB).

Olfactory stimuli were presented via ports in the chamber ceiling, using a previously described procedure (Cousens & Otto, 1998; Herzog & Otto, 1997, 1998; Otto, Cousens, & Rajewski, 1997; Otto & Poon, 2006; Parsons & Otto, 2008). A solenoid valve directed clean air (1.5 l/min) to a 20 ml bottle containing 3 ml of either strawberry extract (McCormick, Hunt Valley, MD) or 15% pyridine in propylene glycol. Odorized air was then introduced to the conditioning chamber through Tygon tubing (inner diameter 1/8 in) connected to an outlet port in the chamber ceiling. An exhaust fan mounted outside the sound-attenuating enclosure provided ventilation, directing odorized air from the chamber out to a vacuum pump within 20 s of solenoid closure. Chambers were cleaned between conditioning sessions with commercially available cage cleaner (Research Laboratories, Inc.).

Testing sessions took place in a separate behavioral chamber located in a distal room and cleaned between sessions with a 10% alcohol solution. Although the dimensions of training and testing chambers were identical, the testing chamber was distinguished from the training chamber by a diagonally striped back wall and solid black Plexiglas floor. A video camera mounted in a corner of the outer sound-attenuating chamber recorded all behavioral and paradigmatic events for off-line analysis.

2.3. Surgical methods

Subjects were anesthetized with an i.p. injection of a Ketamine (80 ml/kg)–Xylazine (12 ml/kg) mixture. The subject's head was shaved and mounted in a stereotaxic frame (Kopf Instruments,

Tujunga, CA), and the scalp was cleaned with Betadine and alcohol. Marcaine (0.15 ml) was injected in multiple subcutaneous sites along the midline. The scalp was incised and retracted, and fascia was removed from the skull. Bilateral burr holes were drilled through the skull at sites overlying the DH (A/P −3.8 mm, M/L ±2.5 mm from bregma), and four additional burr holes were drilled at sites nearby. Small stainless-steel screws (Small Parts, MX-0080-02Fl-M) were inserted into the latter four holes and tightened. A double guide cannula (Plastics One, Roanoke, VA) was then implanted to a depth of 2.2 mm ventral to dura. Dental acrylic was applied both to the cannula and the screws, securing the cannula complex to the skull. The incision was closed with stainless-steel surgical staples, and an obturator was inserted into the guide cannula. The subject was returned to his home cage and monitored carefully for several days.

2.4. Drug infusion

Subjects recovered from surgery for 7 day before behavioral procedures. During this time, subjects were brought twice, individually, to the infusion room while the infusion pump ran to facilitate acclimation to the sounds and procedures associated with infusions. Infusion acclimation also took place once per week during training–testing intervals. Subjects received an infusion 30 m prior to each training and testing session. The subject was transported to the infusion room in a clear plastic holding box and the guide cannula obturator was removed from the cannula. A 30-gauge injection cannula attached via polyethylene tubing (PE-10 to 10-μl Hamilton) to syringes fixed in an infusion pump (Harvard Apparatus, South Natick, MA) was then inserted into the guide cannula. The infusion cannula extended to a depth 1 mm below the guide. Bilateral microinfusion of saline (0.25 μl, 0.9%, pH = 7.4) or muscimol ((0.25 μl, 1 μg/μl) dissolved in 0.9% saline; Sigma Aldrich, St. Louis, MO) occurred over a 1.5-min period. Muscimol for all replications was prepared in a single batch, and aliquots of 20 μl were kept frozen −4 °C for later use. Rats were held during the infusion period to prevent dislodged tubing, and for an additional 2 min to allow diffusion of the drug prior to removal of the injection cannula. Following infusion, the obturator was replaced into the guide cannula and the subject was placed in the holding box for an additional 28 min before being transported to the training or testing room. In all cases, 30 min separated the offset of the infusion procedure and the onset of behavioral training or testing.

2.5. Histological methods

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

3. Behavioral methods

3.1. Experimental design

All subjects were trained 30 min after a saline infusion, and then randomly assigned to one of two infusion groups that would receive either muscimol (MUS) or saline (SAL) before the contextual fear test. Within each primary group (MUS or SAL), subjects were randomly assigned to one of three subgroups that were differentiated by the interval between training and testing (7 days, 28 days, or 42 days). Therefore there were three MUS groups (MUS 7 ($n = 9$), MUS 28 ($n = 8$), and MUS 42 ($n = 6$)), and three SAL groups (SAL 7 ($n = 9$), SAL 28 ($n = 8$), and SAL 42 ($n = 7$)). The training–testing intervals of 28 and 42 days were chosen based on the results of previous studies examining hippocampal participation in systems consolidation (Anagnostaras et al., 1999; Kim & Fanselow, 1992; Maren et al., 1997; Wang et al., 2009).

3.2. Olfactory contextual/auditory delay fear conditioning

Training procedures were adapted from those described in Otto and Poon (2006) and identical to those described in Parsons and Otto (2008). Training occurred 10 days after surgery in one of two identical chambers (counterbalanced for each group). As described previously, each subject received a saline infusion 30 min prior to conditioning. Contextual stimulus presentations consisted of 5-min presentations of either strawberry extract or pyridine odor, designated as either "safe" or "unsafe". The two contextual stimuli alternated throughout the session and were separated from each other by 1-min inter-context intervals. Each contextual stimulus was presented three times. During presentation of each 5-min "unsafe" context, three fear conditioning trials in which a 3.9 kHz pure tone CS (∼80 dB, 20 s) co-terminated with a footshock (2 s, 0.5 mA), were given, separated from each other by 1-min inter-trial intervals. Thus, a total of nine CS–US pairings occurred, each during the presentation of an "unsafe" unimodal contextual stimulus. No explicit conditioned stimuli were presented during "safe" context presentations.

3.3. Testing following 7, 28, or 42 day training–testing intervals

Testing sessions assessed discriminative contextual fear either 7, 28, or 42 days after training. All test sessions (10 m) were preceded by an infusion of either muscimol or saline, as described earlier. Test sessions occurred on two consecutive days. A 2-min baseline period began the test session, followed by presentation of the unsafe or safe contextual stimulus during minute 3, and presentation of the explicit CS during minute 10. The specific contextual stimulus tested on either the first or second test day was counterbalanced within groups.

3.4. Behavioral measures

The primary measure of conditioned fear was freezing scored by a trained human observer. The observer scored the onset and offset of freezing behavior by operating a switch that was sampled continuously by the computer controlling stimulus presentations. Freezing scores were transformed into a percentage of each minute of the testing session. Secondary observers performed off-line video analysis of freezing behavior using the continuous recording method described above.

3.5. Statistical analysis

Freezing scores during testing sessions were transformed to percent of time spent freezing during presentation of each of the relevant stimuli. Relevant testing blocks included the 2-min baseline period, the unsafe and safe contextual stimulus test periods (1 min each), and the explicit conditioned stimulus test period (1 min). Contextually conditioned freezing among muscimol and saline groups was examined using two-way repeated measures analyses of variance with timepoint (7, 28, or 42 days) as the between-subjects factor and period (safe contextual stimulus and unsafe contextual stimulus) as the within-subjects factor. Freezing during the baseline period and during presentation of the explicit conditioned stimulus were examined using two-way analyses of

variance with infusion condition and timepoint as factors. All main effects and interactions were considered statistically significant at $p < 0.05$. Student–Newman-Keuls post-hoc comparisons were used to further explore significant interaction effects.

4. Results

4.1. Histology

Fig. 1 shows the targeted location of guide cannula within dorsal hippocampus. Subjects were retained for data analysis only if the tip of the guide cannula was located within, but not below, the dorsal region of hippocampus. Three subjects were removed from the statistical analysis because of improper cannula placement, leaving 49 subjects in the study.

4.2. Freezing elicited by the olfactory contextual stimuli after various training–testing intervals

The effect of training–testing interval on contextually conditioned freezing behavior for subjects receiving infusions of saline is shown in Fig. 2 (left panel). A two-way repeated measures ANOVA performed on freezing behavior during presentation of the safe and unsafe contexts revealed a main effect of context ($F(7, 154) = 30.864$, $p < 0.0001$), but neither the effect of interval duration ($F(2,22) = 0.428$, $p > 0.05$) nor the interaction between interval duration and context ($F(14,154) = 0.88$, $p > 0.05$) was statistically significant. This pattern was confirmed with subsequent post-hoc comparisons (Student–Newman–Keuls), which revealed that freezing during the unsafe contextual stimulus was significantly different from freezing during the safe contextual stimulus at all intervals durations (all p's < 0.05).

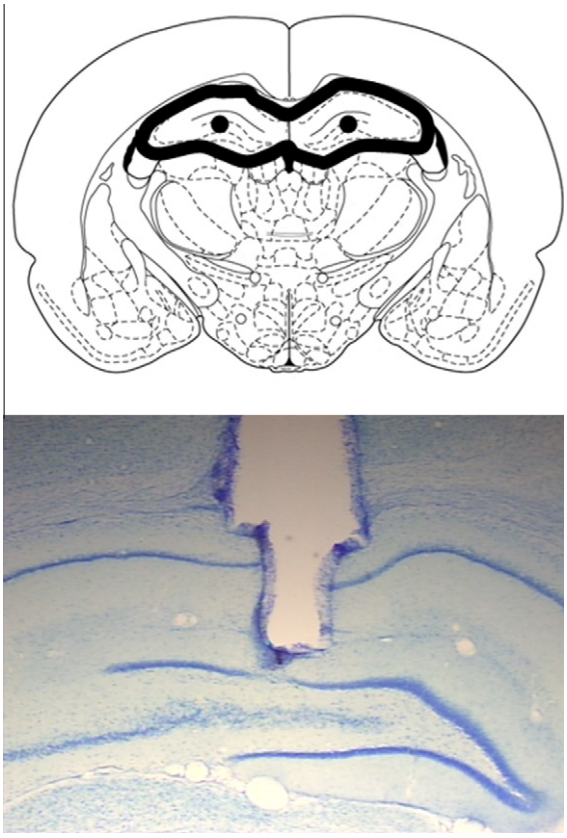

Fig. 1. Data were included only if the guide cannula were observed to terminate within, but not below, the DH region. Top: schematic representation of cannula location; black dots indicate the typical location of the tip of the infusion cannula. Bottom: photomicrograph of typical cannula placement in DH.

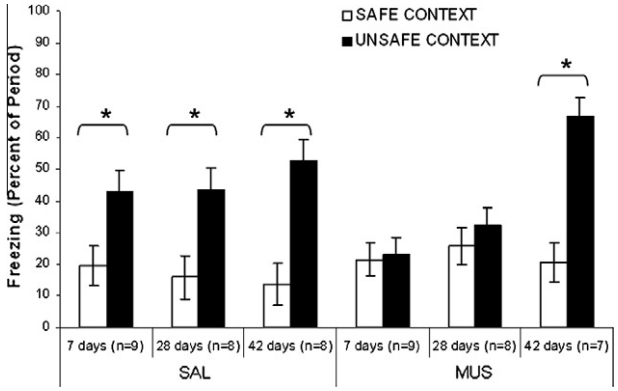

Fig. 2. Mean (±1s.e.m.) freezing elicited by the safe or unsafe contextual stimulus during the retention test for groups infused with saline (left panel) or muscimol (right panel). Saline groups exhibited contextual discrimination at all retention intervals tested; contextual discrimination was only observed in muscimol groups at the 42 day retention test.

The effect of interval duration on contextually conditioned freezing behavior for subjects receiving infusions of muscimol is also shown in Fig. 2 (right panel). A two-way repeated measures ANOVA performed on freezing behavior during presentation of the safe and unsafe contexts revealed a significant main effect of context ($F(7, 147) = 40.63$, $p < 0.0001$) and a statistically significant interaction between context and interval duration ($F(14,147) = 3.00$, $p = 0.0005$). The main effect of interval duration was not significant ($F(2, 21) = 1.86$, $p > 0.05$. Post-hoc (Student–Newman–Keuls) revealed a significant difference between freezing during the safe vs. unsafe context only at the 42 day interval ($p < 0.05$).

4.3. Freezing elicited by the explicit auditory CS at various interval durations

Freezing behavior for both groups of subjects during the 2 min baseline period and during presentation of the explicit conditioned stimulus are shown in Fig. 3. A two-way ANOVA on freezing during the baseline period revealed insignificant main effects for interval duration ($F(2,43) = 1.74$, $p = 0.187$), and infusion condition ($F(1,43) = 0.5380$, $p = 0.4673$), as well as an insignificant interaction between interval duration and infusion condition ($F(2,48) = 0.0231$, $p = 0.9771$). Similarly, a two-way ANOVA on freezing behavior during

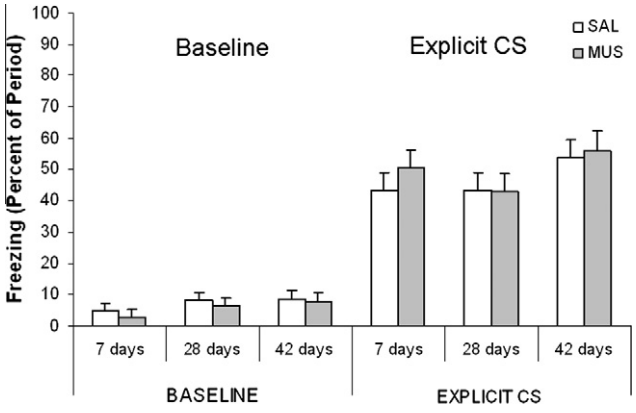

Fig. 3. Mean (±1s.e.m.) freezing during the 2 m baseline period and during presentation of the explicit CS. No differences were observed between animals infused with saline and those infused with muscimol.

the explicit CS revealed insignificant main effects for interval duration ($F(2,43) = 2.048$, $p = 0.1413$), infusion condition ($F(1,43) = 0.431$, $p = 0.515$), and the interaction between timepoint and infusion condition ($F(2,48) = 0.227$, $p = 0.797$).

5. Discussion

5.1. Post-training inactivation of DH results in a time-limited retrograde deficit in discriminative contextual fear conditioning but not in conditioning to an explicit CS

Temporary inactivation of the dorsal hippocampus resulted in a robust and time-limited retrograde impairment of nonspatial discriminative contextual fear memory, but did not affect memory for fear conditioned to an explicit auditory CS. The presence of intact contextual discrimination in subjects infused with muscimol at the 42 day timepoint but not at earlier time points, together with the results of previous research in our laboratory (Otto & Poon, 2006; Parsons & Otto, 2008), suggests that the observed behavioral deficits cannot be due to sensory or perceptual deficits at the time of testing.b Moreover, the freezing levels observed during baseline conditions and during presentations of the explicit CS were stable across infusion condition and unaffected by interval duration, ruling out a performance account of the attenuated contextual discrimination performance in subjects treated with muscimol and further supporting the established involvement of extra-hippocampal areas in delay fear conditioning (e.g., Kim et al., 1993; Maren & Fanselow, 1997; Rudy, Barrientos, & O'Reilly, 2002; Sutherland & McDonald, 1990). Finally, the use of temporary inactivation techniques like those used in the present studies is a powerful and significantly more specific method of examining hippocampal participation in systems consolidation (cf. Sutherland et al., 2010), as it avoids damage to distal regions that are themselves important in remote memory (Anagnostaras et al., 2000; Wiltgen et al., 2006). It should be noted that in the present study inactivation was limited to the dorsal aspect of the hippocampus and did not binclude ventral hippocampus. Thus, it is possible that, at the 42 day timepoint, the storage or retrieval of memory in this paradigm may be supported by ventral hippocampus. Nonetheless, the present study provides strong support for the notion that the dorsal hippocampus plays a prominent role in the temporary maintenance of memory for discriminative contextual conditioning prior to its ultimate consolidation elsewhere.

5.2. Time course of hippocampal participation in discriminative, nonspatial contextual fear conditioning

The extent to which the hippocampus participates in systems consolidation in a time-limited manner is currently the subject of considerable debate. Specifically, while the present data and those from several prior studies (Anagnostaras et al., 1999; Kim & Fanselow, 1992; Maren et al., 1997) provide strong support for a time-limited role for the hippocampus in contextual fear conditioning, Sutherland et al. (2010) suggest that there is currently little support for the notion that manipulations (typically lesions) of the hippocampus result in a temporally-graded deficit in most paradigms. In one recent study, Sutherland, O'Brien, and Lehmann (2008) found that excitotoxic lesions of the hippocampus caused both recent and remote contextual fear deficits using signaled or unsignaled footshock paradigms. In their comparison of reports of flat (see Lehmann, Lacanilao, & Sutherland, 2007) vs. time-limited (Anagnostaras et al., 1999; Kim & Fanselow, 1992) gradients of dorsal hippocampal participation in contextual fear conditioning, Sutherland, O'Brien, and Lehmann (2008) concluded that signaled vs. unsignaled foot shock is not likely to be the key factor explaining gradient differences, but that the hippocampus remains necessary to express remote contextual fear if it participated in its acquisition. Our results, on the other hand, support the idea that a shock-related cue functions differently when it occurs in the context of a discrimination paradigm (wherein subjects learn the distinction between contexts differentially associated with shock). It is likely that such discriminative contextual conditioning paradigms, in contrast to simple or cued contextual fear conditioning, engage temporally-sensitive hippocampal processing (e.g., Fanselow & Baackes, 1982; Frankland et al., 1998; Wang et al., 2009).

The specific time course of hippocampal participation in systems consolidation varies widely across studies, paradigms, and the sensory and temporal nature of stimuli used (see Sutherland et al., 2010). With respect to contextual fear conditioning, temporary inactivation of DH resulted in impaired contextual discrimination up to 28 days after training in our paradigm, whereas Kim and Fanselow (1992) observed that performance of DH-lesioned animals was similar to that of controls 28 days after simple spatial contextual fear conditioning. Interestingly, and consistent with this observation, it has been argued that the time course of hippocampal involvement in consolidation may be more pronounced in tasks that odors or flavors as stimuli (Sutherland et al., 2010). However, even in tasks in which odors or flavors serve as discriminative cues, the retrograde gradients observed following hippocampal manipulations vary dramatically. Specifically, four previous studies over the past decade have reported retrograde amnesia following hippocampal lesions in odor- or flavor-guided tasks, with gradients varying between 1 and 4 days (significantly shorter than that reported here). Three of these studies examined the temporal dependence of hippocampal processing in the retention of appetitively-motivated, socially-transmitted food preferences (Clark, Broadbent, Zola, & Squire, 2002; Ross & Eichenbaum, 2006; Winocur, McDonald, & Moscovitch, 2001), and one in the retention of appetitively-motivated flavor/place paired associates (Tse et al., 2007). While a complete characterization of the factors influencing the temporal limitations on hippocampal participation in memory in general is beyond the scope of the present study, it is likely that the discrepancies in the retrograde gradients between these studies reflect, at least in part, differences in the extra-hippocampal brain areas subserving these forms of learning, and in the timing and complexity of plasticity mechanisms underlying the ultimate consolidation of appetitively vs. aversively motivated memories. Other possibilities include differential effects of lesions vs. temporary inactivation, variability in the time-dependent nature of hippocampal processing across different stimulus modalities, or a difference between the time course of simple vs. discriminative conditioning paradigms.

5.3. Generalization among contexts does not increase across time

Our contextual fear testing sessions took place in a novel chamber, where the observation of increased freezing during baseline or the safe context presentation could be interpreted as evidence of fear generalization to a novel spatial context or to the nonspatial context associated with safety, respectively. Unlike recent reports of increased contextual fear generalization as a function of increased interval duration (Biedenkapp & Rudy, 2007; Houston, Stevenson, McNaughton, & Barnes, 1999; Riccio, Ackil, & Burch-Vernon, 1992; Wiltgen & Silva, 2007; Winocur et al., 2007), the present study reports two distinct and temporally stable patterns of behavior that are inconsistent with the notion of increased contextual generalization over time. First, saline-treated control animals exhibited high and stable levels of discrimination between the safe and unsafe contexts at all time points (see Fig. 2, left), suggesting that generalization between the nonspatial contextual stimuli did not increase over

time. Second, all subjects exhibited low and stable levels of freezing during baseline periods prior to CS presentations (see Fig. 3, left), suggesting that they did not generalize the training and testing chambers. Together these patterns suggest that generalization did not increase with time, and that the attenuated contextual discrimination observed in subjects treated with muscimol reflects a selective, time-dependent role of the DH in the expression of explicitly nonspatial contextual information.

The absence of increased contextual generalization over time may appear inconsistent with the notion that contextual memory becomes less specific with increasing retention intervals (e.g., Balogh, Radcliffe, Logue, & Wehner, 2002). If so, these results could be viewed as incongruous with some contemporary models of the consolidation of contextual fear, in which a loss of detail is proposed to accompany the migration of contextual memory away from the hippocampus (McClelland, McNaughton, & O'Reilly, 1995; Nadel, Winocur, Ryan, & Moscovitch, 2007; O'Reilly & Rudy, 2001). However, a more parsimonious explanation for the intact discrimination at long training–testing intervals may lie in the distinction between discriminative vs. nondiscriminative conditioning paradigms. That is, emerging evidence suggests that paradigms with an explicit discrimination training component (e.g., Wang et al., 2009) do not result in generalization between contexts. Although in the present experiments training occurred in a single spatial environment, the procedures constituted an explicit discrimination training paradigm which may account for the intact discrimination between both the spatial (training chamber) and nonspatial (olfactory) contextual stimuli observed after 42 days.

5.4. Summary and conclusions

The results of this study support a key but time-limited role of DH in explicitly nonspatial contextual discrimination, and are consistent with the idea that discriminative contextual conditioning is particularly sensitive to manipulations of the hippocampus (e.g., Fanselow & Baackes, 1982; Frankland et al., 1998). These data contribute to a growing body of data suggesting that the hippocampus may play an important role in the temporary maintenance of some forms of memory prior to its ultimate consolidation elsewhere, but that the specific time course of this hippocampal processing depends on the nature of the stimulus materials or training paradigm (Wang et al., 2009).

Acknowledgments

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