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Hippocampal Lesions Produce Both Nongraded and Temporally Graded Retrograde Amnesia in the Same Rat

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ABSTRACT: Rats were administered contextual fear conditioning and trained on a water-maze, spatial memory task 28 days or 24 h before undergoing hippocampal lesion or control surgery. When tested postoperatively on both tasks, rats with hippocampal lesions exhibited retrograde amnesia for spatial memory at both delays but temporally graded retrograde amnesia for the contextual fear response. In demonstrating both types of retrograde amnesia in the same animals, the results parallel similar observations in human amnesics with hippocampal damage and provide compelling evidence that the nature of the task and the type of information being accessed are crucial factors in determining the pattern of retrograde memory loss associated with hippocampal damage. The results are interpreted as consistent with our transformation hypothesis (Winocur et al. (2010a) Neuropsychologia 48:2339–2356; Winocur and Moscovitch (2011) J Int Neuropsychol Soc 17:766–780) and at variance with standard consolidation theory and other theoretical models of memory. © **2013 Wiley Periodicals, Inc.**

KEY WORDS: hippocampus; memory; retrograde amnesia; spatial memory; contextual fear

INTRODUCTION

It is well established that hippocampal lesions in humans (e.g., Marslen-Wilson and Teuber 1975; Reed and Squire, 1998) and animals (e.g., Winocur, 1990; Kim and Fanselow, 1992) can produce a temporally graded retrograde amnesia in which memories acquired shortly before damage was sustained are lost, whereas older memories are relatively well preserved. This effect has been taken as important evidence for the Standard Consolidation Theory of memory, which attributes a time-dependent function to the hippocampus (Squire, 1992; Squire and Alvarez, 1995). According to this view, memories are represented in the hippocampus until they are ultimately consolidated in neocortical regions, at which point the hippocampus is no longer required for their retention and retrieval. A central premise of Standard Consolidation Theory is that the consolidated memory in neocortex is identical to that which was represented initially in the hippocampus.

In contrast to the numerous reports of temporally graded retrograde amnesia, many investigators have reported extensive, nongraded retrograde amnesia in people with hippocampal damage (e.g., Cermak and O'Connor, 1983; Bright et al., 2006). Indeed, a recent survey of studies of remote memory following hippocampal damage in humans (Winocur and Moscovitch, 2011) revealed that, since Scoville and Milner's (1957) seminal work implicating the hippocampus in memory, the two patterns of amnesia have been reported equally often. This result is clearly incompatible with Standard Consolidation Theory and we have proposed a different interpretation that emphasizes transformations that memories undergo over time.

According to the transformation view, which derives from Nadel and Moscovitch's (1997) Multiple Trace Theory, contextually rich, episodic memories that are initially represented in the hippocampus, transform into schematic (semantic) versions that capture the essential features of the event but few of the contextual details. Our reviews suggest that nongraded retrograde amnesia is typically seen for contextually rich memories which always depend on the hippocampus (Rosenbaum et al., 2001; Bayley et al., 2003; Winocur et al., 2010a; Winocur and Moscovitch, 2011). In contrast, temporally graded retrograde amnesia is more often exhibited for semantic or schematic memories, which are represented extra-hippocampally (see also McKenzie and Eichenbaum, 2011). A central premise of the transformation hypothesis is that, in principle, both context-specific (episodic) and schematic (semantic) memories are available in the normal brain, but only schematic memories are available following hippocampal damage (Winocur et al., 2010a; Winocur and Moscovitch, 2011).

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In animals, studies of retrograde memory have been conducted with tests considered to be analogous to those used with humans. These studies have yielded the same pattern of results, with nongraded and temporally graded retrograde amnesia, once again occurring equally often following hippocampal damage (Winocur et al., 2010a). Thus, for example, rats with hippocampal lesions reliably exhibit extensive nongraded retrograde amnesia on conventional land- (e.g., Clark et al., 2005a,b; Winocur et al., 2005a) and water-based (Sutherland et al., 2001; Martin et al., 2005) tests of spatial memory. As with episodic memories in humans, spatial memories are context-specific and depend on the ability to remember associations between target locations and contextual cues in the environment. When these contextual associations are disrupted, by changing the position of the maze in the room or relocating the maze to a different room, memory for specific locations is impaired (see Winocur et al., 2005b, 2010b).

On other tasks, such as contextual fear conditioning (Kim and Fanselow, 1992; Anagnostaras et al., 1999; Winocur et al., 2009; but see Sutherland and Lehmann, 2010; Wiltgen et al., 2010), hippocampal lesions typically produce temporally graded retrograde amnesia,. In contextual fear conditioning, both contextually specific and nonspecific, schematic information can be used to recall the fear response, with the former dominant shortly after learning when hippocampal lesions disrupt performance, and the latter dominant at longer intervals when the response generalizes to other contexts and the memory no longer requires the hippocampus for expression (Winocur, 1990; Biedenkapp and Rudy, 2007; Wiltgen and Silva, 2007; Winocur et al., 2007).

A crucial prediction that follows from the transformation account is that, depending on the task and the type of memory assessed, individuals with hippocampal damage could exhibit both temporally graded and nongraded patterns of retrograde amnesia, for context-specific and generalized (transformed) memories, respectively. There are examples of this in the human literature (e.g., Cermak and O'Connor, 1983; Barr et al., 1990; Steinvorth et al., 2005; Rosenbaum et al., 2008), but uncertainties related to lesion characteristics and preoperative experience, which are impossible to control effectively in humans, raise questions about these results (see Sutherland et al., 2008). As a strong test of the transformation hypothesis, the present research was conducted to determine if this intra-individual dissociation can be demonstrated under controlled conditions afforded by an animal model.

Twenty-eight days or 24 h before undergoing hippocampal or control surgery, rats completed training on the Morris water maze spatial memory test and contextual fear conditioning. After recovery from surgery, spatial memory and memory for the contextual fear response were assessed. In contrast to Standard Consolidation Theory and other competing views (see "Discussion" section), the transformation hypothesis predicts that both patterns of retrograde amnesia will be observed in the same hippocampally damaged rat when tested on context-specific (spatial) and schematic (contextual fear conditioning) memory at short and long delays.

MATERIALS AND METHODS

Subjects

Thirty-eight male, adult Long-Evans rats, ~5-month old at the beginning of the study, obtained from the Charles River Laboratories (Saint-Constant, Québec, Canada), served as subjects. The rats were housed individually in shoebox cages with unlimited access to standard lab chow and water and maintained on a 24-h cycle with two 12-h phases (lights on at 1800h and off at 0600h). All testing took place during the high activity, dark phase of the cycle.

The experimental protocol and all handling procedures conformed to guidelines set out by the Canadian Council on Animal Care and were approved by the Trent University Animal Care Committee. Throughout the research, the rats were examined regularly by a veterinarian.

Apparatus

The spatial memory test was administered in a circular pool (130 cm diameter and ~ 30 cm high), located in the center of a standard testing room. The pool was filled with opaque water and maintained at room temperature (21°C). An inverted flower pot, situated a few centimeter below the surface, served as a platform on which the rat could climb to escape the water. Throughout testing, the water was cleaned after each trial and changed every 2–3 days.

The pool was divided into six zones of approximately equal size. Swimming patterns and escape latencies were monitored by an overhead video camera connected to a recorder and data processing system. The system enabled computation of the time required to mount the platform and the number of errors made in the process. As well, records were kept of the animals' swimming routes that were used to count errors and are available on request.

Contextual fear conditioning was conducted in a chamber ($50 \times 40 \times 18$ cm^3) that consisted of four clear Plexiglas walls, a hinged clear Plexiglas roof with holes to allow ventilation, and a floor that consisted of metal rods, spaced 1.3 cm apart. As part of the fear-conditioning procedure, a tone, presented through a centrally mounted speaker attached to the roof of the box, was paired with a 1 s, 1.5 mA foot shock that was delivered by a TechServe (Model 452A shock generator).

The fear conditioning chamber was positioned on a table, 1.3 m above the floor, and situated in the center of a large standard laboratory room (6.3×6.1 m^2). The room contained standard furniture (e.g., desk, table, bookshelf along one wall, etc.), as well as pictures, light fixtures, etc. on the walls. Illumination was provided by overhead fluorescent lights under rheostatic control. All cues were located in the same positions throughout the experiment.

Design

Before the beginning of behavioral training, all rats were handled for 5 min per day for 5 days. Following this, all rats

received spatial memory training in the water maze over 8 days. Two to three hours after the final spatial memory training session, the rats underwent contextual fear conditioning. Half the rats received hippocampal or control surgery within 24 h of fear conditioning (Short-delay [SD]) and the other half were operated on 28 days later (Long-delay [LD]). Thus, the experiment was conducted on four groups: Hippocampal (HPC)—Short-delay (HPC-SD); HPC—Long-delay (HPC-LD); Control (CON)-SD; CON-LD).

Given the nature of this study, it was necessary to vary the time between surgery and postoperative testing in the short- and long-delay conditions. The control rats were tested 24 h after surgery, a delay that allowed sufficient time to recover from the minor surgery and to establish the type of memory representation that governed their behavior at that point. A longer delay could have resulted in changes in the representation of the memory in these rats. The hippocampal groups were lesioned at the same times as control groups but, as in similar, previous studies (e.g., Debiec et al., 2002; Winocur et al., 2009), they were tested 7 days later to allow for recovery from the more invasive lesion surgery. Importantly, this procedure did not bias the results in favor of any theoretical prediction. As described below, the behavior of the respective groups was affected by the type of memory tested and not by the amount of time between surgery and testing.

Testing on both tasks was identical at the short and long delays. All rats were first tested on the contextual fear task followed within a few hours by testing on the spatial memory task. Training and testing procedures, as well as surgical and histological methods, are described in detail below.

The experimental design, group numbers, and general timelines are presented in Figure 1. All behavioral testing was conducted by an experimenter who was blind to the subject's lesion group.

Spatial Memory

The spatial memory task began with 2 days of orientation to the maze (5 trials/day) in which rats were placed in the pool and allowed to swim to the platform, which was visible. The start point and the location of the platform were randomized on each trial. By the end of the second day, all rats were swimming directly to the platform.

Spatial memory training began the following day. The platform was now positioned a few centimeters below the surface and always located in the center of the north-east zone of the pool. For each trial, the rat was placed in the water, facing the wall at the edge of one of five zones of the pool. The start zones were determined pseudorandomly but the rats were never placed in the north-east zone, where the platform was located. Each trial continued until the rat mounted the platform with all four paws, or until 60 s elapsed. If the rat failed to find the platform in the allotted time, it was guided to the platform. After 20 s on the platform, the rat was placed under a heat lamp to await the next trial. Each rat received 5 such trials/day, with an approximate inter-trial interval of 4–5 min, for 5 consecutive days. Escape latency and errors were recorded for each trial of Days 1–5. The escape latency was the time required to reach and mount the platform. An error was counted each time the mouse entered a zone not containing the platform. If the mouse failed to find the platform within 60 s, it was given an error score of 15 and a latency score of 60 s for that trial.

On the sixth day of training, trials 1 and 2 and 4 and 5 were conducted in the usual manner. The third trial served as a probe trial. For the probe trial, the platform was removed, and the amount of time the rat spent swimming in the platform target zone (north-east zone) was calculated and used as an index of the rat's memory of the platform's location. After 30 s, the rat was removed and placed in the holding cage to await the fourth trial.

Postoperative spatial memory testing, including the probe trial, was conducted in the same way as preoperative training.

Contextual Fear Conditioning

The procedure for this task was adapted from Anagnostaras et al. (1999) and similar to that routinely followed in our lab (e.g., Winocur et al., 2007, 2009). Two to three hours after the last spatial memory training session, each rat was given a familiarization trial in which it was placed in the contextual fear conditioning chamber for 30 min and allowed to explore. The next day it received one fear conditioning trial that began with the rat being placed in the chamber and allowed to explore freely for 5 min. It then received 10 tones (CS)—shock pairings at variable intervals. Freezing behavior was recorded every 8 s for the period immediately before and after shock was administered. Following Anagnostaras et al. (1999), freezing was defined by an immobilized crouching response in which the only detectable movement was the rat's breathing. Freezing scores were obtained from video records taken during the training sessions. Following familiarization and training sessions, the rat was removed from the chamber and returned to its home cage.

Postoperative testing consisted of a single trial in which the rat was placed in the chamber, initially in the absence of the tone (Context [CXT]-only). After 8 min, the tone was presented for another 8 min (CXT + CS). No shock was presented during testing. In each test session, freezing behavior was recorded every 8 s for a total of 60 observations. Immediately after testing, the rat was removed from the chamber and returned to its home cage.

Surgical and Histological Procedures

During surgery, rats were maintained on oxygen and isoflurane respiratory anesthetic. Stereotaxic coordinates for the hippocampal lesions were based on the Paxinos and Watson (1997) atlas and located in relation to bregma and the horizontal skull surface. The procedure for making hippocampal lesions was identical to that routinely practiced in our lab (Winocur et al., 2005a,b, 2007, 2010b). A small incision (2 cm) was made in the scalp along the midline of the skull. Using a small dental burr, 8 holes were drilled through the skull directly above the hippocampus in each hemisphere. Hippocampal lesions were produced by 10 intra-cranial micro-injections of a solution containing the cellular

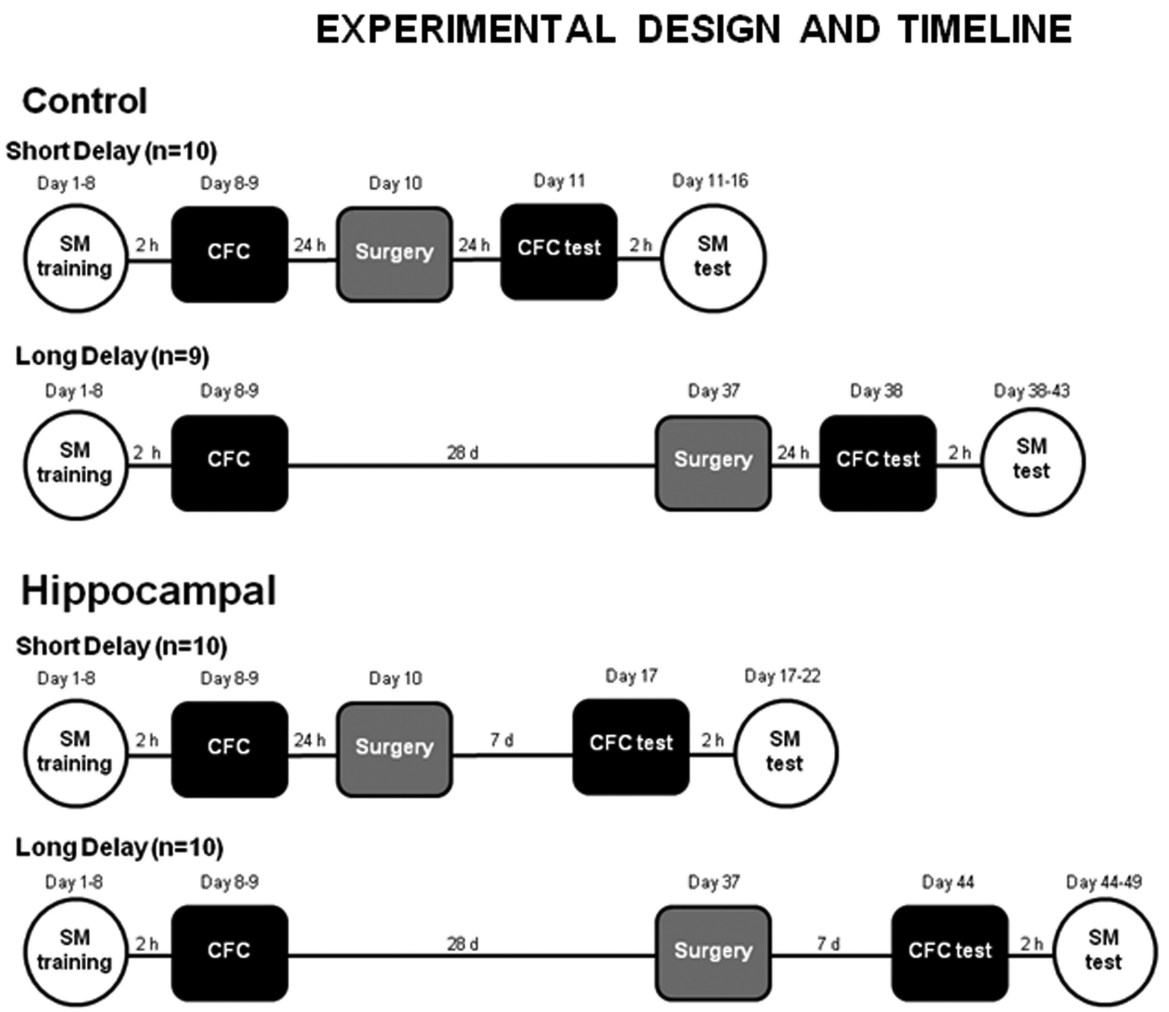

FIGURE 1. Schematic representations of timelines for experimental procedures. The numbers in parentheses represent the number of rats in the various groups. Abbreviations: SM, spatial memory; CFC, contextual fear conditioning; h, hours; d, days.

neurotoxin, *N* -methyl-D-asparate (NMDA) (5 mg/μl phosphate buffer solution (PBS) per site) into each hemisphere. The injection coordinates were calculated from a level head with respect to bregma: 3.1 mm posterior (p), ±1 mm lateral (l), and 3.6 mm ventral (v); 3.1 (p), 2 (l), 3.6 (v); 4.1 (p), 2 (l), 4 (v); 4.1 (p), 3.5 (l), 4 (v); 5 (p), 3, (l), 4.1 (v); 5 (p), 5.2 (l), 5 (v); 5 (p), 5.2 (l), 7.3 (v); 5.8 (p), 4.4 (l), 4.4 (v); 5.8 (p), 5.1 (l); 6.2 (v); 5.8 (p), 5.1 (l), 7.5 (v). The solution was infused at a rate of 0.4 μl/min through a 30-gauge stainless steel cannula for 38 s, using a 10-μl syringe attached to a motorized infusion pump. The last two ventral hippocampal sites were injected for 2 min each. The cannula remained in place for 2 min after each infusion to allow NMDA diffusion away from the cannula tip before removal. In the sham surgery (control) procedure, scalp incision and burr holes were identical to the lesioned animals with the exception that there was no penetration of brain tissue. To facilitate recovery from surgery, all rats were given an intraperitoneal (ip) injection of diazepam (10 mg/kg).

Following testing, rats were deeply anesthetized with sodium pentobarbital (65 mg/kg) via ip injection and perfused intracardially with phosphate-buffered saline followed by 10% formalin. The fixed brains were removed from the skull and stored in 10% formalin. Brains were transferred to a 30% sucrose solution 48 h before sectioning. The brains were sectioned coronally at 40 μm, using a cryostat, and every 5th section was mounted on a gel coated slide and stained with cresyl violet. The sections were dried in a fume hood, then cover-slipped using Permount mounting medium.

The sections were photographed with a Nikon D90 digital camera at 2× magnification using an Olympus BH-2 microscope with a Diagnostic Instruments PA1-10A adapter. Every second section corresponding to figures 28–44 in Paxinos and Watson's (1997) rat brain atlas was used to estimate the area of surviving hippocampal tissue in each brain (nine sections per brain). The volume of spared hippocampal tissue was digitally scored using Image J (NIH). For each section, surviving hippocampal tissue was traced, and measurements were automatically calculated. Measurements were obtained separately for dorsal and ventral portions of the left and right hippocampus in each section. Values for surviving tissue in the left and right hippocampi were then combined to generate a final value of spared hippocampal tissue per brain.

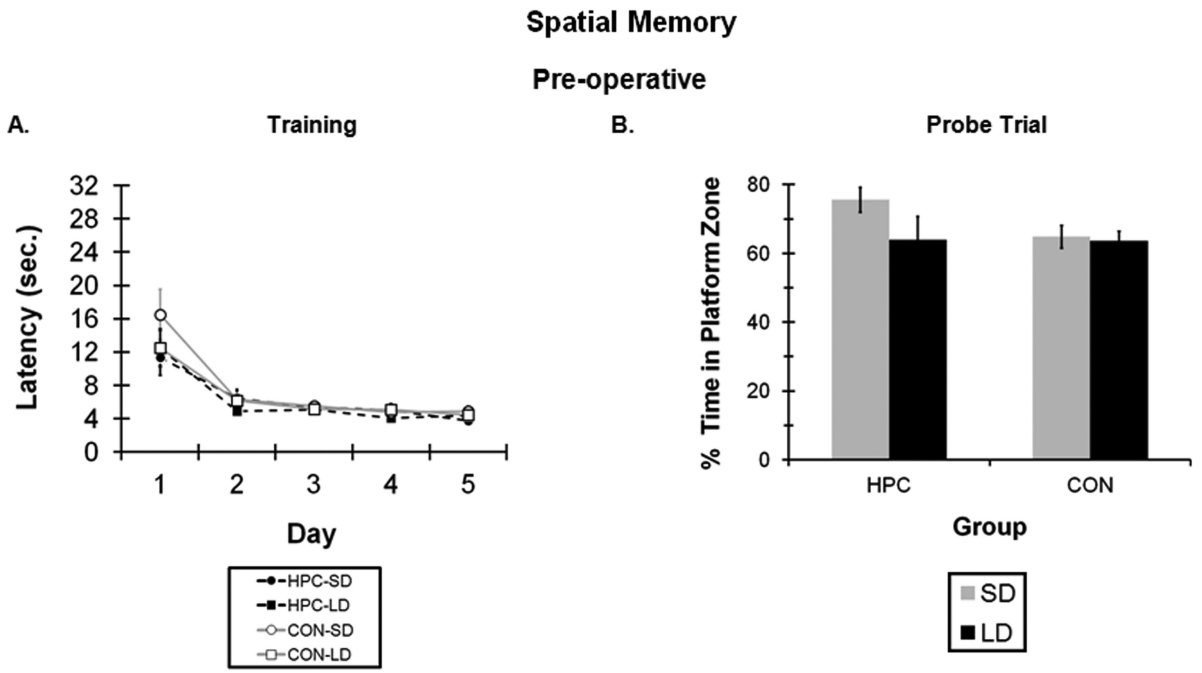

FIGURE 2. Preoperative spatial memory training. A: Average daily escape latency (in seconds) to locate submerged platform for all groups during 5 days of training on the spatial memory task. B: Percent time spent by all groups in the platform zone during the probe trial of Day 6 during preoperative training. Error bars represent ± SEM. Abbreviations: HPC, hippocampal; CON, control; SD, short delay; LD, long delay.

Control measurements were obtained by tracing the entire hippocampus from a subset of control brains. Control values for the dorsal and ventral planes of the left and right hippocampi were obtained in every second section corresponding to Figures 28–44 in the rat brain atlas. For each experimental rat, the surviving volume of tissue in each section was divided by the average total volume in the corresponding control section and multiplied by 100 to obtain the percentage of surviving tissue. That value was subtracted from 100 to report the percent of lesioned tissue.

Data Analysis

Behavioral data were analyzed by analysis of variance to examine between-subjects factors of lesion group and length of delay, and the within-subjects factor of days in the spatial memory test. Correlation coefficients were calculated to examine the association between lesion size and performance on each test. Hypothesis testing was performed at an alpha-level of 5%.

RESULTS

Spatial Memory

All groups readily mastered the task during preoperative training and there was no difference in rate of learning, F (1,35) = 1.80, P = 0.19 (Fig. 2A), or on the probe trial on Day 6, F (1,35) = 1.50, P = 0.23 (Fig. 2B).

Figure 3A shows the average latency for all groups to find the platform on Trial 1 of the first day of postoperative spatial memory testing, a measure of the animals' memory that is uncontaminated by relearning. A main effect of lesion, F (3,35) = 5.91, P = 0.02, indicates that rats with hippocampal lesions were significantly impaired on this measure. Neither the effect of delay ($F < 1$) nor the lesion × delay interaction, F (1,35) = 1.51, P = 0.22, was significant, indicating lack of evidence for a differential lesion effect between short and long delays.

Figure 3B shows the average latency scores for all groups across the 5 days of spatial memory testing. Once again, there was a large effect of lesion, F (1,35) = 70.56, $P < 0.0001$. There was also a significant main effect of days, F (4,140) = 17.19, $P < 0.0001$, indicating relearning of the spatial location, and a significant lesion × day interaction, F (4,140) = 5.71, P = 0.005. The significant interaction was due to the large difference between HPC and CON groups on Day 1 and the substantial recovery of the HPC groups during subsequent testing. It is noteworthy that, over the 5 days of testing, the HPC groups did not re-establish preoperative performance levels. The lesion × delay interaction was not significant, $F < 1$.

Figure 3C shows the percent time that all groups spent in the platform zone during the probe trial on Day 6 of postoperative testing. There was a strong lesion effect, F (1,35) = 139.92, $P < 0.0001$, indicating that rats with hippocampal lesions spent less time than controls in that zone. The lesion × delay interaction, $F < 1$, and the main effect of delay, F (1, 35) = 1.34, P = 0.20, were not significant.

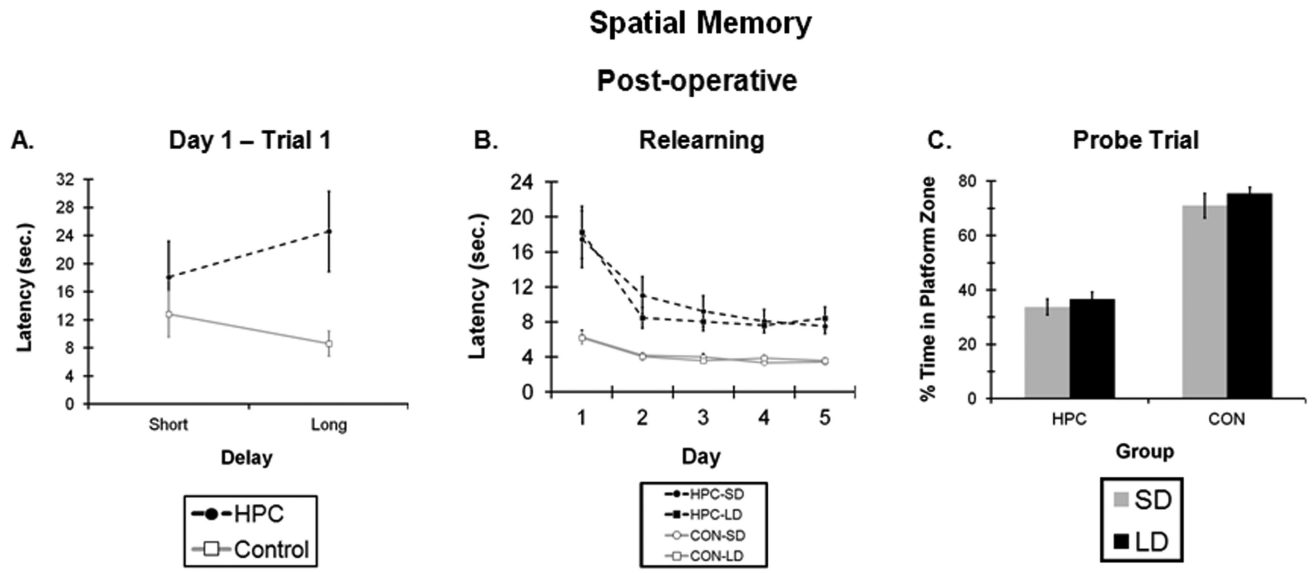

FIGURE 3. Postoperative spatial memory testing. A: Average latency (in seconds) for all groups to locate submerged platform on Trial 1 of the first day of postoperative spatial memory testing. B: Average daily latency (in seconds) to locate submerged platform for all groups during 5 days of postoperative spatial memory testing. C: Percent time spent by all groups in the platform zone during the probe trial on Day 6 testing. At both delays, HPC groups were impaired at recall, overall relearning and spent less time in the platform zone during the probe trial. Error bars represent ± SEM. Abbreviations: HPC, hippocampal; CON, control; SD, short delay; LD, long delay.

Contextual Fear Conditioning

As can be seen in Figure 4A, all groups exhibited considerable freezing following the series of tone-shock pairings during preoperative contextual fear conditioning. Analysis of variance applied to the post-shock freezing scores yielded nonsignificant main effects of group, $F < 1$, and delay, $F\,(1, 35) = 1.89$, $P = 0.18$, as well as a nonsignificant group × delay interaction, $F < 1$.

In the postoperative CXT-only test (Fig. 4B), rats with hippocampal lesions froze significantly less than controls at the short delay, but there was little difference between the groups at the long delay. This was confirmed by a significant lesion × delay interaction, $F\,(1,35) = 6.02$, $P = 0.02$. The main effects

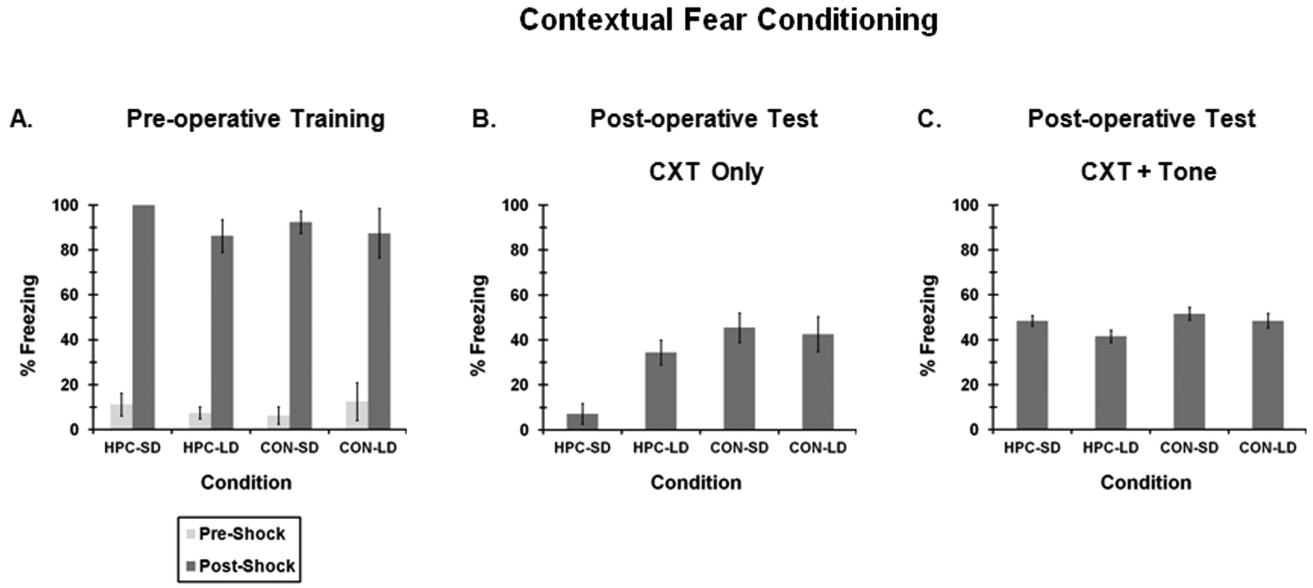

FIGURE 4. Contextual fear conditioning. A: Percent time spent freezing by all groups before (pre) and after (post) shock during preoperative contextual fear conditioning. B: Percent time spent freezing by all groups during postoperative testing of contextual fear response. C: Percent time spent freezing by all groups in the presence of the conditioned stimulus (tone) during postoperative testing. The HPC group was impaired at recalling the contextual fear response at the short delay, but not the long delay. There was no difference between HPC and CON groups in responding to the tone at either test delay. Error bars represent ± SEM. Abbreviations: HPC, hippocampal; CON, control; SD, short delay; LD, long delay; CXT, Context.

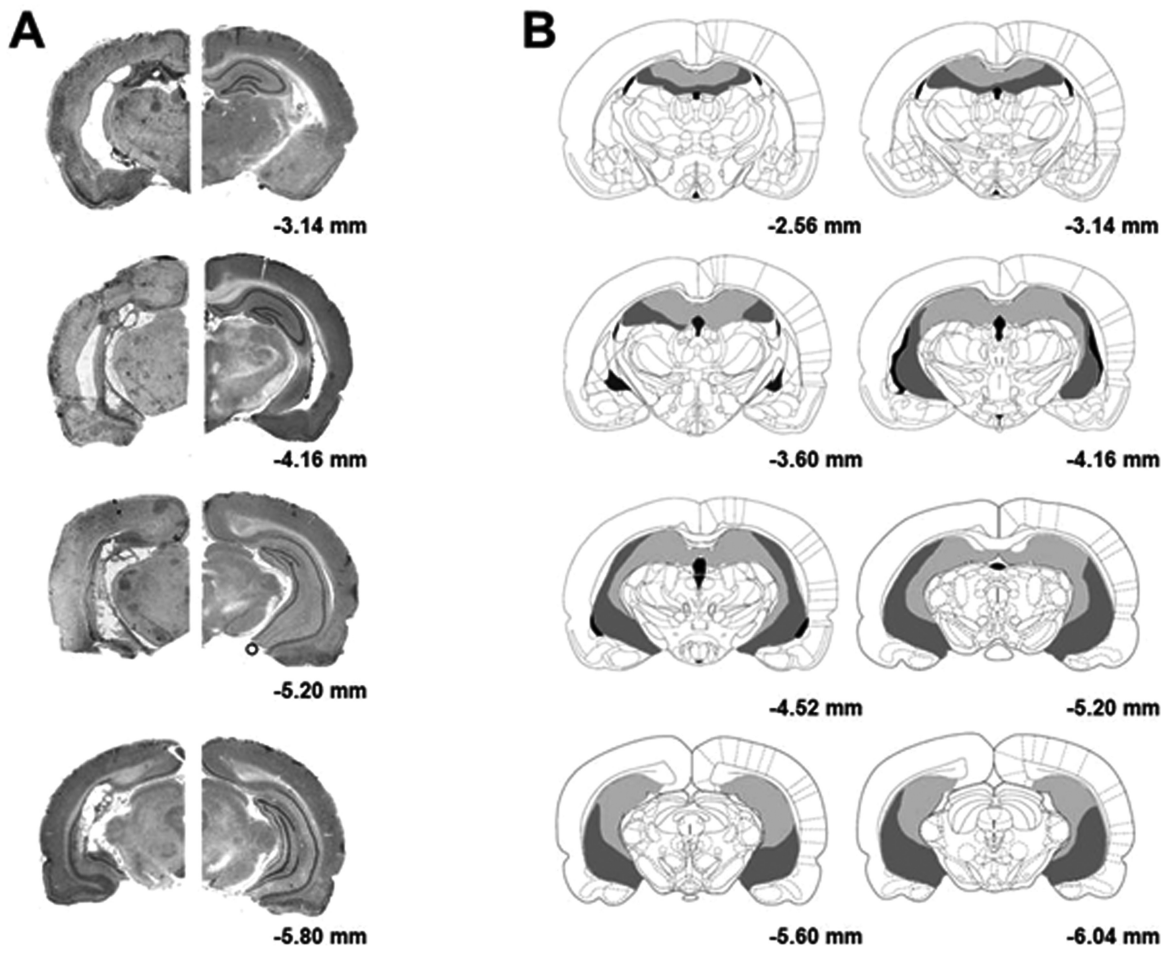

FIGURE 5. Histology. A: Photomicrograph of representative hippocampal lesion (left) in relation to a normal brain (right). B: Schematic drawings of minimal (light gray) and maximal (dark gray) extents of hippocampal lesions included in the experiment.

of lesion, $F(1,35) = 14.18$, $P = 0.001$, and delay, $F(1,35) = 3.93$, $P = 0.05$, were also significant.

In the CXT + CS test, all groups exhibited similar levels of freezing (Fig. 4C). There were no main effects of lesion, $F(1, 35) = 3.15$, $P = 0.08$, or delay $F(1, 35) = 3.12$, $P = 0.09$, and the lesion × delay interaction also was not significant, $F < 1$.

Anatomical

Figure 5 provides photomicrographs of coronal sections of (A) a representative hippocampal lesion in which 80% of the hippocampus was destroyed (left) and a normal brain at approximately the same plane. Figure 5B provides schematic drawings of minimal (light gray) and maximal (dark gray) extents of lesions.

The nature and extent of lesions were similar to those reported in recent studies (e.g., Winocur et al., 2009, 2010b). In all the rats with hippocampal lesions, damage extended bilaterally to dorsal and ventral regions of the structure. Ten of the 20 rats had very large lesions that affected 80% or more of the hippocampus proper (Mean = 91.2%), 5 rats had damage to 70–79% of the structure (Mean = 74.9%), 3 rats had damage to 69% of the hippocampus, and in 2 rats 55.6% and 60% of the hippocampus was destroyed. Overall, the average amount of hippocampal destruction was 80.4%, with the extent and pattern of damage to dorsal and ventral regions similar in all groups. In all rats, the lesions included extensive damage to all the subfields (CA1–CA3, dentate gyrus) and extra-hippocampal damage was minor or nonexistent. The two rats with 55.6% and 60% damage were retained because their performance fell within 1 standard deviation of the average of their respective group on both the contextual fear conditioning and spatial memory tasks. A *t*-test compared the size of the lesions in the two independent hippocampal groups and yielded no significant difference, $t < 1$.

Scatter plots of each rat's performance on the spatial memory (Fig. 6) and contextual fear conditioning (Fig. 7) tests are presented against percent damage to the hippocampus at the short or long delay. Pearson-product correlation coefficients were calculated to investigate associations between total lesion size and performance. On the spatial memory test, there were no statistically significant correlations on any of the measures but, in some instances, this may reflect the effect of an outlier and/or lack of power. When an outlier was removed from the Day 1 latency data at the short delay (Fig. 6A), a significant correlation was observed ($r = 0.82$, $P = 0.006$). On the contextual fear conditioning test, no significant relationship between total lesion size and freezing behavior was revealed at either delay,

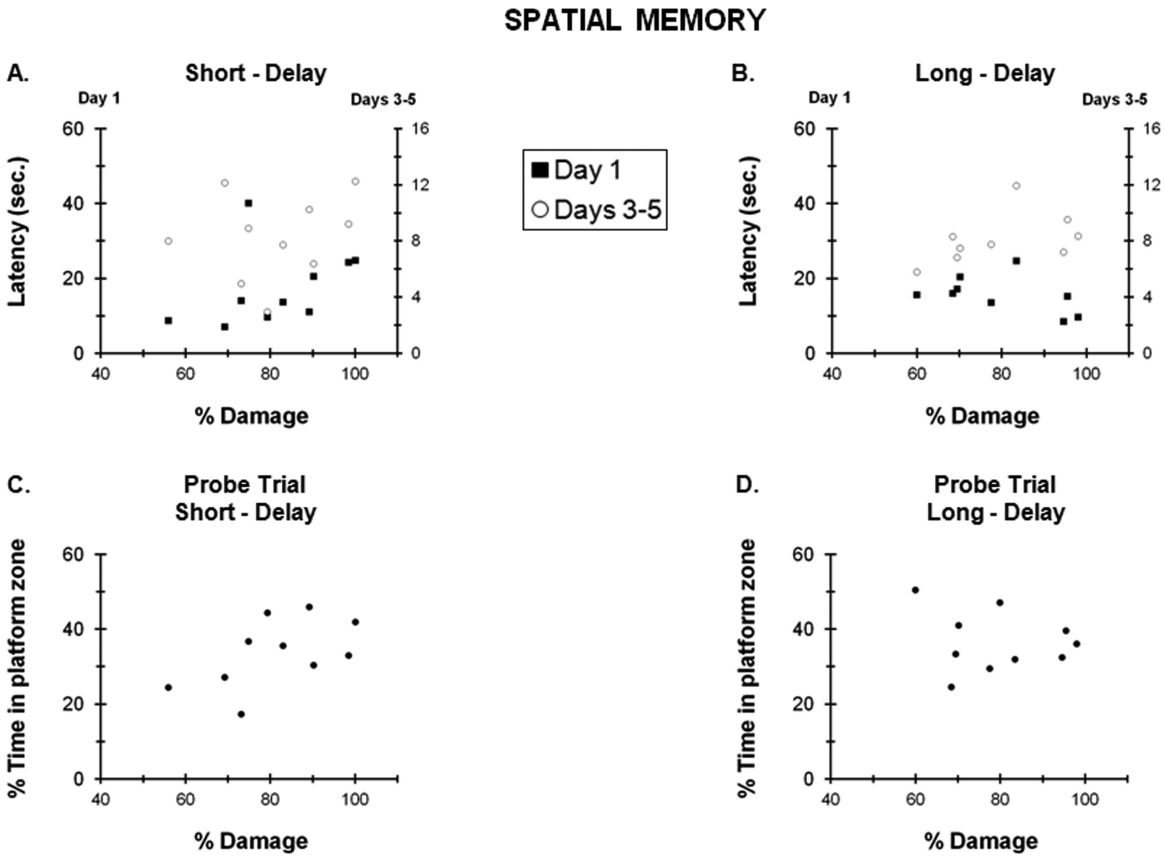

FIGURE 6. Scatter plots for each rat's performance on the first 5 days of spatial memory testing against percent damage to the total hippocampus at the (A) short delay and (B) long delay. Day 2 was not included because it was considered to be a transition period between recall on Day 1 and subsequent expression of relearning. The image also presents scatter plots for the amount of time spent by each rat in the platform zone on the probe test of Day 6 against percent damage to the hippocampus at the short (C) and long (D) delay. There was little evidence of a relationship between total lesion size and performance on the spatial memory test.

although a floor effect may have been a factor at the short delay test (Fig. 7A).

Analysis of performance in relation to damage in dorsal or ventral regions of the hippocampus also generally yielded nonsignificant results. On the spatial memory test, the only exception occurred at the short delay when an outlier was removed from the Day 1 data during relearning and a significant correlation emerged between dorsal hippocampal lesion size and latency ($r = 0.74$, $P = 0.02$). On the contextual fear conditioning task, the only significant relationship between regional lesion size and performance was a negative correlation between lesion size in the dorsal hippocampus and freezing at the long delay ($r = -0.69$, $P = 0.03$). Scatter plots relating performance on the spatial memory and contextual fear conditioning tests to lesions in the dorsal and ventral hippocampus are provided in Supporting Information.

DISCUSSION

The results confirm that hippocampal lesions produce nongraded retrograde amnesia for a context-specific, hippocampus-dependent, spatial memory task but temporally graded retrograde amnesia for a fear response which, at long delays, is known to be relatively context-free (Wiltgen and Silva, 2007; Winocur et al., 2007) and independent of the hippocampus. As has been reported by others (Phillips and LeDoux, 1994; Moses et al., 2007), memory for the conditioned fear response to the tone, which is acquired independently of context, and thought to rely on the amygdala, was not affected by hippocampal lesions. In demonstrating both patterns of amnesia in the same animals, the results parallel similar observations in human amnesics with hippocampal damage and provide compelling evidence that the type of information being accessed is a crucial factor in determining the pattern of retrograde memory loss associated with hippocampal damage.

The present results have important implications for current theories of hippocampal function in memory. For example, they would not be predicted by Standard Consolidation Theory, which maintains that hippocampal involvement is time-limited and that lesions to the structure should have no effect if they are made after the memory has become established in extra-hippocampal brain regions. With respect to the nongraded retrograde amnesia seen in rats with hippocampal lesions on the spatial task, it could be argued that the 28-day

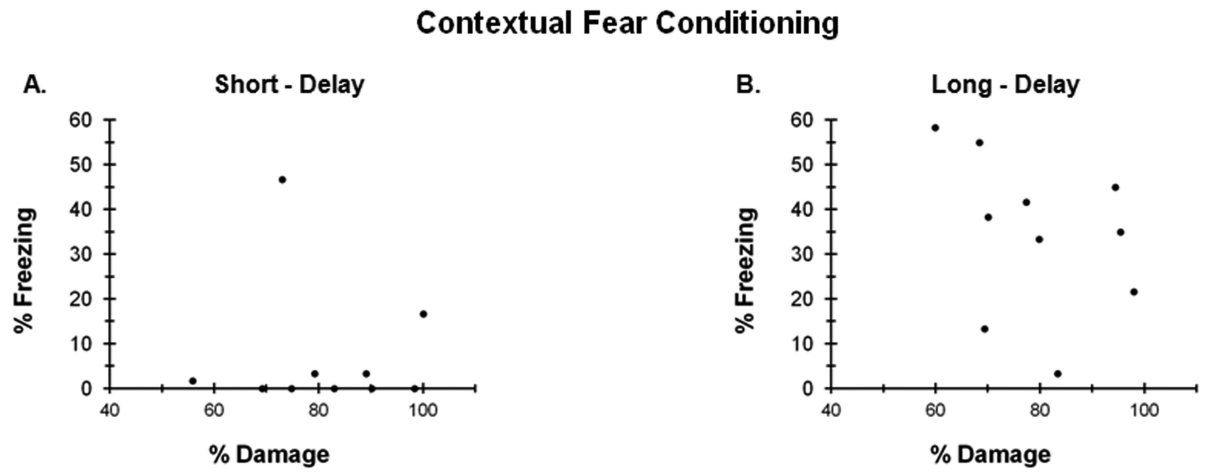

FIGURE 7. Scatter plots for each rat of time spent freezing in the contextual fear conditioning test against percent damage to the total hippocampus at the (A) short delay and (B) long delay. There was no evidence of a relationship between lesion size and freezing behavior at either delay.

training-surgery interval was insufficient to allow consolidation of the spatial memory. Arguing against this possibility is consistent evidence of extensive retrograde amnesia following hippocampal lesions on various water- and land-based spatial mazes, where training-surgery intervals were 100 days (Clark et al., 2005a,b) and even as long as 270 days (Winocur et al., 2005a).

In addressing this issue, proponents of Standard Consolidation Theory have suggested that the extended retrograde amnesia observed in hippocampally damaged animals on many spatial memory tasks may reflect a failure in navigation rather than loss of the specific memory (Clark et al., 2005a,b). The argument is that, in order to find a particular location in a complex environment, the animal must continually update spatial relationships that are constantly changing as the animal moves through the environment; it is this updating process that is disrupted by hippocampal lesions. This notion implies a working memory deficit not unlike that proposed some time ago by Olton et al. (1979). However, this interpretation is problematic in that it can be applied only to tasks that require spatial processing. There is longstanding evidence that animals with hippocampal damage are quite adept at recognizing and updating considerable amounts of information in nonspatial tests of memory (Winocur and Breckenridge, 1973; Jarrard, 1978; Nadel and MacDonald, 1980). A second problem with this interpretation relates to the finding that rats with hippocampal lesions can remember spatial locations and navigate effectively toward them if, preoperatively, they had become very familiar with the general environment (Winocur et al., 2005a,b, 2010b). According to Standard Consolidation Theory, although the representation of a consolidated memory in the brain changes over time, the memory itself remains invariant. It follows that, postoperatively, animals would recruit the same navigational strategies which should include the same amount of online updating. It is unclear why rats with hippocampal lesions would be able to engage in successful updating in one instance but not the other.

Sutherland and colleagues take a different theoretical position (see reviews by Sutherland et al., 2010; Sutherland and Lehmann, 2011). They maintain that the hippocampus is part of one of several brain systems capable of supporting new learning on various tasks. They argue that, in the normal brain, the systems compete for control and that in spatial learning and contextual fear conditioning, the hippocampal system is dominant and overshadows participation by other learning systems. Thus, lesions to the hippocampus may obliterate memory for a preoperatively acquired response without necessarily affecting new learning of that response, which could be taken over by a nonhippocampal system.

In support of their position, they cite evidence that rats with hippocampal lesions exhibit nongraded retrograde amnesia for preoperatively learned spatial memories and contextual fear responses performed after learning a spatial location or a contextual fear response exhibit nongraded retrograde amnesia. The present results are consistent with this position with respect to spatial memory (see also Clark et al., 2005a,b; Martin et al., 2005; Winocur et al., 2005a), but the finding of preserved remote memory for the contextual fear response is not. The latter result is in accord with numerous studies from other labs showing that animals with hippocampal lesions exhibit temporally graded retrograde amnesia for a contextual fear response acquired at training-surgery intervals varying between 28 and 200 days (Kim and Fanselow, 1992; Anaganostaras et al., 1999; Debiec et al., 2002; Wiltgen and Silva, 2007; Quinn et al., 2008; Winocur et al., 2009). The reason for the discrepancy in the results is unclear although it may be important that, in Sutherland's procedure, contextual fear conditioning typically is conducted in a single session with the first shock delivered 3 min after the animal was placed in the training chamber. In our procedure, the animal is placed in the apparatus on the day before conditioning for 30 min to explore and become familiar with it. In our experience, this reduces novelty effects and promotes contextual fear conditioning (see also Phillips and Ledoux, 1994). Conceivably, in Sutherland

et al.'s studies, the animals do not learn the fear response as well and, as a result, the memories are more vulnerable to the effects of hippocampal lesions. Whatever the explanation, as far as the primary issue in this article is concerned, the important point is that the overshadowing model would not predict nongraded retrograde amnesia for a spatial memory and temporally graded retrograde amnesia for contextual fear in the same animal.

With respect to the suggestion that other neural systems are capable of supporting learning and memory in the absence of the hippocampus, there was evidence of improvement by the hippocampal groups on relearning the spatial task at both delays. However, the lesioned group failed to re-establish preoperative performance levels and, at both delays, the hippocampal groups were impaired relative to controls. In this study, it cannot be determined if the hippocampal groups postoperative performance reflected relearning based on partial recall of the learned response or new learning, possibly using other systems, in the face of amnesia for preoperative training. The latter would be consistent with the notion of multiple memory systems (Packard et al., 1989) and the overshadowing hypothesis (Sutherland et al., 2010).

It has been suggested that temporally graded retrograde amnesia for a contextual fear response is more likely to occur in animals with hippocampal lesions if the lesions are relatively small. In this study, of the hippocampal group exhibiting spared remote memory on the contextual fear conditioning, 35% sustained more than 95% damage and all sustained more than 75% damage. Moreover, consistent with previous work in our lab (Winocur et al., 2007, 2009), scatter plot analysis revealed little evidence of a relationship between total lesion size and remote memory of a contextual fear response. On the spatial memory test, which is always dependent on the hippocampus, there was a positive relationship between the amount of total hippocampal damage and latency to find the platform's location at the short delay, but only when an outlier was removed from the analysis. The relatively small numbers of subjects and the resultant lack of power may have precluded reliable statistical demonstrations of this relationship.

There is considerable evidence that the dorsal and ventral hippocampus are functionally distinct regions (see reviews by Bannerman et al., 2004; Fanselow and Dong, 2010). On the suggestion that the dorsal hippocampus is selectively involved in tests of spatial memory (Moser et al., 1995) and contextual fear conditioning (Kim and Fanselow, 1992), the amount of tissue damage in the dorsal and ventral hippocampal regions were related to performance on both tasks in this study. This analysis yielded little statistical evidence of functional dissociation with respect to postoperative performance on either task, although, here again, lack of power may be a factor.

As indicated in the "Introduction" section, we take a different view of the role of the hippocampus in memory. Along with Standard Consolidation Theory, we endorse the view that the hippocampus is essential for the creation of detailed, contextually rich, episodic memories. However, following considerable evidence in the animal and human literatures (see reviews by Winocur et al., 2010a; Winocur and Moscovitch, 2011), we depart from Standard Consolidation Theory in arguing that, as long as a memory retains its episodic quality, the hippocampus is required for it to be recalled regardless of how long ago the memory was acquired (see also Nadel and Moscovitch, 1997). When the hippocampus is damaged, context-specific details are no longer available to the individual. Following Multiple Trace Theory's premise that, in contrast to episodic memories, semantic memories may be spared if they had time to consolidate, we further proposed that, over time, hippocampus-based memories transform into schematic (semantic) versions that retain the essential features but lose most of the contextual associations. Schematic memories are believed to reside in distributed cortical networks outside the hippocampus and, as a result, are resistant to the effects of hippocampal lesions. With respect to the present study, accurate performance in the spatial memory task requires precise contextual information without which the hidden platform cannot be located efficiently. By contrast, the response that assesses memory in contextual fear conditioning does not require the same type of precision, and can be elicited by nonspecific, schematic information.

A central assumption of the transformation hypothesis, which follows from Multiple Trace Theory, is that in the normal brain, context-dependent, episodic memories that are represented in the hippocampus, and context-independent, schematic memories that are represented outside the hippocampus can exist in parallel. The transformation hypothesis, as part of Multiple Trace Theory, is the only theoretical account that predicts that hippocampal damage can result in either temporally graded or ungraded retrograde amnesia, depending on the task and the type of memory tested. In asking whether both patterns of retrograde amnesia can be demonstrated in the same hippocampally damaged animal, this study poses a critical test of this prediction. This effect has been reported in human amnesics with medial-temporal lobe damage (Cermak and O'Connor, 1983; Barr et al., 1990; Steinvorth et al., 2005; Rosenbaum et al., 2008), but studies of animals have yielded only one or the other pattern, creating the impression of conflicting evidence. In showing both patterns of retrograde amnesia in rats with hippocampal lesions, the present results explain an apparent contradiction in the animal literature and, in using tests that can be considered analogous to those used in human studies, they provide strong support for the transformation hypothesis.

At this stage, an important question is to ask which structures are implicated in the representation of schematic memories. Convergent evidence from studies using different methodologies points to such structures as the ventro-medial prefrontal cortex, anterior cingulate gyrus, and retrosplenial cortex as possible candidates (Frankland and Bontempi, 2005; Goshen et al., 2011; Weible et al., 2012). This results make it clear that, in addressing this issue, the role of these and other regions must be investigated in relation to whether context-specific or schematic memory is being expressed.

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