HIPPOCAMPUS 15:260–272 (2005)

Hippocampus and Remote Spatial Memory in Rats

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ABSTRACT: Damage to the hippocampus typically produces temporally graded retrograde amnesia, whereby memories acquired recently are impaired more than memories acquired remotely. This phenomenon has been demonstrated repeatedly in a variety of species and tasks. It has also figured prominently in theoretical treatments of memory and hippocampal function. Yet temporally graded retrograde amnesia has not been demonstrated following hippocampal damage in spatial tasks like the water maze. We have assessed recent and remote spatial memory following hippocampal lesions in three different tests of spatial memory: (1) the standard water maze; (2) the Oasis maze, a dry-land version of the water maze; and (3) the annular water maze, where training and testing occur within a circular corridor. Training protocols were developed for each task such that retention of spatial memory could be expressed after very long retention intervals. In addition, retention in each task was assessed with single probe trials so that the assessment of remote memory did not depend on the ability to relearn across multiple trials. The findings were consistent across the three tasks. In the standard water maze (Experiment 1), spatial memory was impaired after training–surgery intervals of 1 day, 8 weeks, or 14 weeks. Similarly, in the Oasis maze (Experiment 2), spatial memory was impaired after training–surgery intervals of 1 day and 9 weeks. Finally, in the annular water maze (Experiment 3), spatial memory was impaired after training–surgery intervals of 9 weeks and 14 weeks. Dorsal hippocampal lesions impaired performance to the same extent as complete lesions. The impairment in remote spatial memory could reflect disruption of previously acquired spatial information. Alternatively, it is possible that in these tasks hippocampal lesions might produce an impairment in performance that prevents the expression of an otherwise intact spatial memory. Published 2004 Wiley-Liss, Inc.†

KEY WORDS: water maze; annular maze; retrograde; Oasis maze

INTRODUCTION

Descriptions of human memory impairment have long emphasized that remote memory is typically spared relative to recent memory (Ribot, 1881). This phenomenon, termed temporally graded retrograde amnesia, has been taken to support the idea that the structures damaged in amnesia have a temporary role in the formation and storage of memory (Squire and Alvarez, 1995). Formal studies have clarified the phenomenon considerably. Thus, retrograde amnesia tends to be temporally graded when damage is limited to the hippocampal region (Manns et al., 2003; Kapur and Brooks, 1999) and extensive and ungraded when the damage includes lateral temporal neocortex (Kapur, 1993; Squire and Alvarez, 1995).

During the 1990s, retrograde amnesia began to be studied prospectively in experimental animals. Through 2003, one can identify as many as 20 studies in which animals were given equivalent amounts of training on two or more occasions before damage to the hippocampus, fornix, or entorhinal cortex. Of these, 16 studies found temporally graded retrograde amnesia. The typical extent of the retrograde amnesia gradient was about 30 days (Squire et al., 2005). Interestingly, in the four cases in which a temporal gradient of retrograde amnesia was not observed following hippocampal damage, three involved the water maze (Bolhuis et al., 1994; Mumby et al., 1999; Sutherland et al., 2001). In these three studies, memory was impaired similarly regardless of the interval between training and surgery. The water maze requires the animal to swim to a specific point in space, using distal cues as guides. Studies with the water maze can be contrasted with three others that also required the animal to use spatial information, but only to discriminate between two (Cho et al., 1993; Cho et al., 1995) or three (Ramos, 1998) arms of a maze rather than to find a specific location. In these latter studies, temporal gradients of retrograde amnesia were observed, that is, remote memory was relatively preserved.

It is unclear why remote memory was impaired by hippocampal lesions in a task that requires the animal to remember a specific point in space. One possibility is that this type of spatial memory always requires the hippocampus because the hippocampus forms and stores the essential spatial maps needed for task performance (O'Keefe and Nadel, 1978). Alternatively, it is possible that a hippocampal lesion interferes with the ability to perform the spatial task, rather than the ability to remember a specific location. Thus, the lesion might impair navigational abilities, or the lesion might impair new learning that is required so that the animal can update its position in space during the performance test (Knowlton and Fanselow, 1998). One should also consider the possibility that some peculiarity of the water maze task itself underlies the finding that remote spatial memory was impaired by hippocampal lesions. In this respect, it is

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Grant sponsor: Medical Research Service of the Department of Veterans Affairs; Grant sponsor: National Institute of Mental Health (NIMH); Grant number: 24600; Grant sponsor: Metropolitan Life Foundation; Grant sponsor: National Institute of Aging; Grant number: P50 AG05131; Grant sponsor: National Science Foundation (NSF); Grant number: 0237053; Grant sponsor: James S. McDonnell Foundation; Grant number: 21002077; Grant sponsor: National Alliance for Research on Schizophrenia and Depression Effie Beeman Investigator Award.

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Accepted for publication 15 September 2004
DOI 10.1002/hipo.20056
Published online 2 November 2004 in Wiley InterScience (www.interscience.wiley.com).

notable that some sparing of remote spatial memory was reported in a dry land version of the water maze task (Kubie et al., 1999).

It is also notable that patient E.P., who developed profound amnesia at the age of 70 following extensive bilateral damage to the medial temporal lobe, demonstrated intact spatial memory for the neighborhood in which he grew up (Teng and Squire, 1999). Perhaps spatial memory requires quite a long time to become independent of the hippocampus. Interestingly, it has been difficult to assess spatial memory in rodents after very long retention intervals, because intact animals often do not remember for longer than a few weeks (e.g., Sutherland et al., 2001), or they perform near chance levels (e.g., near 30% when chance = 25%; Bolhuis et al., 1994).

These considerations suggest that the time is ripe for a thorough examination of the effects of hippocampal lesions on recent and remote spatial memory. Accordingly, we have assessed spatial memory after long retention intervals in each of three different spatial memory tasks: (1) the water maze, which has been the benchmark task of spatial memory in the rat (Experiment 1); (2) the Oasis maze, which is a dry land version of the water maze task (Experiment 2); and (3) the annular water maze, which is a task of spatial recognition memory that removes the need for spatial navigation (Experiment 3).

We gave animals extensive training to improve the long-term retention of spatial memory and also to provide a long period during which memories might become independent of the hippocampus. Further, the entire hippocampus was removed at different times after training so that results would not be complicated by sparing of hippocampal tissue. Finally, retention of spatial memory was assessed with probe trials, in order to obtain a direct measure of memory that was not confounded by the difficulty animals would be expected to have in relearning the tasks.

EXPERIMENT 1: WATER MAZE

In the water maze, a rat learns to escape water by using spatial cues to locate a platform hidden just below the water surface (Morris, 1984) (Fig. 1A). This task has proved to be quite sensitive to hippocampal damage, and even a partial lesion is sufficient to impair performance (e.g., Moser et al., 1993, 1995; de Hoz et al., 2003; Broadbent et al., 2004). We developed a training protocol that would allow spatial memory to be expressed on a single probe trial even after long retention intervals. We then compared the performance of control animals and animals with large hippocampal lesions made 1 day, 8 weeks, or 14 weeks following training. For comparison, an additional group was included that received dorsal hippocampal lesions 8 weeks after training.

Subjects

We tested 73 experimentally naive, male Long-Evans rats weighing 300–350 g at the beginning of the experiment. Rats were housed in pairs and maintained on a 12:12-h light/dark cycle. All rats had free access to food and water.

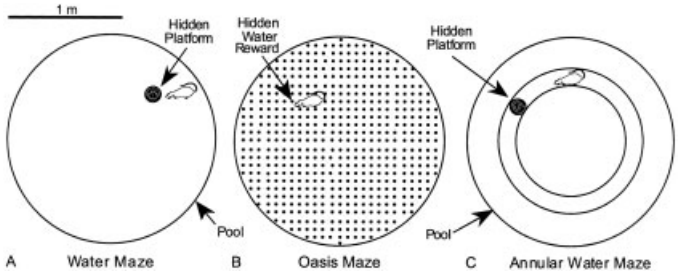

FIGURE 1. Top view of the three spatial tasks. All three tasks require the use of distal spatial cues (e.g., posters located on testing room walls). A: Water maze (Experiment 1). Rats were trained to find an escape platform hidden just below the surface of the water. B: Oasis maze (Experiment 2). Water-deprived rats were trained to find a single well containing a 0.3 ml drop of water among 426 wells. C: Annular water maze (Experiment 3). Rats were trained to find an escape platform hidden just below the surface of the water. The swim path was restricted by clear walls that formed an annulus, such that rats necessarily encountered the platform location in the course of swimming. Recognition of the platform location during probe trials was indicated by a slowing of swim speed in the vicinity of the platform. Scale bar = 1 m for all three mazes.

Apparatus

Water maze testing was conducted in a pool of water (1.8-m diameter at the water level) that was rendered opaque by the addition of powdered milk. The room was illuminated by four 30-W spotlights pointed at a white ceiling. The water was maintained at room temperature. The testing room contained a number of constant, salient visual cues (posters, objects, and equipment). A video camera was mounted on the ceiling directly above the pool and was used in conjunction with a video tracking system (San Diego Instruments) to record the swim path of each rat.

We used an Atlantis platform which could be raised or lowered remotely (Spooner et al., 1994). When the platform (12.7-cm diameter) was in the lowered position, the rat could neither detect the platform nor escape from the water. When the platform was in the raised position (1.5 cm below the surface of the water), it remained invisible to the rat but provided a means to escape the water. The Atlantis platform provides two advantages over the standard, static platform. First, it provides the opportunity to present reinforced probe trials. That is, a probe trial can be presented (to assess retention) with the platform in the lowered position. When the probe trial ends, the platform is raised so that the rat can escape and be reinforced for searching in the correct location. Second, the rats can be shaped to "dwell" over the correct platform location. This procedure trains the rat to be both accurate and persistent and it substantially improves the retention performance of normal rats on probe trials (i.e., it increases the percentage of time spent in the correct location; Spooner et al., 1994).

Behavioral Training

Phase I. Platform training

During this phase of training, curtains surrounded the pool and blocked the distal spatial cues. An object was suspended 20 cm

above the hidden platform and served as a beacon for the rats to find the platform. To begin each trial, the rat was placed in the water, facing the pool wall, at one of 4 start points around the pool (i.e., North, South, East, West). Two trials were given each day from each start point (eight trials/day for 3 days). The hidden platform was raised to within 1.5 cm of the water surface only when the rat was both accurate (<20 cm from the platform) and persistent in searching at the correct location (dwell time of 0.5 s on the first day, 1.5 s on the second day, and 2.5 s on the third day). After escaping, the rats remained on the platform for 30 s before being removed. If the rat failed to find the platform within 90 s, it was guided to the platform where it remained for 30 s. This procedure shaped a focused and sustained search at the platform location.

Phase II. Spatial training

Following platform training, the curtains were removed to reveal the distal spatial cues. Rats were given eight trials each day for 10 days (intertrial interval approximately 8 min; 2 trials/day from each of the starting points and with the sequence of starting points counterbalanced within groups). The platform location was the same for all animals. The platform was raised to permit escape from the water when the rat dwelled within 20 cm of the platform for 2.5 s. After escaping, it remained on the platform for 30 s. The first and fifth trial of each training day were 30-s, reinforced probe trials. During these trials, the platform was in the lowered position. After 30 s the platform was raised. This procedure allowed spatial learning to be tracked during the 10 training days.

Surgery and histology. Anesthesia was maintained throughout surgery with isoflurane gas (0.8–2.0% isoflurane delivered in O_2 at 1 L/min). The animals were placed in a Kopf stereotaxic instrument, and the incisor bar was adjusted so that bregma was level with lambda. Thermocoagulation lesions were made with a radiofrequency electrode and generator (Radionics model RF-4A). The electrode was first lowered to the surface of the dura, and a small puncture was made in the dura just below the electrode tip. The electrode was then lowered to the target and left in place for 1 min before heating the tissue to 80–90°C (depending on the target site) for a period of 1 min. The current to the electrode was then turned off, and the electrode was removed after the tip temperature fell to 41°C (see Clark et al., 2000 for additional details). Lesions were intended to damage the dorsal and ventral hippocampus (H-RF) or only the dorsal hippocampus (DH-RF). Lesions were made at multiple locations: All coordinates are in millimeters and relative to bregma (leveled to lambda). AP −2.4, ML ± 1.0, DV −3.5; AP −3.2, ML ± 1.4, DV −2.7; AP −3.2, ML ± 3.0, DV −2.7; AP −4.0, ML ± 2.5, DV −2.3; AP −4.0, ML ± 3.7, DV −2.7; AP −4.8, ML ± 4.9, DV −6.8*; AP −4.8, ML ± 4.3, DV −7.4*, −3.5; AP −5.4, ML ± 4.2, DV −4.2*; AP −5.4, ML ± 5.0, DV −6.5*, −5.5*, −4.5*. Asterisks indicate those locations that were omitted when the surgery was designed to damage only the dorsal hippocampus. The wounds were then closed, and the rats recovered from anesthesia on a water-circulating heating pad. All animals were allowed to recover for 14 days before behavioral testing.

For the rats given complete hippocampal lesions (H-RF), surgery was scheduled 1 day (n = 8; control group, n = 8), 8 weeks (n = 9; control group, n = 16), or 14 weeks (n = 12; control group, n = 12) after training. For rats given dorsal hippocampal lesions (DH-RF), surgery was scheduled 8 weeks after training (n = 8).

At the completion of behavioral testing, rats were administered an overdose of sodium pentobarbital and perfused transcardially with buffered 0.9% NaCl solution followed by 10% formaldehyde (in 0.1 M phosphate buffer). The brains were then removed and cryoprotected in 20% glycerol/10% formaldehyde. Coronal sections (50 μm) were cut with a freezing microtome. Every fifth section was mounted and stained with thionin to assess the extent of the lesions.

Phase III. Retention probe

Three 60-s probe trials were administered 14 days after surgery. The trials began by placing the rat in the water, facing the pool wall, at one of the 4 start points (counterbalanced within groups). The platform remained lowered for 60 s before being raised to provide escape. Each rat remained on the platform for 30 s before being removed. Performance on the probe trials was calculated by measuring the percentage of time that each rat spent in the quadrant of the pool where the platform had been located during training (chance performance = 25%). We also used an alternative method for analyzing probe trial performance (Moser et al., 1993) by calculating the percentage of time that a rat spent in a circular zone (30 cm diameter) around the point where the platform had been located during training. Chance performance = 4% (i.e., a 30-cm circle represents 4% of the total area of the pool).

Results

Histological findings

Figure 2A illustrates the extent of the largest and smallest lesion in each group.

Hippocampal damage H-RF groups. All animals sustained extensive bilateral damage to all the cell fields of the hippocampus, including the dentate gyrus. The average percentage damage to the hippocampus was 85.3% (range 74.5–91.0%). The primary areas of spared hippocampal tissue were located along the midline of the dorsal hippocampus and involved primarily the most medial aspects of CA1 and the crest of the dentate gyrus. All rats had sparing of the most ventromedial portion of the ventral hippocampus. Occasionally, small islands of spared hippocampal tissue were observed within the damaged areas of the hippocampus. All animals had some damage to the alveus and to the fimbria on the dorsal edge of the dorsal hippocampus. Most animals sustained at least minor damage to the dorsal and ventral subiculum. When present, the damage tended to occur in the ventral subiculum just below the ventral hippocampus. The large portion of the subiculum posterior to the ventral hippocampus was spared.

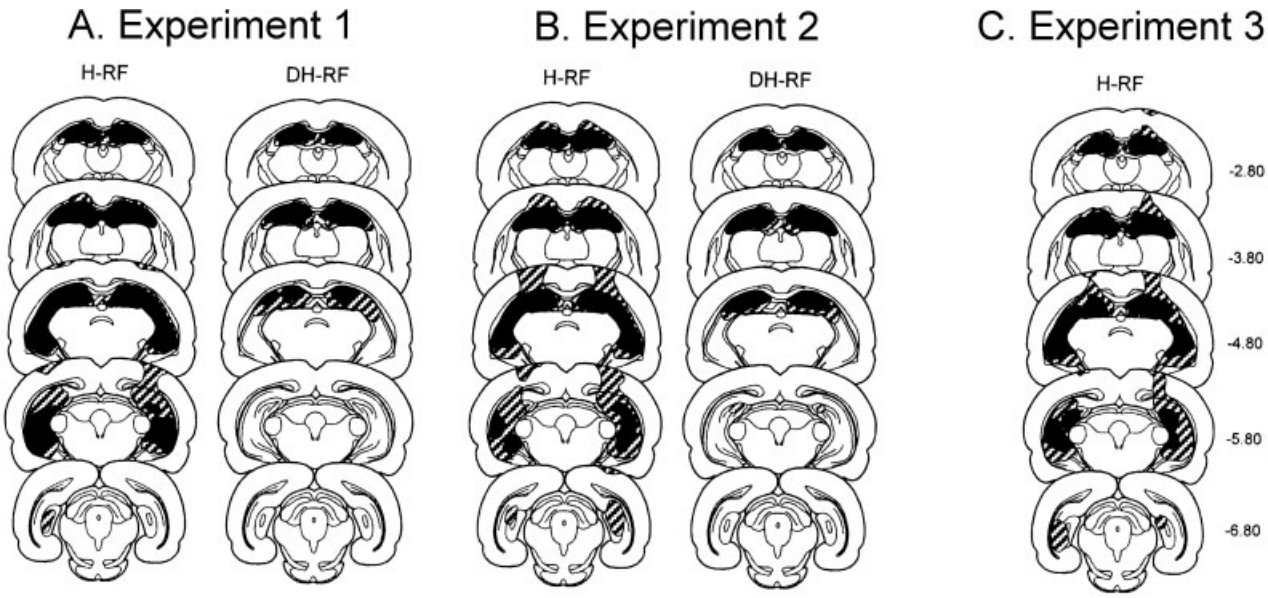

FIGURE 2. Reconstructions of coronal sections showing the largest (striped) and smallest (black) area of damage for rats with large hippocampal lesions (H-RF) and dorsal hippocampal lesions (DH-RF). Reconstructions from rats in Experiment 1(A), Experiment 2 (B), and Experiment 3 (C). Each series of sections progresses (top to bottom) from anterior to posterior levels. Numbers represent the distance in millimeters posterior to bregma.

Other damage. Three animals sustained minor damage to the entorhinal cortex that amounted to less than 10% of total entorhinal volume. The entorhinal cortex was entirely spared in all other animals. In most cases, there was some detectable damage to the cortical regions directly dorsal to the dorsal hippocampus. Most frequently this damage was in the form of cortical thinning, but a few cases involved direct tissue damage. No animal had any damage to the perirhinal cortex or the amygdala.

Hippocampal damage DH-RF group. All animals sustained extensive bilateral damage to all the cell fields of the dorsal hippocampus, including the dentate gyrus. The average percentage damage to the hippocampus was 37.9% (range 31.3–41.7%). The primary areas of spared hippocampal tissue were located along the midline of the dorsal hippocampus and involved primarily the most medial aspects of CA1 and the crest of the dentate gyrus. All rats had sparing of the ventral hippocampus and the ventral subiculum. Several of the animals had detectable damage to the alveus and to the fimbria.

Other damage. There was no substantial extrahippocampal damage in this group, and no cortical thinning as noted in the H-RF groups.

Behavioral Findings

Acquisition. All rats readily learned the platform location within the first few days of training. During the first probe trial on the tenth and final day of training, rats spent 50.4 ± 3.8% of the time in the target quadrant and 24.3 ± 2.6% in the circle around the platform. Both of these scores were well above chance levels (25% and 4% respectively; $t > 6.7$, $P < 0.0001$). Based on these scores, the rats were divided into equivalent lesion and control groups.

Initial retention probe: quadrant analysis. Figure 3 illustrates the percentage of time that each group spent in the training quadrant during the initial 60-s retention probe trial (chance = 25%). For the three control groups, the scores were 67.6 ± 5.2%, 50.9 ± 5.6%, and 31.2 ± 6.7% for the 1-day, 8-week, and 14-week intervals, respectively. These scores were above chance for the 1-day and 8-week groups ($t > 4.5$, $P < 0.001$), but not for the 14-week group ($t[11] = 0.9$, $P > 0.10$). Thus, forgetting was evident in the control group as time passed after training (one-way analysis of variance [ANOVA], ($F[2] = 7.2$, $P < 0$ .01).

By contrast, rats with hippocampal lesions performed similarly to each other ($t < 0.7$, $P > 0.10$) and at chance at all training–surgery intervals ($t < 1.9$, $P > 0.09$). The scores were 30.2 ± 4.2%, 29.6 ± 2.5%, and 26.1± 4.7% for the 1-day, 8-week, and 14-week H-RF groups, respectively (chance = 25%), and the score for the DH-RF group was 27.0% ±4.5%. The 1-day H-RF group performed more poorly than the 1-day control group ($t[14] = 5.6$, $P < 0.0001$), and both the H-RF and DH-RF groups at 8 weeks performed more poorly than the 8-week control group ($t > 2.7$, $P < 0.05$).

Initial retention probe: circle analysis. The results were the same as in the quadrant analysis when retention was measured by the percentage of time that rats spent in a small circle around the platform location (chance = 4.0%). For the three control groups, the scores were 41.2 ± 7.1%, 21.1 ± 3.8%, and 8.2 ±

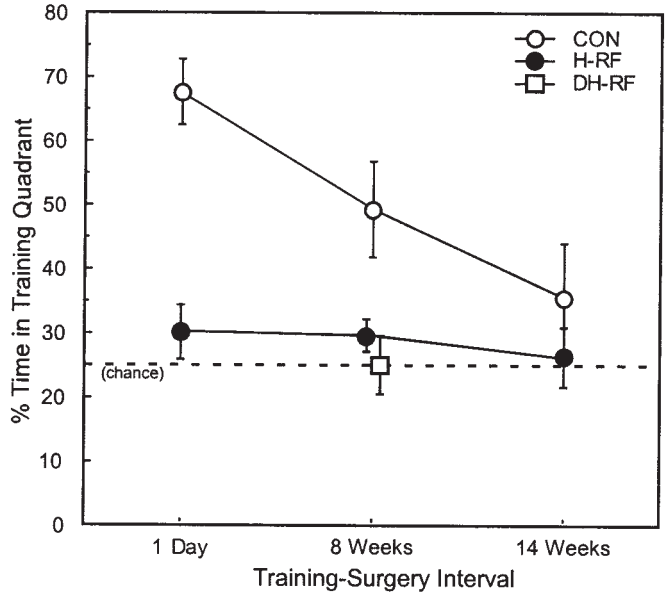

FIGURE 3. Water maze performance (Experiment 1). Percentage time in the training quadrant during a retention probe given 14 days after surgery. Control rats (CON), rats with complete hippocampal lesions (H-RF), and rats with dorsal hippocampal lesions (DH-RF) were tested for retention after a 1-day (CON, n = 8; H-RF, n = 8), 8-week (CON, n = 16; H-RF, n = 9; DH-RF, n = 8), or 14-week (CON, n = 12; H-RF, n = 12) training–surgery interval. None of the lesion groups performed above chance (25%). The 1-day and 8-week control groups performed above chance and better than the corresponding lesion group. Error bars = SEM.

2.5% for the 1-day, 8-week, and 14-week intervals, respectively. These values were above chance for the 1-day and 8-week groups ($t > 4.4$, $P < 0.01$), but not for the 14-week group ($t[11] = 1.6$, $P = 0.13$). By contrast, the lesion groups performed similarly to each other ($t < 1.5$, $P > 0.10$) and at chance at all training–surgery intervals ($t < 1.5$, $P > 0.10$). The scores were 6.0 ± 3.0%, 4.7 ± 2.0%, and 6.9 ± 2.1% for the 1-day, 8-week, and 14-week H-RF groups, respectively, and the score for the DH-RF group was 4.0% ± 1.2%. The 1-day H-RF group performed more poorly than the 1-day control group ($t[14] = 4.6$, $P < 0.001$), and the H-RF and DH-RF groups at 8 weeks performed more poorly than the 8-week control group ($t > 3.0$, $P < 0.01$).

Retention probes 2 and 3: quadrant analysis. On the second probe trial (administered approximately 15 min after the initial probe trial), all the control groups increased their percentage time in the target quadrant (probe 2 performance for control groups; 1-day = 80.4 ± 3.8%; 8-week = 72.8 ± 4.1%; 14-week = 54.5 ± 10.1%). This improvement might have been due to a reminder effect from probe trial 1 or to relearning (the probe trial was reinforced). Interestingly, the 1-day and 8-week H-RF groups did not improve on probe 2 and remained at chance levels, but both the 8-week DH-RF group and the 14-week H-RF group improved to above chance levels (8-week DH-RF = 53.9 ± 8.8%; 14-week H-RF = 39.9 ± 4.6%, $t > 3.3$, $P < 0.05$). Indeed, there was no difference in performance on probe 2 between the 14-week control group and the 14-week H-RF group (54.5 ± 10.1% vs. 39.9 ± 4.6%, respectively; $t = 1.3$, $P > 0.1$). On the third probe trial, the control groups continued to increase their percentage time in the target quadrant (all scores >70%). However, among the lesion groups, only the 8-week DH-RF group remained above chance (47.8 ± 5.6%; $t = 4.1$, $P < 0.01$). For this probe trial the 14-week H-RF group spent only 33.6 ± 6.1% in the target quadrant.

Retention probes 2 and 3: circle analysis. The findings from the circle analysis were the same as for the quadrant analysis with the following exception: The 14-week H-RF group did improve on probe trial 2 (from 6.9 ± 2.1% to 11.3 ± 2.8%; $t[11] = 2.1$, $P = 0.06$) but was still impaired relative to the 14-week control group on the second probe trial (26.7 ± 5.9%; $t[24] = 2.4$, $P < 0.05$).

Discussion

The control groups exhibited forgetting as the retention interval lengthened, and there was scant evidence of retention in the 14-week group. Performance of the lesion groups was identical on the initial probe trials irrespective of the retention interval. Further, none of the lesion groups exhibited evidence of memory retention, either by an analysis of percentage time in the target quadrant (Fig. 3) or by an analysis of percentage time in a small circle around the target.

On the second probe trial, the performance of all of the control groups improved, as did the performance of the DH-RF 8-week group and the H-RF 14-week group. The good performance of the 14-week H-RF group on probe trial 2 could be evidence of a reminder effect from the first probe trial that built on a weak spatial memory. If so, some spatial memory was present in this group, but not in the other groups where large hippocampal lesions were made sooner after training. However, it is also notable that performance of the 14-week H-RF group declined to chance on the third probe trial.

EXPERIMENT 2: OASIS MAZE

To date, previous studies using the water maze (Bolhuis et al., 1994; Mumby et al., 1999; Sutherland et al., 2001; Experiment 1 present study) have found remote spatial memory to be impaired following hippocampal damage. However, the single study to use a dry version of the water maze, where the rat must locate a specific point in space, found some evidence of spared remote memory after hippocampal lesions (Kubie et al., 1999). Accordingly, in this experiment we used a probe trial to assess recent and remote spatial memory for a land-based task following large hippocampal lesions.

We developed a land-based spatial memory task (Oasis maze) in which a thirsty rat uses distal spatial cues to search an open field for a specific location (Oasis) containing water (Fig. 1B). This task, which is conceptually similar to a previously described, food motivated task (Kesner et al., 1991), was designed to approximate the spatial memory demands that are required by the water maze task (e.g., the open field of the Oasis maze was the same diameter as the water maze pool). We compared the performance of control ani-

mals and animals with large hippocampal lesions made 1 day or 9 weeks after training. For comparison, an additional group was included that received dorsal hippocampal lesions 9 weeks after training.

Subjects

We tested 106 experimentally naive, male Long-Evans rats weighing 300–350 g at the beginning of the experiment. Rats were housed individually or in pairs and maintained on a 12:12-h light/dark cycle. All rats had unlimited access to food (see water restriction below).

Apparatus

The Oasis maze was a circular, acrylic board (1.8 meters in diameter) that was painted flat white and raised 76 cm from the floor by a table that allowed the board to be freely rotated on its center axis. The surface of the board contained 426 evenly spaced wells (2.5 cm in diameter, 1.3 cm in depth) in which small amounts of water (0.3 ml) could be hidden. Water was used as a reward because, unlike food rewards, rats cannot locate the water using olfactory cues (see results from RANDOM group below).

Phase I: Pretraining

Rats were water deprived by removing their water for 23 h/day for 3 days. Next, water was placed randomly in approximately 50% of the wells (0.3 ml per well), and the rats were given 5 min to find and drink as much water as they could on two occasions separated by 30 min. The next day this procedure was repeated with only 25% of the wells containing water. On the final day, only five of the wells contained water. Pretraining on this day ended when the rats located and consumed water from all five baited wells.

Phase II: Spatial training

Following pretraining, rats were given eight trials a day for 10 days. On each trial, water was placed in a single well. For each rat, the water was always located in the same position relative to the testing room and distal spatial cues. Four different target well locations were used, one in the center of each quadrant, to control for any position biases the rats might have. The target well location each rat was assigned to was balanced within each experimental group. Local cues were made irrelevant (including odor trails) by rotating the entire apparatus a predetermined distance and direction after each trial. However, the water was always placed in the same location relative to the testing room and distal spatial cues.

In addition to the groups trained to find water by using spatial cues, another group (RANDOM) was given an equivalent amount of training, except that the location of the water was varied randomly from trial to trial and could appear in any of the 426 wells. Thus, this group could develop optimal strategies for finding the water efficiently, but memory of specific locations was irrelevant. The RANDOM group provided a measure of where rats with no spatial memory of a trained location will spend their search time during probe trials. The probe trials for this group were administered 14 days after the end of training.

At the beginning of each acquisition trial, the rats were placed at one of four start points (North, South, East, and West) around the edge of the board. Across the eight trials of each day (intertrial interval was approximately 15 min), two trials were given from each of the four start points, and the sequence was counterbalanced within each group. A trial was terminated when the rat located the water or after 5 min. If the rat failed to find the water within 5 min, it was guided to the correct well and allowed to drink. Once the water was located, the rat was allowed to drink the water before being returned to the home cage. Following each training session, the rats were allowed to drink freely for 1 h.

Following acquisition, rats in the spatial group were divided into equivalent surgery and control groups based on performance on day 10, with the constraint that the animals in the surgery and control groups were equally likely to have been trained to find water in each quadrant.

Surgery and histology. The surgical procedures and coordinates for the complete hippocampal lesion were the same as in Experiment 1. For rats given complete hippocampal lesions (H-RF), surgery was scheduled 1 day ($n = 16$; control group $n = 16$) or 9 weeks ($n = 22$; control group $n = 32$) after training. The surgical procedures and coordinates for the dorsal hippocampal lesions were the same as in Experiment 1. For rats given dorsal hippocampal lesions (DH-RF), surgery was scheduled 9 weeks ($n = 8$) after training. The RANDOM group ($n = 12$) did not undergo surgery. Following surgery, all rats were allowed 14 days to recovery before retention probe testing. The neurohistological methods were the same as in Experiment 1.

Phase III: Retention probe

A 5-min probe trial was given 14 days after surgery. The trial began by placing the rat at one of two start points around the edge of the board. Start points were always in one of the two quadrants adjacent to the quadrant that contained the training well location. For example, for rats with training wells in the North, start points for the probe trial were either West or East (counterbalanced within each group). No water was available during the probe trial.

Probe trials on the Oasis maze differed in two important ways from probe trials in the water maze. First, 5-min probe trials were used, rather than 1-min probe trials, to insure that rats explored a substantial portion of the Oasis maze. Second, in the Oasis maze rats had a strong tendency to explore the edges of the board and also to return to the start point. For these reasons, chance performance cannot be calculated in a straightforward way. We estimated chance performance by assessing the performance of the RANDOM group (because rats in this group could not acquire a memory for a specific spatial location). Specifically, we compared the percentage of time that a trained rat spent within a 58-cm-diameter circle around the trained well with the percentage of time that rats in the RANDOM group spent in this same region (target locations for the RANDOM group were counterbalanced across rats). A 58-cm circle was the diameter of the largest circle that could be completely contained within one quadrant of the maze.

Results

Histological findings

Figure 2B illustrates the extent of the largest and smallest lesion in each group.

Hippocampal damage H-RF groups. All animals sustained extensive bilateral damage to all the cell fields of the hippocampus, including the dentate gyrus. The average percentage damage to the hippocampus was 82.7% (range 70.5–93.0%). The pattern of sparing was highly similar to the pattern reported in Experiment 1.

Other damage. Five animals sustained minor unilateral damage to the entorhinal cortex that amounted to less than 10% of total entorhinal volume. The entorhinal cortex was entirely spared in all other animals. As in Experiment 1, in most cases there was some detectable damage to the cortical regions directly dorsal to the dorsal hippocampus. Most frequently this damage was in the form of cortical thinning, but a few cases involved direct tissue damage. Most animals sustained at least minor damage to the dorsal and ventral subiculum. One animal had bilateral damage to the medial and lateral habenula. No animal had any damage to the perirhinal cortex or the amygdala.

Hippocampal damage DH-RF group. All animals sustained extensive bilateral damage to all the cell fields of the dorsal hippocampus, including the dentate gyrus. The average percentage damage to the hippocampus was 35.7% (range 33.5–39.5%). The pattern of spared tissue was highly similar to the pattern described in Experiment 1.

Other damage. There was no substantial extrahippocampal damage in this group, and no cortical thinning as noted in the H-RF groups.

Behavioral Findings

Acquisition

The average distance that each group traveled to locate the water across the first eight trials of training day 1 did not differ between rats given spatial training and the rats trained with random locations (trained groups = 1,035 ± 75 cm; RANDOM group = 1,349 ± 75 cm, t[54] = 1.6, $P > 0.1$). By training day 2 however, the trained groups were already performing better than the RANDOM group (562 ± 30 cm vs. 1324 ± 127 cm, t[54] = 8.5, $P > 0.0001$). By the tenth and final day of training, the average distance traveled during all eight trials was 198 ± 8 cm for the trained groups and 640 ± 110 cm for the RANDOM group. The improvement exhibited by the RANDOM group reflects the development of more efficient search strategies. The substantial improvement exhibited by the trained group reflects the learning of specific information about the location of the water.

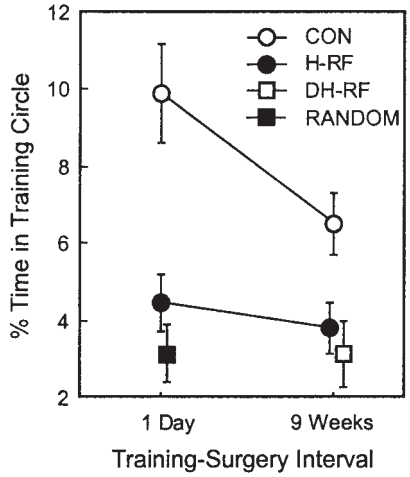

FIGURE 4. Performance on the Oasis maze (Experiment 2). Percentage of time in the training circle during a retention probe trial that was given 14 days after surgery. Control rats (CON) and rats with complete hippocampal lesions (H-RF) or dorsal hippocampal lesions (DH-RF) were tested for retention after a 1-day (CON, n = 16; H-RF, n = 16) or 9-week (CON, n = 24; H-RF, n = 22; DH-RF, n = 8) training–surgery interval. None of the lesion groups performed better than rats that were presented on each trial with random water locations (RANDOM, n = 12). Mean performance of the RANDOM group is represented by the filled square. The 1-day and 9-week control groups performed better than the corresponding lesion groups and better than the RANDOM group. Error bars = SEM.

Retention probe: circle analysis

Figure 4 illustrates the percentage of time that each group spent in the training circle during the 5-min retention probe trial that was given at different times after the completion of training. The control groups exhibited forgetting across the intervals tested (1 day, 9.8 ± 1.3% vs. 9 weeks, 6.4 ± 0.9%; t[46] = 2.2, $P < 0.05$.). Both scores were much better than the 3.1 ± 0.7% score obtained by the RANDOM group ($t > 2.2$, $P < 0.05$). By contrast, rats with complete hippocampal lesions (H-RF) or restricted dorsal lesions (DH-RF) performed similarly to each other ($t < 1.1$, $P > 0.10$) and no better than the RANDOM group ($t < 0.6$, $P > 0.10$). The scores for the lesion groups were as follows: 1-day H-RF = 4.4 ± 2.9%; 9-week H-RF = 3.7 ± 0.7%, 9-week DH-RF = 3.1 ± 0.8%.

Discussion

The control groups exhibited forgetting as time passed after training and performed better than the RANDOM group at both retention intervals. Each lesion group performed more poorly than the corresponding control group and no better than the RANDOM group. Thus, the lesion groups failed to demonstrate retention at either a short or long training–surgery interval.

These findings are consistent with Experiment 1 and with previous studies that have used the water maze task (Bolhuis et al., 1994; Mumby et al., 1999; Sutherland et al., 2001). However, these findings are at odds with the only previous study that used a dry land version of the water maze task (Kubie et al., 1999). This

study reported spared remote spatial memory when hippocampal lesions were made 14 weeks after training, but not 3 days after training. In that study, rats had to search a large arena (1.83-m diameter) to locate food buried in sawdust. One difference between the two studies is the length of the training–surgery interval (9 weeks in our study vs. 14 weeks in the earlier study) and this difference might be an important factor.

EXPERIMENT 3: ANNULAR MAZE

In the annular water maze (Hollup et al., 2001), the rat is confined to a circular corridor within a pool of water (Fig. 1C). The rat is trained to swim around the annulus, and an escape platform is subsequently raised to just below the water level. A normal rat will learn the location of the hidden platform relative to distal landmarks and will swim rapidly until it reaches the platform where it can escape the water. Memory for the platform location can be measured with probe trials while the platform is inaccessible. In that circumstance, memory is indicated when the rat decreases its swim speed (i.e., spends more time) in the platform location compared to other zones of the annulus. The tendency to remain near the platform location reveals that the rat has recognized the target location. Rats with hippocampal lesions made prior to training do not acquire this tendency (Hollup et al., 2001). Using the annular water maze, we compared performance of control animals and animals with large hippocampal lesions made 9 weeks or 14 weeks following training.

Subjects

We tested 28 experimentally naive, male Long-Evans rats weighing 300–350 g at the beginning of the experiments. Rats were housed individually or in pairs and maintained on a 12:12-h light/dark cycle. All rats had free access to food and water.

Apparatus

The annular maze consisted of a clear Plexiglas annulus (outer wall = 103-cm diameter, inner wall = 75-cm diameter) placed in the center of the same pool of water used for the standard water maze task. The walls of the annulus extended 14 cm above the water line and created a circular corridor that was 14 cm wide. The distal spatial cues were the same as in Experiment 1. Two pneumatically controlled Atlantis platforms were placed on opposite sides of the pool within the annulus. When one platform was used as a training location, the other remained in the lowered and inaccessible position. Each rat received spatial training using one of the two platform locations (counterbalanced within groups).

Phase I: Pretraining

During pretraining, curtains surrounded the pool and blocked the distal spatial cues. Each rat received four trials per day for 3 days. For each trial, the rat was placed at the North, South, East, or West starting points and allowed to swim around the annulus for 2 min, while the platform was not available. This procedure encouraged the rats to swim around the entire annulus.

Phase II. Annular spatial training

Following pretraining, the curtains were removed to reveal the distal spatial cues. Rats were given eight trials each day for 10 days (2 trials each day from the North, South, East, and West starting points and with the sequence of starting points counterbalanced within groups). Rats were tested in squads of 8 (approximate intertrial interval = 8 min). To escape the water, a rat was required to swim one complete lap around the pool. Once the lap was completed, the escape platform was made available. After escaping, the rat remained on the platform for 30 s before being removed (or it was removed when it climbed onto the annulus walls and moved away from the platform). The first and fifth trials of each day were 60-s reinforced probe trials. During these trials, the platform was initially in the lowered and inaccessible position. After 60 s elapsed and the rat had completed at least one lap, the platform was raised to just below the water surface so that escape was possible.

Following acquisition, rats were divided into equivalent surgery and control groups based on performance on the first probe trial on day 10, with the constraint that each group had the same distributions of platform locations.

Surgery and Histology

The surgical procedures and coordinates for the complete hippocampal lesion were the same as in Experiment 1. For this lesion, surgery was scheduled 9 weeks (n = 7; control group n = 7) or 14 weeks (n = 7; control group n = 7) after training. Rats were allowed 14 days to recover before retention probe testing. The neurohistological methods were the same as in Experiment 1.

Phase III: Retention probe testing

A probe trial was given 14 days after surgery. For probe trials, the annulus corridor was divided into 12 zones of 30° arcs (Fig 5A). The platform was located in the center of one of the 30° zones (target zone). We measured the percentage of time that the rat spent in the target zone and the time spent in each of 11 other zones (chance performance = 8.3% per zone; Fig. 5A). The probe trial began by placing the rat immediately opposite the trained platform location and allowing the rat to swim for 2 min. The timer began when the rat was placed in the pool and began to swim away from the start point. During the probe trial, spatial memory for the platform location was expressed as a reduction in swim velocity when the rat was in the vicinity of the target zone. As a result, the rat spent more time in this zone than in other zones.

Results

Histological findings

Figure 2C illustrates the extent of the largest and smallest lesion in each group.

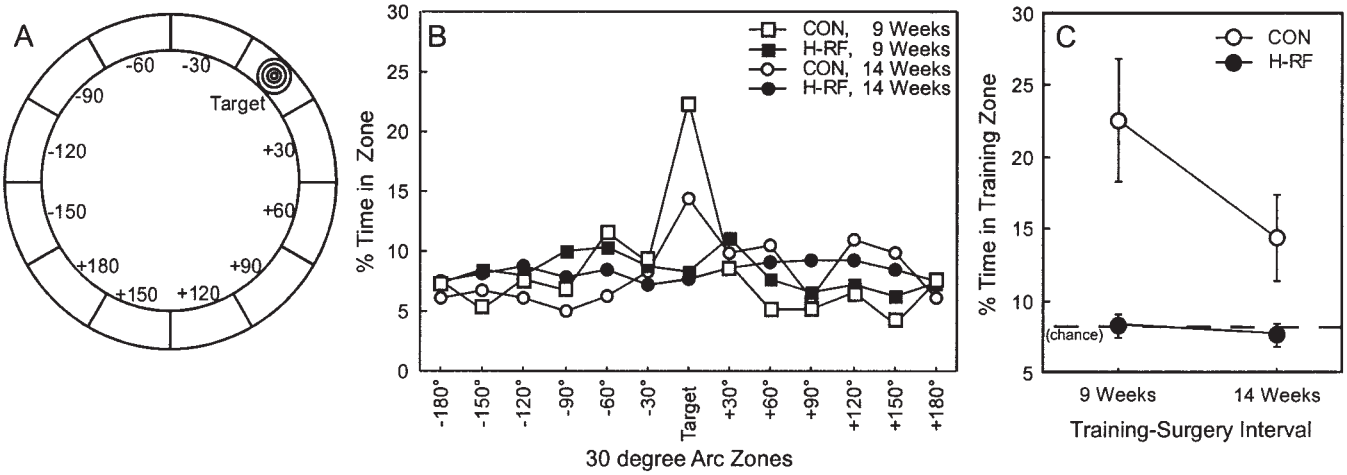

FIGURE 5. Performance on the annular water maze (Experiment 3). A: For scoring, twelve 30° arc zones were constructed around the training (platform location) zone. Chance performance is 8.3% (100% divided by 12 zones). B: Percentage time spent by each group in the 12 zones during the retention probe trial that was given 14 days after surgery. Memory of the platform location is indicated as increased percentage time in the training zone. C: Percentage time in the training zone replotted as a function of training–surgery interval. For rats with complete hippocampal lesions (H-RF), surgery occurred 9 weeks or 14 weeks after training (n = 7 for all groups). None of the lesion groups performed above chance (8.3%). Both the 9-week CON and the 14-week CON groups performed better than the corresponding lesion groups. Error bars = SEM.

Hippocampal damage H-RF groups. All animals sustained extensive bilateral damage to all the cell fields of the hippocampus, including the dentate gyrus. The average percentage damage to the hippocampus was 87.4% (range 76.4–91.9%). The pattern of sparing was highly similar to the pattern reported in Experiment 1.

Other damage. One animal sustained minor unilateral damage to the entorhinal cortex that amounted to less than 5% of total entorhinal volume. The entorhinal cortex was entirely spared in all other animals. As in Experiments 1 and 2, most cases showed some detectable damage to the cortical regions directly dorsal to the dorsal hippocampus. Most frequently this damage was in the form of cortical thinning. Most animals sustained at least minor damage to the dorsal and ventral subiculum. In two cases, there was some minor unilateral damage to the lateral dorsal nucleus of the thalamus. No animal had any damage to the perirhinal cortex or the amygdala.

Behavioral findings

Acquisition. On the first probe trial (trial 1) of spatial training, rats spent 9.8 ± 1.0% of the time in the target zone. This score was not above chance (chance = 8.3%, t[27] = 1.5, $P > 0.10$). On the first probe trial on the tenth and final day of training, rats spent 19.7 ± 1.9% of the time in the target zone. This score was well above chance (chance = 8.3%, t[27] = 6.0, $P < 0.0001$) and better than performance on the first probe trial on the first day of training (t[27] = 5.1, $P < 0.0001$).

Retention probe. Figure 5B shows the percentage of time that each group spent in each zone during the 2-min retention probe trial. Notice the elevated percentage of time the control rats spent in the target zone. Figure 5C shows the percentage of time each group spent in the target zone as a function of training–surgery interval. The control groups spent 22.6 ± 4.3% and 14.4 ± 3.0% of the time in the target zone in the 9-week and 14-week conditions, respectively. The 9-week control group performed above chance (t[6] = 3.3, $P < 0.05$) and better than the 9-week H-RF group (8.3 ± 0.6%; t[12] = 3.2, $P < 0.01$). The 14-week control group performed marginally above chance (t[6] = 2.0, $P = 0.09$) and better than the 14-week H-RF group (7.7 ± 0.7%; t[12] = 2.2, $P = 0.05$). The two groups with hippocampal lesions performed similarly to each other and no better than chance ($t < 0.13$, $P > 0.10$).

Discussion

The control groups exhibited forgetting as time passed after learning. Each lesion group performed more poorly than the corresponding control group and no better than chance. Thus, the lesion group exhibited no evidence of retention at either training–surgery interval (Fig. 5C). It was previously reported that hippocampal lesions impair anterograde memory on this task (Hollup et al., 2001). The present work extends these findings and shows that hippocampal lesions also impair retrograde memory.

GENERAL DISCUSSION

The findings were clear and consistent across three experiments that examined recent and remote spatial memory following damage to the hippocampus. The results in the standard water maze (Experiment 1) revealed no evidence of spared spatial memory with training–surgery intervals of 1 day, 8 weeks or 14 weeks. Similarly, the results in the Oasis maze (Experiment 2) revealed no evidence of spared spatial memory with training–surgery intervals

of 1 day or 9 weeks. Finally, in the annular maze (Experiment 3), there was no evidence of spared spatial memory with training–surgery intervals of 9 weeks or 14 weeks. In summary, there was no tendency for the lesion groups, in any of the experiments, to perform well at the longer training–surgery intervals. Further, relatively small, dorsal hippocampal lesions impaired performance to the same extent as complete lesions.

Two points merit discussion. First, our lesion method (radiofrequency) damaged both cell bodies and fibers. We were concerned that excitotoxic lesions might damage structures, and possibly impair remote memories, outside of the hippocampus as the result of the sustained hyperactivity of hippocampal neurons following the injection of a fiber-sparing excitotoxin (e.g., Anagnostaras et al., 2001). Further, because most fiber bundles within the hippocampus have hippocampal neurons as their origin or target (Amaral and Witter, 1995), most of these fiber bundles would have been compromised by either a radiofrequency lesion or an excitotoxic lesion. Nonetheless, the possibility remains that our results might have differed if fiber-sparing lesions had been used. Second, the effects of dorsal hippocampal lesions (as opposed to complete lesions) were studied only at an 8-week (water maze, Experiment 1) and 9-week (oasis maze, Experiment 2) training–surgery interval. It is possible that had the effects of dorsal hippocampal lesions been studied in all three tasks and also at shorter or longer training–surgery intervals, some sparing of remote memory might have been observed. Nonetheless, the results following complete hippocampal lesions as well as the results following dorsal hippocampal lesions were unambiguous and provided no evidence of spared remote spatial memory.

These findings can be contrasted with the pattern of results that have typically been observed following hippocampal damage on tests of nonspatial memory, such as trace eyeblink conditioning (Kim et al., 1995; Takehara et al., 2002, 2003). In these cases, hippocampal lesions made soon after training impaired performance, and lesions made one month after training had no effect. A number of other similar findings have been reported (for review, see Squire et al., 2004). For example, in the odor-odor association task known as the social transmission of food preference (Galef and Wigmore, 1983), temporal gradients of retrograde amnesia covering 1–5 days have been observed following lesions of the dorsal hippocampus (Winocur, 1990) and following larger lesions that damaged the dorsal and ventral hippocampus (Winocur et al., 2001). When the damage was even more extensive, including dorsal and ventral hippocampus and substantial portions of the dorsal and ventral subiculum, a 1–30-day gradient of retrograde amnesia was observed (Clark et al., 2002).

On first examination, the present findings appear broadly consistent with the view, which grew out of the discovery of hippocampal place cells in the rat, that the hippocampus is essential for forming and storing spatial maps (O'Keefe and Nadel, 1978). By this view, temporally graded retrograde amnesia should not occur for spatial memory because spatial maps (i.e., spatial memories) thought to be stored in the hippocampus are destroyed when the hippocampus is damaged. Accordingly, a hippocampal lesion should impair spatial memory, no matter how long after learning the lesion is made. However, there are other possible ways to understand the present results, and there are also other findings that must be considered in any discussion of remote spatial memory and hippocampal function.

One important finding is that in humans, remote spatial memory was spared even following large medial temporal lobe lesions. Thus, patient E.P. was able to recall the spatial layout of the region where he grew up and from which he moved away as a young adult more than 50 years earlier. He could mentally navigate, construct novel routes, and point correctly to landmarks while imagining himself at various locations. Yet E.P. has no knowledge of the neighborhood where he has lived since 1993, the year after he became amnesic (Teng and Squire, 1999). In another study, patient K.C., who has bilateral hippocampal damage, as well as other significant damage, can retrieve remotely formed spatial memories of major landmarks, construct routes, and estimate distances and directions in the neighborhood in which he grew up and has lived for approximately 40 years (Rosenbaum et al., 2000). These findings show that the human medial temporal lobe is needed to acquire new spatial knowledge (Teng and Squire, 1999), but that it is not the repository of remotely acquired spatial maps (Teng and Squire, 1999; Rosenbaum et al., 2000). One difference between these studies in humans and our studies in rats is that spatial learning in the rats occurred during a limited period of time when the animals were adults, whereas the spatial learning studied in the patients was acquired beginning at an early age and the learning continued over many years. Perhaps spatial memories must be very well learned over a long period of time, or perhaps they must be acquired early in life (or both) if they are to be spared following hippocampal damage (for a thoughtful discussion of these possibilities, and others, see Rosenbaum et al. (2001).

Although there was little or no evidence of spared remote spatial memory in the present study, other studies have clearly demonstrated spared remote memory following hippocampal lesions in rats in tasks that appear to require some form of spatial memory. For example, spared remote (and impaired recent) memory has consistently been reported when spatial information must be used to guide a simple two-choice (Cho et al., 1993, 1995) or three-choice (Ramos, 1998) spatial discrimination between arms of a maze. Additionally, in an 8-arm spatial discrimination task in mice, brain imaging ([^{14}C]2-deoxyglucose uptake) revealed greater hippocampal activation during a retention test 5 days after learning (recent spatial memory) than during a retention test 25 days after learning (remote spatial memory) (Bontempi et al., 1999). Finally, infusion of lidocaine into dorsal hippocampus 1 day after training impaired memory for the trained arm of a 5-arm maze, but infusion after 30 days had no effect (Maviel et al., 2004). These results are not consistent with the view that spatial memory is permanently stored in the hippocampus.

Contextual fear conditioning also involves spatial memory, because the rat explores a novel environment and encodes numerous stimulus features, including visuospatial information, which must be integrated in order to represent a context (Fanselow, 2000). Temporal gradients of retrograde amnesia (i.e., spared remote memory and impaired recent memory) have consistently been observed following hippocampal damage in the case of classically conditioned fear to a context (Kim and Fanselow, 1992; Maren et

al., 1999; Anagnostaras et al., 1999). In support of these studies, findings with α-CaMKII$^{+/-}$ mutant mice and activity-dependent genes suggest that both contextual fear conditioning and spatial learning in the water maze become progressively more dependent on the neocortex as time passes after learning (Frankland et al., 2001, 2004).

One feature that may distinguish tasks where remote spatial memory is spared following hippocampal lesions from tasks where remote spatial memory is abolished involves how animals use spatial information. Interestingly, only in those tasks where the expression of memory requires the animal to move to a specific spatial location has remote spatial memory been impaired following hippocampal lesions (see Kubie et al., 1999, for the single exception). In tasks where animals must use spatial information to discriminate between two or three maze arms, rather than to find a specific location, remote memory is spared (Cho et al., 1993, 1995; Ramos, 1998; Maviel et al., 2004). Here we consider two possible reasons why remote spatial memory is impaired by hippocampal lesions in the first case, but not in the second case.

First, remote spatial memory might survive hippocampal lesions, but be reduced in quality or quantity. By this account, the surviving spatial information is sufficient to guide a spatial discrimination between maze arms, or to reactivate conditioned fear of a context (Kim and Fanselow, 1992; Maren et al., 1999; Anagnostaras et al., 1999), but not sufficient to guide navigation to a specific point in space. This idea is consistent with the view that the hippocampus stores the rich spatial details (maps) that are critical for guiding an animal to a specific location (O'Keefe and Nadel, 1978).

Yet, another possibility is that remote spatial memory fully survives hippocampal damage, but that the lesion impairs the expression of spatial memory (i.e., the ability to perform the task). By this view, the navigational demands of expressing spatial memory require an intact hippocampus, perhaps because navigating to a specific point requires new learning. In other words, the animal must be able to update (encode) its position in space continually in order for a specific spatial memory to be expressed (Knowlton and Fanselow, 1998). In the same sense, an amnesic patient would not be expected to be able to express an otherwise intact spatial memory, if the performance test required executing a number of turns and moving along several routes. In this case, the memory demands of the performance test would exceed what can be maintained in immediate memory.

In contrast, the ability to accomplish new learning would not be required for spatial discrimination tasks where the animal needs only to choose the correct arm of a maze. Once the correct arm is chosen, the animal needs only to proceed along the arm to obtain food. Similarly in the case of contextual fear conditioning, memory is measured by the amount of freezing (no locomotion is required), and the performance test does not require the acquisition of new information. In the same sense, amnesic patients do not need to acquire new information in order to answer questions about their remote spatial memory.

These considerations raise the possibility that hippocampal lesions impair task performance rather than memory itself. This idea is reminiscent of the distinction between "getting there," which can be impaired after fornix lesions, and "knowing where," which can be spared (e.g., Whishaw et al., 1995). Rats with fornix lesions were impaired in learning how to locate a hidden platform as opposed to learning where a platform is located.

Rats with hippocampal damage also exhibited marked impairments in path integration (Whishaw and Maaswinkel, 1998; Maaswinkel et al., 1999). In path integration (also referred to as dead-reckoning; Darwin 1873), an animal continually updates and computes its position in space relative to some starting point by integrating the body movement cues generated by physical locomotion (for review, see Etienne and Jeffery, 2004). In addition, many hippocampal neurons exhibit physiological properties that would be useful for navigation. Thus, in addition to place cells, the hippocampus possesses head direction cells, and the firing properties of these cells are influenced by body movements (for review, see Redish, 2001; Etienne and Jeffery, 2004). These observations provide additional reasons for supposing that hippocampal lesions might impair performance whenever a task requires navigation.

At first glance, it might appear that the findings of Experiment 3 count against this proposal that hippocampal lesions impair performance by impairing the new learning that is needed to navigate and express a remote spatial memory. In Experiment 3, remote spatial memory was impaired even though the annular maze would seem to minimize the need for navigation. Animals must simply swim until they encounter (and recognize) the target location. Yet, it is unknown how animals actually accomplish this task. It is true that the annular maze task is less sensitive to hippocampal disruption than the standard water maze task (Brun et al., 2002), but this finding is consistent with the possibility that normal animals in this task demonstrate their spatial memory by continually updating their position in space relative to distal spatial cues, just as in the standard water maze. By this view, animals with hippocampal lesions are unable to update their position in space, do not know where they are, and thus do not recognize the target location when they approach it.

It will be difficult to test the idea that hippocampal lesions impair performance, i.e., the expression of memory, in animals with permanent hippocampal lesions. However, in other paradigms, reversible lesions have been successful in distinguishing between impaired memory and the impaired expression of memory (Clark et al., 1992; Clark and Lavond, 1993; Krupa et al., 1993). Reversible lesions afford the important advantage that retention can be tested after the lesion has been reversed and during a time when the hippocampus is functional. Some progress has been made using reversible lesions (e.g., with an AMPA antagonist) in studies of spatial memory (Riedel et al., 1999; Maviel et al., 2004).

Acknowledgments

The authors thank Laura Entwistle, Marie-Claire Doré, Gene Delay, Natalie Shanks, Daniel Guadarrama, Darwin-Dean Castillo, and Stuart Zola for assistance.

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