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Retrograde amnesia and selective damage to the hippocampal formation: memory for places and object discriminations

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Abstract

Using a within-subjects design, rats were trained on two place-memory problems and five object-discrimination problems at different intervals prior to receiving either ibotenate lesions of the hippocampal formation or sham surgery. Places # 1 and 2 were fixed-platform water-maze tasks that were run in different rooms and they were learned during the 14th and 2nd week before surgery, respectively. Object-discrimination problems # 1–5 were learned during the 13th, 10th, 7th, 4th, and 1st week before surgery, respectively. Rats with hippocampal lesions displayed impaired retention of both Place problems with no evidence of a temporal gradient to the impairment. In contrast to their retrograde place-memory deficits, the hippocampal rats displayed normal retention of the five object-discriminations that were learned before surgery. Hippocampal lesions had similar consequences for anterograde learning, as the lesioned rats were impaired in acquisition of a new water-maze problem that was run in a third room (Place # 3), whereas they showed normal acquisition of two new object-discriminations. The findings indicate that the hippocampal formation is not required for long-term consolidation of information underlying accurate performance of object-discriminations, and that its critical role in memory for places persists for at least 14 weeks, and probably for as long as those memories exist.

Keywords: Retrograde amnesia; Spatial memory; Object discrimination; Hippocampal formation; Consolidation; Water maze; Rats

1. Introduction

Studies in humans and laboratory animals suggest that damage to the hippocampal formation can produce retrograde amnesia in which information that was acquired recently prior to the injury is more severely affected than information acquired long before the injury (see ref. [24] for a review). One interpretation of such temporally-graded retrograde amnesia is that the hippocampus has a time-limited role in information storage. Purveyors of this view [12,28,37] have not been clear in describing this role, at times seeming to suggest that information is temporarily stored within the hippocampus as more permanent representations are gradually established in another brain area, and at other times implying that the hippocampus never actually stores information but somehow enables dispersed neocortical representations to communicate with each other until, at some point, this can be accomplished without the hippocampus. According to either interpretation, eventually the hippocampus is no longer needed for storage or retrieval of the information.

Hippocampal damage can also produce anterograde learning and memory deficits. The findings from studies of anterograde amnesia in human and nonhuman primates with medial-temporal-lobe damage have led many investigators to view the hippocampal formation as part of a 'temporal-lobe memory system' that also includes the entorhinal cortex, parahippocampal gyrus, and the perirhinal cortex [5,28,40]. This system is thought to support the representation of relational information about individual items or events that the subject experiences (i.e. *declarative* memory). The severity of both anterograde and retrograde memory impairment worsens as the extent of damage to medial temporal lobes increases [24], and most lesion studies

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today are concerned with characterizing impairments as well as spared memory abilities following lesions restricted to specific temporal-lobe structures.

Recent evidence of double-dissociations on tests of object-recognition and place-memory suggests that some components of the putative 'temporal-lobe memory system' are specialized to deal with specific kinds of information about the environment [1,15]. Neither rats [7,9,21,22,26] nor monkeys [23] with hippocampal lesions were impaired on an object-recognition task when their lesions did not also include the rhinal cortex (entorhinal and perirhinal cortices). Lesions of the perirhinal cortex that spared the hippocampal formation produced object-recognition deficits both in rats [1,7,19] and monkeys [14,39]. The opposite pattern of results has been observed on tests of place-memory, including various water-maze tasks [4,17,30]. Hippocampal lesions in rats impair performance on such tasks (see ref. [2] for a review), whereas perirhinal cortex lesions do not [7,8,11,33,34]. Thus, at least with respect to the formation of new long-term memories, the hippocampal formation seems to play a more important role in memory for places than for objects, and the opposite appears to be true for perirhinal cortex.

One of the goals of the present experiment was to determine whether a dissociation involving impaired place-memory and spared object-memory occurs in the retrograde direction following damage restricted to the hippocampal formation. The answer has important theoretical implications. For instance, it is frequently asked whether anterograde amnesia and retrograde amnesia reflect disruption of the same memory processes or different processes [25]. If anterograde and retrograde amnesia reflect the same underlying functional impairment, amnesic subjects should display anterograde and retrograde amnesia for the same kinds of information. To the extent that hippocampal lesions produce anterograde amnesia for information about places but not for information about objects, the same dissociation should also be observed in retrograde memory.

Accordingly, the present experiment examined retrograde memory for places and object discriminations in rats with ibotenate lesions of the hippocampal formation. Rats learned five simple object-discrimination problems and two fixed-platform water-maze problems, each at a different time-point prior to surgery, and we assessed their retention of those problems following surgery. We examined anterograde memory in the same rats by assessing their ability to learn new object-discrimination and place-memory problems following surgery.

Retrograde amnesia caused by temporal-lobe damage in humans is sometimes extensive and without a temporal gradient [3]. It has been proposed that the presence or slope of a temporal gradient in retrograde amnesia depends upon the kind of information the subject is asked to remember [13]. The present experiment was designed with the potential to provide evidence bearing on this hypothesis because each subjects' memory was probed for both place information and object information that was acquired over a similar range of intervals prior to surgery. Thus, if rats with hippocampal formation lesions displayed retrograde amnesia for both kinds of information, the slope of the corresponding temporal gradients potentially could be compared.

Monkeys with large lesions of the medial temporal lobes have displayed both temporally-graded [37] and ungraded [27] retrograde amnesia for object discriminations that were learned between 2 and 16 weeks prior to surgery. In both studies the lesions were made by aspiration and included most of the hippocampal formation, but also the rhinal cortices and parahippocampal gyrus, the temporal stem and other white matter, and in the study by Salmon et al. [27], the amygdala. It is, therefore, unclear whether damage to the hippocampal formation contributed to the monkeys' impairments.

There have been reports of temporally-graded retrograde amnesia [29], of ungraded retrograde amnesia [4], and of no retrograde amnesia [18] for water-maze place-memory tasks in rats with hippocampal lesions. The colchicine lesions in the study by Sutherland et al. [29] selectively damaged the dentate gyrus, whereas the ibotenate lesions in the study by Bolhius et al. [4] included the dentate gyrus and cornu Ammonis. It may be that the retrograde amnesia in the former study was temporally graded because there was less damage to the hippocampal formation than in the latter study. Support for this hypothesis comes from reports of temporally-graded retrograde amnesia of fear conditioning [10] and food preference [35] in rats with lesions of the dorsal hippocampal formation that included dentate gyrus and CA cell-fields but spared much of the caudoventral portions of the hippocampal formation. In the present experiment, we used ibotenic acid to make relatively complete and selective lesions of the hippocampal formation (cornu Ammonis, dentate gyrus, and subiculum).

2. Materials and method

2.1. Subjects

The subjects were 16 experimentally naive, male, Long–Evans rats (Harlan Breeding Farms, St. Loius, MO) that were between 8 and 10 weeks old at the beginning of the experiment. They were housed individually with continuous access to water under a 12:12 light–dark cycle, with light onset at 07:00 h. Their body weights were reduced to approx. 85% of presurgery

levels by giving them daily rations of rat chow. They received approx. 25 g of rat chow per day throughout the remainder of the experiment, which allowed them to gradually gain weight while ensuring that they were sufficiently motivated to work for food reward on the discrimination task. Training began after the rats had been on the restricted feeding regimen for 14 days.

2.2. Apparatus

Three different rooms were used for the place-memory testing. The same water maze [16] was used in each room. It was approx. 150 cm in diameter and filled with water to a depth of approximately 22 cm. The water (22°C) was made opaque by adding instant skim milk powder. A movable Plexiglas platform (20.5 cm high × 13 cm^2) extended from the floor of the pool to approx. 1.5 cm below the surface of the water. There were no visible cues within the pool that the rats could use to locate the hidden platform; thus, they were required to learn the location of the platform relative to distal room cues.

The apparatus for object-discrimination training has been described in detail elsewhere [20]. Briefly, it consisted of an elevated runway, which was separated from identical goal areas at each end by opaque guillotine doors. Each goal area contained two food wells into which food pellets (45 mg Bio-Serv, Inc., Frenchtown, NJ) could be delivered by hand through plastic tubes that were mounted on the outside of the apparatus. A short divider wall protruded from the center of the end wall to separate the two food wells.

The test stimuli for the object-discrimination problems were 14 objects of various shapes, sizes, textures, and colors. Each object was large enough to cover a food well but small enough and light enough to be easily displaced by the rats. No objects with obvious scents were included. The objects were washed with water after every two sessions and with a solution of diluted chlorine bleach at the end of each day to remove any extraneous scents that they might have acquired during displacement by the rats or handling by the experimenter.

2.3. Procedure

All testing occurred during the light phase of the light–dark cycle, between 14 and 24 h after the rats' most recent meal. The time-line in Fig. 1 shows the general design of the experiment. The rats were trained on two place problems and five object-discrimination problems at different time points prior to surgery. Places # 1 and 2 were learned during the 14th week prior to surgery and the 2nd week prior to surgery, respectively. Object-discriminations # 1, 2, 3, 4, and 5 were learned during the 13th, 10th, 7th, 4th, and 1st week prior to surgery, respectively. After surgery, the rats were tested for retention and reacquisition of the presurgery problems and for acquisition of two new object-discrimination problems (object-discriminations # 6 and 7) and a new place problem (Place # 3). To counterbalance certain training and testing conditions, the rats were divided into two squads; behavioral training was identical for all of the cohorts within each squad.

2.3.1. Presurgery place-memory training

The procedures for Place problems # 1 and 2 were identical, but they were conducted in different rooms (room A and room B); thus, the two problems required the rats to learn about different sets of spatial cues. For one of the squads of rats, Place problem # 1 was conducted in room A and Place problem # 2 was conducted in room B; for the other squad of rats, the reverse was true.

Each rat received eight trials on each of five daily sessions, for a total of 40 trials. On each trial, the rat was placed into the edge of the pool, facing the wall, at one of the four cardinal compass points, N, E, S, W. Each of the four starting positions was used twice per session in a pseudorandom sequence, which was the same for all rats. The platform was located in the center of the NE quadrant on every trial. A trial continued until the rat climbed onto the platform or until 60 s had elapsed. The rat was left on the platform for 10 s; if it failed to find the platform within the 60-s maximum, it

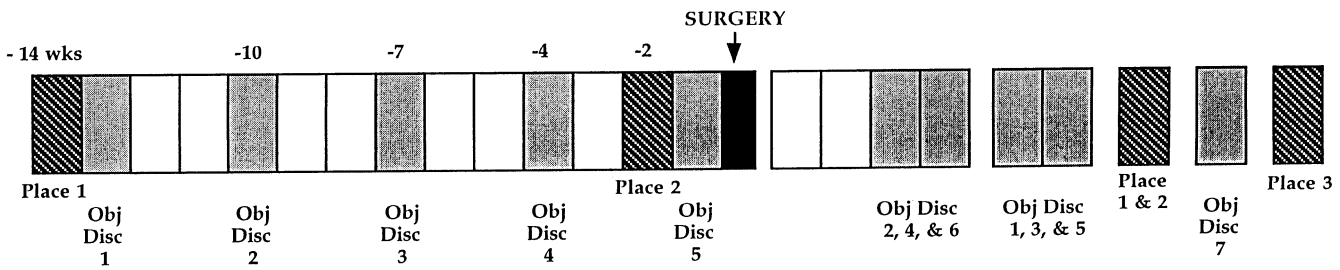

Fig. 1. A time-line showing the various phases of the experiment, their durations, and the intervals between them. Each rectangle represents one week, except for the black rectangle, which denotes when surgery occurred and represents only 4 days. The spaces between postsurgery testing phases denote that 2–4 days intervened between the end of one phase and the beginning of the next for some rats. Each rat received surgery between 24 and 72 h following their final presurgery session of object discrimination # 5.

was placed onto the platform and left there for 10 s. The dependent measure was the latency to find the platform.

A probe trial was conducted on trial 38 (i.e. the third-to-last trial of the fifth session). The platform was removed from the pool and the rat placed into the pool at the South starting position and allowed to swim for 20 s. The dependent measure was the proportion of the swim path that was in the quadrant of the pool that had previously contained the platform.

2.3.2. Presurgery object-discrimination training

The rats were habituated to the apparatus and shaped to retrieve food pellets from the food wells [20]. Fourteen objects were divided into seven pairs, which served as the discriminanda for object-discrimination problems #1–7. One of the objects in each pair was designated S+ (rewarded) and the other one was designated S− (not rewarded). For each problem, one of the objects was S+ for one squad of rats, and the other object was S+ for the other squad.

To begin a session, the rat was placed into the center of the apparatus and allowed to explore for approx. 1 min. To begin the first trial, one of the guillotine doors was closed, and the experimenter positioned S+ and S− over the food wells on the other side of the door from the rat. The experimenter opened the door, and the rat approached and displaced one of the objects. If it displaced S+, a food pellet was delivered to that food well; if it displaced S−, no food pellet was delivered. A rat was considered to have made a choice if the object was displaced enough to expose the food well. The experimenter then closed the far door and positioned S+ and S− over the food wells on the other side of it, in preparation for the next trial. The intertrial interval was approx. 15 s. The rats were allowed to correct their errors on the first session of object-discrimination #1, but not thereafter—if the rat displaced S− on these initial correction trials it was allowed to then displace S+ to obtain a reward before the experimenter removed the objects. There were 20 trials per session, and the location of S+ (i.e. left or right well) varied pseudorandomly across trials. Training on each of the five presurgery object discriminations continued for a rat until it reached a criterion of at least 17 correct trials out of 20 (i.e. 85%) during a single session; however, each rat received a minimum of three sessions (i.e. 60 trials) and a maximum of seven sessions (i.e. 140 trials) per problem.

2.3.3. Surgery

Surgery was performed under pentobarbitol anesthesia (65 mg/kg), between 48 and 72 h after a rat's last presurgery object-discrimination session. Rats in group HPC ($n = 7$; three rats from one squad and four from the other squad) received intrahippocampal injections of ibotenic acid (Sigma, St. Louis, MO) at 10 sites bilaterally (Table 1 shows the coordinates), using a concentration of 5 μg/μl, and a flow rate of 0.1μl/min over 2.5 min, for total injection volume of 0.25 μl per site. The cannula was left in place for 2.5 min after each injection. Rats in group SHAM ($n = 9$; 5 rats from one squad and 4 from the other squad) received sham lesions—their scalp was incised and sutured, but they sustained no damage to the skull or brain. Immediately after surgery, each rat received diazepam (approx. 2 mg, i.m.; Hoffmann-La Roche, Mississauga, Ont.) as a prophylaxis against seizures, and antibiotic (penicillin G, 15 000 units, i.m.; G.C. Hanford Co., Syracuse, NY). They were allowed to recover for two weeks before behavioral testing recommenced. The experimenters who collected the behavioral data were blind to the group assignment of individual rats.

Table 1
Cannulae coordinates relative to bregma (in mm) for ibotenate lesions of the hippocampal formation

Anteroposterior (AP)	Mediolateral (ML)	Dorsoventral (DV)
−3.1	±1.0	3.6
−3.1	±2.0	3.6
−4.1	±2.0	4.0
−4.1	±3.5	4.0
−5.0	±3.0	4.1
−5.0	±5.2	5.0
−5.0	±5.2	7.3
−5.8	±4.4	4.4
−5.8	±5.1	6.2
−5.8	±5.1	7.5

2.3.4. Postsurgery testing

There were five phases of postsurgery testing (see Fig. 1). All 16 rats were tested each day, and each successive phase of testing began on the day after the previous phase had been completed by all rats. During the first postsurgery phase, the rats were tested concurrently on presurgery object-discriminations #2 and 4, and on the new object-discrimination #6. During the second phase, they were tested on the two presurgery place problems (Places #1 and 2). During the third phase, they were tested concurrently on presurgery object-discriminations #1, 3, and 5. During the fourth phase, they were trained on the new object-discrimination problem #7. During the fifth phase, they were trained on a new place problem—Place #3.

During the first test phase each session consisted of three blocks of five object-discrimination trials, each block of trials comprising a different object-discrimination problem (#2, 4, or 6). Order of presentation varied in a balanced fashion across sessions, and different orders on each session were also counterbalanced among the subjects in each group. Trial blocks were

separated by a 30-s interval. Training continued for each rat until it reached the criterion of at least 17 correct trials out of 20 over four consecutive sessions on all three problems. All three problems continued to be administered each day, even if a rat reached the criterion on one or two of them.

During the second phase of testing, the rats were retested on the two place-memory problems that they had learned prior to surgery. General procedures were identical to those used for presurgery training. Each rat received 2 consecutive days of testing on each problem. The order of retraining on the two problems was counterbalanced across groups; that is, approximately half the rats in each group were first retrained on Place #1 and then on Place #2, and for the remaining rats the order was reversed. Two probe trials with the platform removed were conducted for each place problem—an 'early' probe, on the fourth trial of the first day (i.e. trial 4) and a 'late' probe, on the final trial of the second day (i.e. trial 16).

The third phase of testing followed the same general procedures as the first phase, except that the rats were now retested concurrently on presurgery object-discrimination problems #1, 3, and 5. All three problems continued to be administered each day, even if a rat reached the criterion on one or two of them.

During the fourth phase of testing, the rats were trained on the new object-discrimination #7. This allowed us to assess their ability to solve a new object-discrimination problem without interference from the concurrent performance of other problems, such as during the first phase of testing when they were trained on the new object-discrimination #6. Procedures were identical to those for presurgery training. Rats were trained until they reached the criterion of at least 17 correct trials out of 20.

During the fifth phase of testing, the rats were trained on a new place problem (i.e. Place #3). General procedures were similar to those for previous place problems, but the water maze was located in a new room (Room C). Each rat received eight trials per day for 2 days, and a probe trial with the platform removed was conducted on the final trial of the second day (i.e. trial 16).

We administered two subsets of presurgery object-discrimination problems separately in phases 1 and 3 because we were concerned the lesions might produce transient performance effects, such as positional biases (e.g. always choosing the object on the left) or a tendency for hasty responses. Such effects could obscure retention and be mistaken for retrograde memory deficits. We reasoned that the rats would have to overcome any such performance effects to remaster the first subset of problems, and therefore, subsequent performance on the second subset of problems would be easier to interpret.

A new object-discrimination problem (#6) was included with the first subset of presurgery problems so the rate at which rats solved the new problem could serve as a baseline against which to compare the rate at which they remastered the presurgery problems, thus teasing apart the anterograde and retrograde effects. If a rat retained any information unique to a particular presurgery problem it should require fewer trials to remaster that problem than to master the new problem. This assumes that normal rats would learn the three problems at similar rates; indeed, there were no significant differences in number of trials to criterion by SHAM rats during original acquisition of discriminations #2, 4, or 6 (Figs. 3 and 6). During the first testing phase all of the rats remastered the presurgery problems much faster than they mastered the new problem, indicating some retention of the presurgery problems. We did not, therefore, include another new problem during the third phase of postsurgery testing.

2.4. Histological procedures

At the completion of behavioral testing all rats were sacrificed using a lethal dose of sodium pentobarbitol (100 mg/kg, ip). They were perfused with 0.1 M phosphate-buffered saline solution followed by 10% formalin in 0.1 M phosphate-buffered saline solution. Their brains were excised and stored in 10% formalin/30% sucrose in 0.1 M phosphate-buffered saline solution until sectioning. The brains were frozen-sectioned at 40 μ, every fifth section through the hippocampal formation was mounted on glass microscope slides, and stained with cresyl violet for microscopic examination.

3. Results

3.1. Histology

Fig. 2 shows the extent of a representative hippocampal lesion. The ibotenic acid injections produced extensive loss of cells in all principle subfields of the hippocampus and dentate gyrus. The fimbria/fornix was largely spared in each rat, and in each rat there was also some minor sparing of dentate granule cells and CA1 pyramidal neurons in the most temporal portions of the hippocampal formation. The extent of damage to the subiculum was variable, but there was some bilateral loss of subicular cells in all rats, which was incomplete in every case. There was no evidence of damage to the thalamus or rhinal cortex in any of the rats with hippocampal lesions. There was, however, some thinning of parietal cortex near the sites where the injection cannulae were inserted.

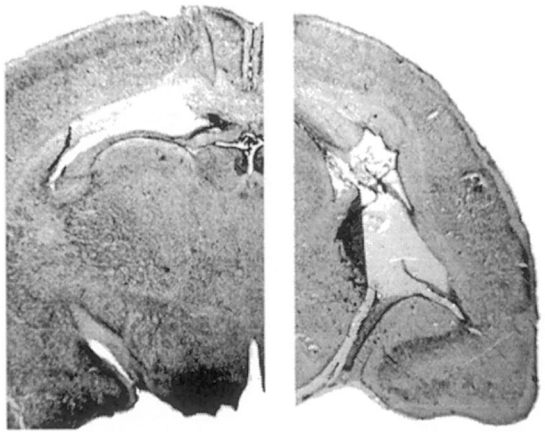

Fig. 2. Coronal sections through planes near the anterior extent (left) and posterior extent (right) of a representative hippocampal lesion. Both slices are from the same rat. Slice thickness = 30 μm, stained with cresyl violet.

3.2. Presurgery training

The rats learned the two presurgery place problems to a similar level of proficiency. The mean proportion of the swim paths that were in the correct quadrant during the probe trial (i.e. trial 38) was 51.5% (SE = 4.23) for Place # 1 and 44.12% (SE = 2.61) for Place # 2. The mean escape latency on the final 3 trials with the platform in place (i.e. trials 37, 39 and 40) was 5.54 seconds (SE = 0.66) for Place # 1 and 5.05 s (SE = 0.48) for Place # 2. There were no significant differences between the groups or between the two place problems.

The groups were also well-matched in acquisition of the five presurgery object-discrimination problems (# 1–5). The mean number of trials to criterion on each of these problems are shown in Fig. 3; the trials of the criterion session were not included in the calculation of this measure. There were nonsignificant effects

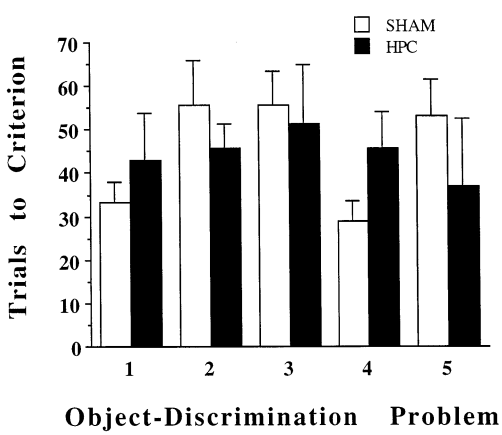

Fig. 3. Mean number of trials to criterion during presurgery acquisition of object-discrimination problems # 1–5. Error bars show standard mean errors.

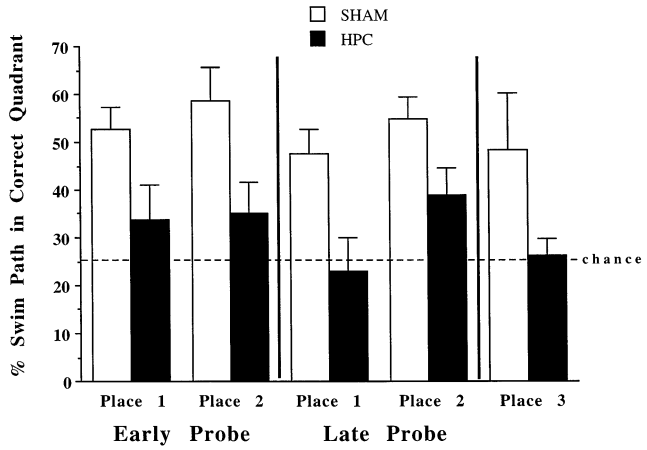

Fig. 4. Mean percentage of swim path that was in the correct pool quadrant on each of the five 20-s probe trials that were conducted following surgery. Rats had been trained on Place # 1 and Place # 2 prior to surgery; Place # 3 was a new problem that was administered only after surgery. The early probes for Place # 1 and Place # 2 were conducted on the fourth postsurgery trial; the late probes were conducted on the sixteenth postsurgery trial. There was only one probe trial for Place # 3, and it was conducted on the sixteenth trial. The chance-level of performance is 25%. Error bars show standard mean errors.

of Problem ($F[4,56] = 1.49$, $P > 0.20$), Group ($F[1,14] < 1$), and a nonsignificant Group × Problem interaction ($F[4, 56] = 1.31$, $P > 0.20$).

3.3. Postsurgery training and testing

3.3.1. Place memory problems

Fig. 4 shows the mean percentage of the swim path that was in the correct quadrant on the postsurgery probe trials. A repeated measures analysis of variance (ANOVA) was performed on the probe trial data for Place # 1 and Place # 2, with Group as a between-subjects factor and with place Problem and Probe (early versus late) as within-subjects factors. There was a significant Group effect ($F[1,14] = 13.52$, $P < 0.003$), but none of the other main effects or interactions were statistically significant (all Ps > 0.05). Thus, the general results were the same for Place # 1 and Place # 2.

Comparing early probes with late probes, it appears that the SHAM group had already reached asymptotic performance by the early probe trial (i.e., after only 3 postsurgery trials). The HPC rats were impaired on the early probes and did not improve over the course of postsurgery training, thus they were still impaired on the late probes. The HPC rats were also impaired in acquisition of the new place problem, Place # 3. They displayed no preference for the correct quadrant on the single probe trial of that problem ($t[14] = 4.55$, $P < 0.001$).

It appeared that the first postsurgery probe trial was conducted early enough during retraining of Place # 1

and Place #2 to provide a valid index of the rats' retention of those problems. Indeed, the asymptotic performance by the SHAM rats on the early probe is more consistent with reactivation of a previously formed representation than with new learning. Still, because there were three postsurgery trials prior to this probe, it is possible that anterograde deficits contributed to the differences between the lesioned rats and SHAM rats. A more 'pure' measure of retrograde memory is performance on the first postsurgery trial.

Fig. 5 shows the mean latencies to find the platform on the first postsurgery trial on each of the three Place problems. A repeated measures ANOVA revealed a significant effect of Group, $F(1,14) = 4.77$, $P < 0.05$; the main effect of Problem and the Group × Problem interaction were not statistically significant. There was a significant difference between the groups on the first postsurgery trial of Place #2 ($t[16] = 2.279$, $P < 0.02$), but not of Place #1 or Place #3 (both Ps > 0.05).

The first time that any of the rats were in room C was on the first trial of Place #3. Thus, their performance on that trial should reflect a lack of knowledge of relevant place information. It follows from this assumption that any retention of the presurgery Place problems should result in shorter latencies on the first postsurgery trial of those problems than on the first trial of Place #3. Within-group comparisons of first-trial latencies on the three Place problems revealed only one significant difference: SHAM rats had significantly shorter latencies on the first trial of Place #2 than on the first trial of Place #1, $t(9) = 2.365$, $P < 0.05$.

3.3.2. Object-discrimination problems

Fig. 6 shows the mean number of postsurgery trials that were required to reach the criterion of 85% correct over 20 consecutive trials on each of the object-discrimination problems. A repeated measures ANOVA on data from the five problems that were learned prior to surgery (#1–5) revealed no significant differences between the groups, $F(1,14) < 1$. There was a significant main effect of Problem ($F[4, 56] = 19.69$, $P < 0.0001$) and a Group × Problem interaction ($F[4, 104] = 2.83$, $P = 0.03$).

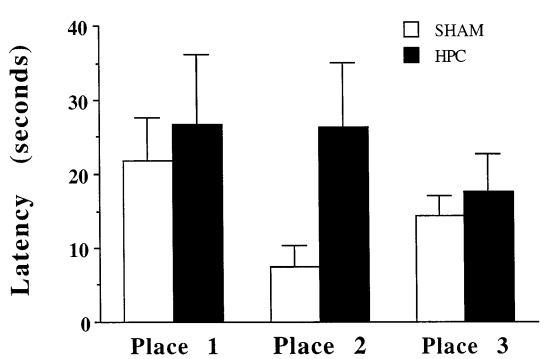

Fig. 5. Mean latency to find the hidden platform on the first postsurgery trial of three place-memory problems. Rats had been trained on Place #1 and Place #2 before surgery; Place #3 was a new problem that was administered only after surgery. Error bars show standard mean errors.

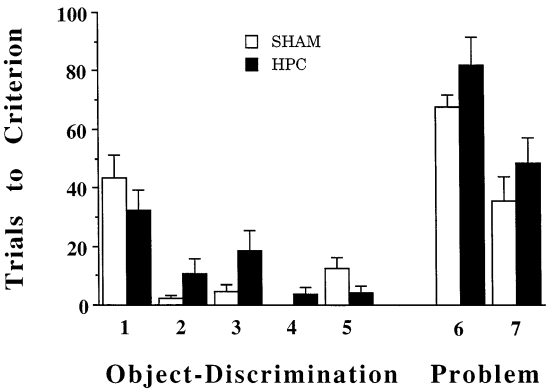

Fig. 6. Mean number of postsurgery trials required to reach the criterion of at least 85% correct over 20 consecutive trials on each object-discrimination problem. Problems #1–5 were originally learned before surgery, whereas problems #6 and 7 were learned after surgery. Error bars show standard mean errors.

The significant effect of Problem appears to be almost entirely due to poor performance by both groups on discrimination problem #1. Overall, the postsurgery scores on discrimination #1 were not significantly different from the presurgery scores obtained on that problem during original training, $t(16) = 0.339$, $P < 0.25$, nor were they significantly different from the scores on the new discrimination problem #7, $t(16) = 0.122$, $P < 0.25$. In contrast, both groups required significantly fewer postsurgery trials to reach criterion on discrimination problems #2, 3, 4, and 5 than they had required during original presurgery training (all Ps < 0.05), and significantly fewer postsurgery trials to criterion on each of these problems than on problem #7 (all Ps < 0.05). These results suggest that most rats showed little or no retention of discrimination problem #1, but good retention of the remaining discrimination problems.

An ANOVA performed on the data from the two new discrimination problems (#6 and #7), with Group as a between-subjects factor and Problem as a within-subjects factor, revealed a significant Problem effect, $F(1,14) = 22.85$, $P < 0.0005$, but a nonsignificant Group effect, $F(1,14) < 1$, and Group × Problem interaction, $F(1,14) < 1$. The number of trials required to reach criterion on discrimination problem #7 was within the range required to reach criterion on the five presurgery problems during original presurgery training (compare Figs. 3 and 6). This contrasts with the observation that in both groups the number of trials to reach criterion on discrimination problem #6 was considerably above this range. Presumably, the concurrent reacquisition of two presurgery problems interfered with

acquisition of problem #6. To the extent that this kind of interference actually occurred, it appeared to affect both groups approximately equally.

In sum, the trials-to-criterion data indicate that: (a) the rats displayed significant retention of discrimination problems #2, 3, 4, and 5, but little or no retention of problem #1, (b) hippocampal lesions did not significantly affect either reacquisition of the problems that had been learned prior to surgery or acquisition of the postsurgery discrimination problems #6 and 7, and (c) lesioned and control rats were not differentially affected by interference effects in acquisition of discrimination problem #6.

The trials-to-criterion measure reflects performance over several trials, and therefore, the absence of an anterograde deficit in the lesioned rats might have obscured the presence of a retention deficit. An alternative index of retention can be obtained by looking at performance on only the first few postsurgery trials of the problems that were learned prior to surgery.

Fig. 7 shows the mean number of correct trials out of the first block of five postsurgery trials for each object-discrimination problem. Mean scores on each of the new discrimination problems (#6 and 7) were not significantly different than chance for either group (one-sample t-tests, $P > 0.05$, two-tailed), which implies that five trials is not sufficient for new learning to become evident in performance of an object-discrimination task. This in turn suggests that complete forgetting of a presurgery discrimination problem should be reflected in a score on the first five postsurgery trials that is no better than chance, and that retention of a presurgery discrimination problem should be reflected in scores that are higher than chance. An ANOVA performed on scores from the five presurgery problems, with scores on each problem as a within-subjects factor, revealed a significant Problem effect ($F[4,56] = 21.45$, $P < 0.0001$), a nonsignificant Group effect ($F[1,14] < 1$) and a nonsignificant Group × Problem interaction ($F[4,56] = 1.678$, $P > 0.15$). Scores on problem #1 did not differ significantly from chance levels, which suggests that most rats did not retain this problem. In contrast, scores on problems #2, 3, 4, and 5 were significantly better than chance (one-sample t-tests, all Ps < 0.05, two-tailed), which suggests that most rats retained some information relevant to the accurate performance of each of these problems.

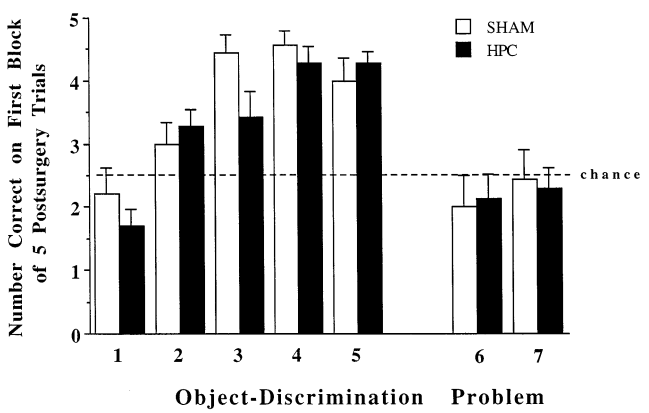

Fig. 7. Mean number of correct trials on the first block of five postsurgery trials on each object-discrimination problem. Problems #1–5 were originally learned before surgery, whereas problems #6 and 7 were learned after surgery. The chance-level of performance is 2.5 correct trials. Error bars show standard mean errors.

4. Discussion

Rats with hippocampal lesions displayed both retrograde and anterograde amnesia on the place-memory task, but there was no evidence of a temporal gradient to their retrograde deficits. Relative to control rats, the rats with hippocampal lesions displayed impaired retention of Place #2, which was learned 2 weeks before surgery, as they had longer latencies to find the platform on the first postsurgery trial and they swam less in the correct quadrant on the early probe trial (trial 4). The hippocampal rats also displayed impaired retention of Place #1, which was learned 14 weeks before surgery, as they swam less in the correct quadrant during the early probe trial. The presence of an anterograde deficit in hippocampal rats was evident from their weaker preferences for the correct quadrant during the probe trial of Place #3, which was conducted on trial 16.

In contrast to their place-memory deficits, the rats with hippocampal lesions displayed no evidence of either anterograde or retrograde amnesia for object discriminations. Hippocampal rats did not differ significantly from controls in either (a) the rate at which they reacquired the discrimination problems that they had learned between 1 week and 13 weeks prior to surgery, (b) their performance accuracy on the first five postsurgery trials of those problems, or (c) the rates at which they learned two new object-discrimination problems.

It has been proposed that retrograde amnesia is temporally graded only when normal subjects display some degree of forgetting over the interval range in which the temporal gradient is observed [27,28]. It should be noted that our SHAM lesioned rats displayed forgetting over the 14-week period of presurgery training on both the place-memory problems and the object-discrimination problems (see Figs. 4 and 6). Our results, therefore, did not reveal any obvious relation between the presence of forgetting in normal subjects and the presence of temporal gradients in retrograde amnesia.

One of the purposes of the present experiment was to examine whether the slope of the temporal gradient in retrograde amnesia depends on the type of information

for which memory is probed. We observed retrograde amnesia on only one of our memory tasks, and therefore, we cannot make inferences about the effects of information type on temporal gradients of retrograde amnesia. Instead, the results are more relevant to the questions about the types of information that are dealt with by a hippocampus-dependent memory system.

The results are consistent with the view that the hippocampal formation is critically involved in encoding and storage of information about places, but we found no evidence to suggest that its role is only temporary. It is important to note, also, that alternative interpretations exist for our finding of temporally-ungraded retrograde effects in the water maze. For instance, hippocampal lesions may impair path integration [31] or some other instinctive behaviour normally required for successful water maze performance. Such an interpretation is just as consistent with our results as the suggestion that the hippocampus is responsible for recent and remote spatial memory in the water maze.

The results also suggest that the hippocampal formation does not a make a critical contribution to encoding, storage, or retrieval of information necessary for accurate performance of an object-discrimination task. If the hippocampal formation plays a critical role in consolidating a representation of this information, such involvement would have a duration shorter than the 3 to 4 days that intervened between acquisition of the final presurgery object discrimination problem and surgery. (Although the rats received surgery between 24 and 72 h after their final session on object-discrimination # 5, the actual interval between original learning and surgery is presumed be somewhat longer because at least some, if not most, of the learning would have occurred during the initial training sessions).

The HPC lesions may have been ineffective because the hippocampal formation had already ceased to be involved in consolidation of relevant information. If this assumption is correct, our observation of a retrograde memory impairment on the place-memory tasks that were learned between approximately 1 and 14 weeks prior to surgery would suggest that the duration of the role of the hippocampal formation in memory consolidation depends on what type of information is involved. At the very least, it would suggest that this duration is quite different for information about places and information about object discriminations, and it would predict that a temporally-graded retrograde amnesia for object discriminations might be observed if the intervals between learning and surgery were shorter than those employed in the present experiment. However, a recent experiment found no evidence of retrograde amnesia for object-discrimination problems learned between 72 h and 2 h prior to bilateral hippocampal lesions [6].

The lack of either anterograde or retrograde amnesia for object discriminations in our HPC rats is inconsistent with two reports in monkeys with hippocampal formation lesions [27,37]. In both of those studies, the monkeys displayed retrograde amnesia for object-discrimination problems that were learned between 2 and 16 weeks prior to surgery. In the study by Zola-Morgan and Squire [37], the retrograde amnesia had a temporal gradient, whereas in the study by Salmon et al. [27] it did not. The monkeys in the Salmon et al. study were also impaired in learning new object-discrimination problems; anterograde object-discrimination learning was not assessed in the Zola-Morgan and Squire study, but deficits were reported earlier in a study from the same laboratory [38]. It is not clear how much, if anything, the hippocampal damage contributed to the object-discrimination deficits that were observed in the previous monkey studies because the lesions also included the entorhinal cortex, parahippocampal gyrus, inferotemporal cortex, various white matter, and in one study [27], the perirhinal cortex and amygdala. Extrahippocampal damage might have contributed to the retrograde deficits, the anterograde deficits, or both. The only obvious extrahippocampal damage sustained by our rats was in posterior partietal cortex—cortical areas homologous to primate medial-temporal cortex were spared. Differences in extrahippocampal damage may account for different findings in monkeys and rats with hippocampal lesions on tests of retrograde memory for object discriminations. It is also possible that the parietal cortex damage sustained by our hippocampal rats contributed to their deficits on place-memory problems.

There has been at least one report of impaired acquisition of an object-discrimination task in rats with bilateral lesions of the hippocampal formation [21], which is consistent with some of the findings in monkeys with large medial temporal lesions but inconsistent with the present findings in rats with neurotoxic lesions. The hippocampal lesions in the former study included damage to the posterior parietal cortex and corpus callosum overlying the dorsal hippocampal formation and to the fibres comprising the alveus, whereas these structures were largely spared by the present ibotenic acid lesions. The extrahippocampal damage in the earlier study might have contributed to the object-discrimination deficits, either alone or in a synergistic interaction with the hippocampal damage. Normal object-discrimination performance has been reported in rats with electrolytic hippocampal lesions [32] and in rats with ischemia-induced hippocampal lesions [36], but in both studies the amount of extrahippocampal damage was considerably less than in the those studies that found object-discrimination deficits [21,27] [38]. Moreover, unlike the impaired rats in the study by Mumby et al. [21], the unimpaired rats in the present

experiment and in previous studies [32,36] received object-discrimination training prior to surgery. It is difficult to interpret acquisition deficits displayed by subjects that received no training prior to surgery because they could reflect impairments in learning procedural aspects of the task rather than impairment of encoding and retention of associations that underlie accurate performance.

The extent of hippocampal involvement in memory for object discriminations may depend on the circumstances under which the discriminations are learned. The rats in the present experiment learned only a single discrimination problem at a time, whereas the monkeys in previous studies learned many problems concurrently at each presurgery time interval [27,37]. In contrast to the evidence that restricted hippocampal damage does not disrupt acquisition of a single object-discrimination problems [24], rats with hippocampal lesions are impaired in acquisition of multiple concurrent object discriminations [21,32].

It has been argued that in order for a demonstration of temporally-graded retrograde amnesia in nonhuman animals to have external validity as a model of clinical phenomena the subjects must display retention of remotely acquired information that is actually *superior* to that for recently acquired information [28]. Our results did not fit that pattern. Moreover, it is unlikely that a temporal gradient would be observed in retrograde amnesia for place-memory problems that were acquired over a different range of intervals prior to surgery than the 1-week to 14-week range that we used, because rats with hippocampal lesions were similarly impaired in retention of a fixed-platform water-maze problem, whether the single acquisition session was 72 h or 2–3 h prior to surgery [6].

In sum, the present findings suggest that the hippocampal formation does not have a short-term role in place-memory, but rather one that lasts for at least 14 weeks, and probably for as long as such memories exist. The findings also suggest that the hippocampal formation does not play a critical role at any time in learning or memory of object discriminations. The dissociation of spared object-discrimination memory and impaired place memory cannot be attributed to differences in the extent of the lesions, because each dissociation occurred *within* subjects. The observation of parallel dissociations in both anterograde and retrograde memory is consistent with the hypothesis that a common functional impairment underlies anterograde and retrograde amnesia following damage to the hippocampal formation.

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