Spatial memory, recognition memory, and the hippocampus

Nicola J. Broadbent*, Larry R. Squire*†‡§, and Robert E. Clark*§¶

*Veterans Affairs Medical Center, San Diego, CA 92161; and Departments of †Psychiatry, ‡Neurosciences, and §Psychology, University of California at San Diego, La Jolla, CA 92093

Contributed by Larry R. Squire, August 27, 2004

There is wide agreement that spatial memory is dependent on the integrity of the hippocampus, but the importance of the hippocampus for nonspatial tasks, including tasks of object recognition memory is not as clear. We examined the relationship between hippocampal lesion size and both spatial memory and object recognition memory in rats. Spatial memory was impaired after bilateral dorsal hippocampal lesions that encompassed 30–50% total volume, and as lesion size increased from 50% to ~100% of total hippocampal volume, performance was similarly impaired. In contrast, object recognition was intact after dorsal hippocampal lesions that damaged 50–75% of total hippocampal volume and was impaired only after larger lesions that encompassed 75–100% of hippocampal volume. Last, ventral hippocampal lesions that encompassed ~50% of total hippocampal volume impaired spatial memory but did not affect object recognition memory. These findings show that the hippocampus is important for both spatial memory and recognition memory. However, spatial memory performance requires more hippocampal tissue than does recognition memory.

The hippocampal region (the CA fields, dentate gyrus, and subicular complex) is part of a system of anatomically related structures in the medial temporal lobe that are important for mammalian memory (1). In humans, monkeys, and rodents, damage to this region impairs performance on a variety of tasks of learning and memory (2). Further, single-cell recording and neuroimaging techniques document changes in the hippocampal region during both learning and retention (3–5).

The hippocampal region (referred to throughout this paper as the “hippocampus”) is the final stage of convergence within the medial temporal lobe, receiving projections from the adjacent perirhinal and parahippocampal/posi10 rithral cortices, as well as the entorhinal cortex (6). Accordingly, there has been interest in the possibility that the hippocampus may be especially important for tasks that depend on relating or combining information from multiple sources, as in certain spatial memory tasks (7). A related idea is that tasks that do not have such requirements, such as tasks of recognition memory for single items, may be supported by cortex adjacent to the hippocampus (8, 9).

Efforts to test these ideas have led to mixed results. On the one hand, selective hippocampal lesions have been reported to impair recognition memory performance in humans (10), monkeys (11–13), and rodents (14–17). Yet, it also has been reported that recognition performance is largely spared by hippocampal lesions (18–21).

In contrast to these conflicting reports, the data are unequivocal that hippocampal damage severely impairs spatial memory. Thus, in the rodent, dorsal hippocampal lesions involving as little as 40% of total hippocampal volume markedly impaired learning in the water maze (22, 23). In another study, small dorsal hippocampal lesions that impaired spatial memory entirely spared recognition memory (24). Such a finding could mean that spatial memory has special status with respect to hippocampal function, and that recognition memory, in at least some circumstances, is independent of the hippocampus. However, an alternative possibility is that the hippocampus is important for both spatial and recognition memory and that the two kinds of memory tasks (as typically administered in the laboratory) differ in how much hippocampal tissue is needed to support performance.

According to this latter idea, the relationship between hippocampal lesion size and task performance is different for spatial memory and recognition memory. Thus, larger hippocampal lesions may be needed to impair recognition memory than are needed to impair spatial memory. The present study tested this prediction. Experiment 1 assessed the effects on spatial memory of hippocampal lesions that varied in size. Experiment 2 compared spatial memory performance and recognition memory performance in the same animals after hippocampal lesions that varied in size.

Experimental Procedures

Details of experimental methods may be found in Supporting Text, which is published as supporting information on the PNAS web site. In experiment 1, 92 Long–Evans male rats received either sham lesions (n = 36) or bilateral ibotenic acid lesions of the hippocampus that varied in septotemporal extent (5–30%, n = 5; 30–50%, n = 24; 50–75%, n = 11; 75–100%, n = 16; all lesions began in the anterior dorsal hippocampus). Spatial memory training was conducted in a standard water maze with a retractable (“Atlantis”) platform. Training consisted of one session daily for 5 consecutive days. Each session began with a single 60-sec reinforced probe trial followed by four training trials. After escaping to the platform, rats remained in place for 30 sec before being removed. Two days after completion of spatial training, rats were trained to escape to a visible platform (two sessions).

Experiment 2 tested 56 Long–Evans male rats that had received sham lesions (n = 30), bilateral ibotenic acid lesions that encompassed 50–75% or 75–100% of total hippocampal volume (with spared tissue located in the ventral hippocampus), or bilateral ibotenic acid lesions of the ventral hippocampus (encompassing ~50% of total hippocampal volume) on a novel object recognition (NOR) task and then a spatial memory task (as described for experiment 1). Training on the NOR task began with habituation to the testing room and chamber. The next day, each rat was placed in the chamber with two identical objects for 15 min and then removed to the home cage. After a 3-h delay, each rat was returned to the chamber, which now contained a novel object and a copy of the previously seen familiar object. Each rat was allowed to explore until a total of 30 sec of object exploration had been accumulated. The percent time spent exploring the novel object served as the measure of recognition memory for the familiar object (see Supporting Text).

At the completion of testing, animals were perfused according to standard methods, and the hippocampal lesions were extensively evaluated and carefully measured to determine the lesion volume. Reconstructions of representative hippocampal lesions

Abbreviation: NOR, novel object recognition.

© 2004 by The National Academy of Sciences of the USA
are shown in Fig. 1. Detailed lesion descriptions and representative photomicrographs may be found in Supporting Text and Figs. 6–8, which are published as supporting information on the PNAS web site.

Results

Experiment 1: Spatial Memory. All groups found the hidden platform more quickly as training progressed (Fig. 2A). The three groups with the largest lesions (30–50%, 50–75%, and 75–100%) were on average significantly slower than the SHAM or 5–30% lesion group (see Supporting Text).

Probe trial performance was measured by percent time that the rat spent inside a small zone (diameter of 30 cm, 4% of the water surface) centered on the trained platform location as well as the time spent in the training quadrant (25% of the small training zone) across sessions 2–5. Repeated-measures ANOVA comparing the time spent in the small training zone for the four dorsal hippocampal lesion groups and the sham-operated group. Probe trials were given at the beginning of each daily session before training (thus, probe trial 1 shows performance before platform training was begun). Brackets show the standard error of the mean.

Fig. 2. Acquisition of spatial memory as a function of hippocampal lesion size. (A) Mean latency (sec) for the four dorsal hippocampal lesion groups (5–30%, 30–50%, 50–75%, and 75–100%) and the sham-operated group (SHAM) to find the platform during five daily training sessions (four training trials per day) and during visual platform training (two sessions, four trials per day). (B) Daily probe trial performance as measured by the percent time spent in the small training zone for the four dorsal hippocampal lesion groups and the sham-operated group. (C) Daily probe trial performance as measured by the percent time spent in the training quadrant for the four dorsal hippocampal lesion groups and the sham-operated group. Probe trials were given at the beginning of each daily session before training (thus, probe trial 1 shows performance before platform training was begun). Brackets show the standard error of the mean.

Fig. 1. Reconstructions of coronal sections through the hippocampus showing the smallest (black) and largest (stippled) lesion for each of the four hippocampal lesion groups (damage extending from the dorsal hippocampus to include 5–30%, 30–50%, 50–75%, and 75–100% of total hippocampal volume) from experiment 1 and the ventral lesion group (damage to 50% of total hippocampal volume) from experiment 2. Note that the locus and extent of hippocampal damage for the 50–75% and 75–100% groups were similar in experiments 1 and 2 (experiment 2 not shown). All rats sustained bilateral damage to the CA cell fields and dentate gyrus. In cases where the lesion was not complete at a particular level of the dorsal hippocampus, the sparing was typically restricted to the most medial aspect of the dentate gyrus or CA1 cell field. There was no evidence of damage to the amygdala or perirhinal cortex. Numbers (right) represent the distance (mm) posterior to bregma. For an additional description of the lesions, see Supporting Text.

Groups (5–30%, 30–50%, 50–75%, and 75–100%) revealed the main effects of Group \( F(4, 87) = 14.6, P < 0.0001 \), Probe trial \( F(3, 261) = 27.8, P < 0.0001 \), and a Group × Probe trial interaction \( F(12, 261) = 2.1, P < 0.001 \). The same findings were obtained for the time in the training quadrant (all \( P \) values < 0.05). Thus, these analyses indicate that time spent in the target zone increased across training, and that the rate of learning differed between SHAM and lesion groups. Indeed, by the end of training (probe trial 5), all groups were performing above chance by both measures (\( P < 0.05 \)), but the 30–50%, 50–75%, and 75–100% hippocampal lesion groups spent less time in the small training zone than did SHAM rats (SHAM, 15.6 ± 1.1%; 30–50%, 10.7 ± 1.7%; 50–75%, 7.1 ± 0.7%; and 75–100%, 7.9 ± 1.1%; \( P < 0.05 \)). Further, the two largest lesion groups also spent less time in the training quadrant than did SHAM controls (SHAM, 45.5 ± 1.7%; 50–75%, 35.3 ± 2.3%; and 75–100%, 34.6 ± 3.5%; \( P < 0.05 \)). These results indicate that all groups with hippocampal damage >30% were impaired relative to the SHAM group.

Acquisition across the training sessions also was examined by averaging performance across probe trials 2–5 (Fig. 3B and D).
We also calculated correlations between the size of the hippocampal lesion and behavioral performance. Fig. 3A and C illustrate the relationship between lesion size and percent time in the small training zone and in the training quadrant. There were significant negative correlations between performance and hippocampal lesion size (small training zone, Pearson correlation coefficient $r = -0.31, P < 0.05$; training quadrant, $r = -0.45, P < 0.001$). Thus, the greater the amount of hippocampal damage, the more poorly the rat remembered the platform location on probe trials.

A notable feature of the scatter plots (Fig. 3A and C) is the appearance of a boundary between rats that performed well and rats that did not. By both measures (percent time in small training zone and training quadrant), the effect of the lesion on performance was first clearly detectable in rats with lesions that damaged 30–50% of total hippocampal volume (and spared the ventral hippocampus; Fig. 3B and D). These rats were impaired relative to the SHAM group (small training zone, 14.1 ± 1.0% vs. 7.6 ± 0.9%; training quadrant, 40.8 ± 1.6% vs. 35.3 ± 1.5%; $t > 2.4, P < 0.05$). Yet, rats with smaller lesions (5–30%) performed nearly as well as the SHAM group (small training zone, mean = 10.2 ± 1.5%; training quadrant, mean = 37.2 ± 2.8%; $P > 0.1$).

The two groups with lesions that damaged 50–100% of total hippocampal volume were impaired relative to the SHAM group and performed within 3% of chance (Fig. 3B and D). Fig. 3A and C also show that increasing the size of the lesion beyond 50% of total hippocampal volume did not increase the impairment (for the small training zone measure, there was a trend in the opposite direction: $r = 0.38, P = 0.05$; for the training quadrant measure, there was no correlation: $r = 0.05, P > 0.1$). Together, these findings suggest that (i) small lesions in the dorsal hippocampus that damage <30% of total hippocampal volume affect performance minimally (at most); (ii) a severe impairment occurs when an intermediate level of damage is reached (when the damage involves 30–50% of total hippocampal volume); and (iii) increasing the amount of damage beyond 50% of total hippocampal volume does not exacerbate the deficit.

**Experiment 2: NOR.** Fig. 4A shows the preference for the novel object exhibited by the SHAM group and the three hippocampal lesion groups. The SHAM group exhibited a strong preference for the novel object, spending $69.9 \pm 2.5\%$ of the test period exploring the novel object (chance $= 50\%$, $t(29) = 8.0, P < 0.001$). Rats in the dorsal 50–75% hippocampal group and the ventral 50% hippocampal group also exhibited a strong (above chance) preference for the novel object (dorsal 50–75%, 71.1 ± 2.3%; ventral 50%, 64.4 ± 5.1%) and did not differ from the SHAM group ($t < 1.1, P > 0.1$). In contrast, the dorsal 75–100% hippocampal group did not exhibit a measurable preference for the novel object ($55.9 \pm 4\%$; chance $= 50\%$, $t(8) = 1.5, P > 0.1$). This group was impaired relative to both the SHAM group and the dorsal 50–75% hippocampal group ($t > 2.6, P < 0.05$), despite taking similar amounts of time to acquire 30 sec of exploration time with the objects (mean = 176.6–208.7 sec; $t < 1.2, P > 0.25$). Further, the same pattern of statistical findings was found after 5 and 15 sec of cumulative object exploration, indicating that the dorsal 75–100% group did not exhibit a
The two dorsal hippocampal groups (50–75% and 75–100%) did not differ from each other in the time spent in the small training zone [dorsal 75–100%, 7.1% ± 1.0%; t(14) = 1.6, P > 0.1].

Note that the dorsal 75–100% hippocampal group (7.1 ± 1.0%) did not itself differ from the SHAM control group [t(35) = 1.0, P > 0.1]. Two lines of evidence suggest that this finding was because of poorer than expected performance of the SHAM group rather than because of the absence of impairment after large hippocampal lesions. First, the SHAM group in experiment 1 performed better than the SHAM group in experiment 2 [14.1 ± 1.1% vs. 8.4 ± 0.6%; t(64) = 4.4, P < 0.001]. Second, the dorsal 75–100% hippocampal lesion group in experiment 2 performed similarly to the dorsal 75–100% hippocampal lesion group in experiment 1 [7.1 ± 1.0% vs. 5.8 ± 0.6%, t(22) = 1.2, P > 0.1]. Thus, the large lesion group in experiment 2 performed about as would be expected.

**NOR vs. Spatial Memory.** To compare performance on the two tasks directly, we transformed the data for the 16 animals with dorsal hippocampal lesions (Fig. 4) into z scores (standardized against the SHAM group in each case) and then calculated a repeated-measures ANOVA with Task (water maze vs. NOR) and Lesion size (50–75% vs. 75–100%) as factors. There was a significant interaction of Task × Lesion size [F(1, 14) = 20.9, P < 0.001], indicating that the extent of damage to the hippocampus had a different effect depending on the task that was used to measure performance. Similar results were obtained when time in the training quadrant, instead of time in the small training zone, was used as the measure of spatial memory performance [Task × Lesion size interaction, F(1, 14) = 6.6, P < 0.05].

To ask whether this finding might have been driven by the unexpected result for the water-maze task that the dorsal 75–100% lesion group performed numerically (but not significantly) better than the dorsal 50–75% lesion group, we also compared NOR performance in experiment 2 with water-maze performance in experiment 1. In this case, the two lesion groups in experiment 2 (Fig. 4B) were compared with animals in experiment 1 (Fig. 3B and C) with similar-sized lesions (dorsal 50–75%, n = 11; dorsal 75–100%, n = 16). Again, there was a significant Task × Lesion size interaction for both time in the small training zone [F(1, 39) = 19.6, P < 0.001] and also time in the training quadrant [F(1, 39) = 11.4, P < 0.01].

Last, we compared the relationship between lesion size and performance on the water-maze task (Fig. 5A) with the relationship between lesion size and performance on the NOR task (Fig. 5B). As lesion size increased from 50% to nearly 100% of total hippocampal volume in experiment 2 (Fig. 5A, gray circles), there was no relationship between lesion size and water-maze performance, because performance was poor across the entire range of lesion size (r = 0.37, P > 0.1). In contrast, performance on the NOR task worsened as the hippocampal lesion became larger (hippocampal damage 50–100%, r = –0.66, P < 0.01). Further, the difference between the slopes of these regression lines was significant [t(28) = 3.3, P < 0.01]. Similarly, the slopes were different when the data from experiment 1 (Fig. 5A, black circles) were compared with the NOR data from experiment 2 [t(39) = 4.6, P < 0.001]. Thus, the effect of increasing the size of the lesion from 50% to 100% had a different effect on the NOR task than on the water-maze task.

**Discussion**

Rats with bilateral hippocampal lesions exhibited impaired spatial memory for the location of a hidden platform. Lesions in the dorsal hippocampus that damaged 30–50% of total hippocampal volume caused severe impairment, and increasing the level of damage beyond 50% did not exacerbate the deficit. Further, ventral hippocampal lesions that encompassed ~50% of total hippocampal volume also impaired performance.

---

**Experiment 2: Spatial Memory.** All groups found the hidden platform more quickly as training progressed. The dorsal 50–75% and 75–100% groups were on average slower than the SHAM group to find the hidden platform, and there was no difference in the latencies of the SHAM and ventral 50% groups (see Supporting Text).

Repeated-measures ANOVA comparing SHAM and hippocampal lesion group (dorsal 50–75%, 75–100%, and ventral 50%) performance (time in small training zone) for probe trials 2–5 revealed main effects of Group [F(3, 52) = 4.2, P < 0.01] and Probe trial [F(3, 156) = 33.6, P < 0.0001] but no Group × Probe trial interaction [F(9, 156) = 1.0, P > 0.4]. Similar findings were obtained for the time spent in the training quadrant.

Fig. 4B shows the performance of the SHAM group and each of the lesion groups in the water maze (percent time in small training zone, probe trials 2–5). SHAM rats spent 8.4 ± 0.6% in the small training zone, better than would be expected by chance (chance = 4.0%, P < 0.001). In contrast, both the dorsal 50–75% hippocampal group and the ventral 50% hippocampal group were significantly impaired relative to the SHAM group (dorsal 50–75%, 5.1 ± 0.7%; ventral 50%, 5.5 ± 0.7%; t > 2.6, P < 0.05).
One reason ventral hippocampal lesions impaired performance on learning under training conditions similar to those in our study (30). Broadbent et al. report that the ventral hippocampus was important for spatial memory impairment observed here was similar to what has been described previously. A recent study did not report a significant effect of lesion size on performance for the NOR task or whether spatial cues were the relevant factor in this negative finding. For example, in our previous study, NOR was tested in a black chamber in a dimly lit room with the objects illuminated by a single light positioned directly overhead (14). Thus, the spatial contextual information in this situation was minimal, yet rats with hippocampal lesions were impaired at NOR. Further, the finding was not due to a failure to respond to novelty altogether or to some other deficiency of exploratory behavior, because performance was intact after a short retention interval and impaired only after long intervals; i.e., the impairment was delay-dependent (14).

The central aim of the present study was to compare the effects of hippocampal lesions of various sizes on spatial memory and visual recognition memory with two tasks that have been used commonly to assess the role of the hippocampus in memory in the rat. Spatial memory proved more vulnerable to hippocampal dysfunction than recognition memory. The results do not mean that testing conditions could not be found that would reveal impaired object recognition after partial hippocampal lesions. Rather, the results show that, under standard testing conditions for spatial memory and recognition memory, spatial memory performance requires more hippocampal tissue than does recognition performance.

It is interesting to consider the fact that spatial memory tasks have much in common with tasks of cued recall. That is, the rat must remember the location of a hidden target based on distal extramaze behavior, because performance was intact after a short retention interval and impaired only after long intervals; i.e., the impairment was delay-dependent (14).

The central aim of the present study was to compare the effects of hippocampal lesions of various sizes on spatial memory and visual recognition memory with two tasks that have been used commonly to assess the role of the hippocampus in memory in the rat. Spatial memory proved more vulnerable to hippocampal dysfunction than recognition memory. The results do not mean that testing conditions could not be found that would reveal impaired object recognition after partial hippocampal lesions. Rather, the results show that, under standard testing conditions for spatial memory and recognition memory, spatial memory performance requires more hippocampal tissue than does recognition performance.

It is interesting to consider the fact that spatial memory tasks have much in common with tasks of cued recall. That is, the rat must remember the location of a hidden target based on distal extramaze behavior, because performance was intact after a short retention interval and impaired only after long intervals; i.e., the impairment was delay-dependent (14).

The central aim of the present study was to compare the effects of hippocampal lesions of various sizes on spatial memory and visual recognition memory with two tasks that have been used commonly to assess the role of the hippocampus in memory in the rat. Spatial memory proved more vulnerable to hippocampal dysfunction than recognition memory. The results do not mean that testing conditions could not be found that would reveal impaired object recognition after partial hippocampal lesions. Rather, the results show that, under standard testing conditions for spatial memory and recognition memory, spatial memory performance requires more hippocampal tissue than does recognition performance.
lesions. In many cases, hippocampal damage may not have been sufficiently complete to reveal a deficit.

We thank Laura Entwistle, Natalie Shanks, Joseph Manns, Daniel Guadarrama, and Stuart Zola for assistance. This work was supported by the Medical Research Service of the Department of Veterans Affairs, the National Institute of Mental Health, the Metropolitan Life Foundation, National Institute on Aging Grant P50 AG05131, the National Science Foundation, the James S. McDonnell Foundation, and a National Alliance for Research on Schizophrenia and Depression Effie Beeman Investigator Award.

Supporting Text

Supporting Methods

Subjects. Experiment 1. The subjects were 92 male Long–Evans rats weighing 300–350 g at the beginning of the experiment. Rats were housed individually and maintained on a 12:12-h light–dark cycle. Food and water were freely available. Rats were assigned to one of five groups, a sham-operated control group (SHAM; \( n = 36 \)) and four groups with bilateral ibotenic acid (IBO) lesions of the hippocampus that varied in septotemporal extent (\( n = 56 \)). Following recovery from surgery, rats were trained on a spatial memory task in the water maze.

Experiment 2. The subjects were 56 male Long–Evans rats weighing 300–350 g at the beginning of the experiment. Rats were housed individually and maintained on a 12:12-h light–dark cycle. Food and water were freely available. Rats were assigned to one of four groups, a sham-operated control group (SHAM; \( n = 30 \)), two groups (each \( n = 8 \)) with bilateral IBO lesions of the dorsal hippocampus that varied in septotemporal extent (50–75% and 75–100% of total hippocampal volume), and one group (\( n = 10 \)) with bilateral IBO lesions of the ventral hippocampus that involved \( \approx 50\% \) of total hippocampal volume. Following recovery from surgery, rats were trained first on the novel object recognition (NOR) task and then on the spatial memory task in the water maze.

Apparatus. Spatial memory testing was conducted in the Morris water maze (1.8-m diameter) with an “Atlantis platform” (12.7-cm diameter; Spooner et al., ref. 1), which could be raised or lowered remotely during a trial. The platform was located in the center of the northeast quadrant of the pool throughout spatial and visual platform testing. The water was rendered opaque by the addition of powdered milk, and 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), and an opaque curtain shielded the experimenter from the view of the rat once the trial began. 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, San Diego) to record the swim path of each rat.

NOR was tested in an opaque plastic chamber [35 cm \( \times \) 41.5 cm \( \times \) 50 cm (height)]. The stimuli were a plastic animal head and a multicolored jewelry box. Three identical copies of each object were available. A video camera mounted on the wall directly above the chamber was used to record the testing session for offline analysis. Overhead fluorescent lighting illuminated the testing area.

Procedures. Spatial training. Each rat received one training session daily for 5 consecutive days. Each daily session began with a single reinforced probe trial, followed by four training trials. For the probe trials, the platform was lowered so that it was inaccessible, and the rat was placed in the water facing the pool wall at one of four start points (north, south, east, or west). The particular start point was counterbalanced across trials for all animals. Upon release into the water, the rat was allowed to swim for 60 sec,
at which point the platform was raised to within 1.5 cm of the water surface. An additional 60 sec was then allowed for the rat to locate the platform and escape from the water. After escaping, the rat remained on the platform for 30 sec before being removed. If the rat failed to escape, it was guided to the platform and remained there for 30 sec.

At the completion of the daily probe trial, four training trials were given with the platform in the raised position (1.5 cm below the water surface), where it remained invisible to the rat but provided a means of escape from the water. The procedure was the same as for the probe trials, except that the rat was allowed 120 sec to find the platform. The intertrial interval for the training trials ranged from 6 to 21 min.

**Visual platform testing.** Two days after completion of spatial platform training, the rats were tested with the platform in the same location but now 1.5 cm above the water and made salient by the addition of high-contrast colored tape. Rats were given four trials daily for 2 consecutive days. These trials were conducted in the same way as the spatial training trials (intertrial interval = 6–8 min).

**NOR.** Rats were acclimated to the testing room and chamber 1 day before testing (45 min in the testing room and 5-min exploration of the empty chamber). On the day of testing, a rat was first acclimated again to the testing room for 45 min and then placed in the empty chamber for 1 min. Then the rat was removed, and two identical objects were placed centrally in the chamber (9 cm apart). The rat was then allowed to explore the chamber and the objects for 15 min. After a delay of 3 h, the rat was returned to an empty chamber for 1 min and then reintroduced to the chamber in the presence of two objects: a novel object and a copy of the previously encountered object. The rat was allowed to explore until it accumulated 30 sec of contact time with the objects (nose within 2 cm of object and vibrissae moving). The score was the percent time (compared with the familiar object) that a rat spent exploring the novel object (see ref. 2 for additional details).

Which object was novel (plastic animal head or jewelry box), and the left/right position of the novel object were counterbalanced within each group. The experimenter was blind to the group membership of the rats during testing and offline data analysis. After completion of the NOR task, the rats were trained on the water maze.

**Surgery and Histology.** Anesthesia was maintained throughout surgery with isoflurane gas (0.8–2.0% isoflurane delivered in O₂ at 1 liter/min). The rat was placed in a stereotaxic instrument (Kopf Instruments, Tujunga, CA), and the incisor bar was adjusted until bregma was level with lambda. For the four lesion groups, bilateral excitotoxic hippocampal lesions were produced by local microinjections of ibotenic acid (IBO, Biosearch). IBO was dissolved in 0.1 M PBS to provide a solution with a concentration of 10 mg/ml, pH 7.4. IBO was injected at a rate of 0.1 µl/min with a 10-µl Hamilton syringe mounted on a stereotaxic frame and held with a microinjector (model 5000, Kopf Instruments). The syringe needle was lowered to the target coordinate and left in place for 1 min before beginning the injection. Following the injection, the syringe needle was left in place for a further 2 min to reduce the spread of IBO up the needle tract.
In experiment 1, four sets of surgical coordinates (modified from Jarrard, ref. 3) were used to create lesions of the dorsal hippocampus and to damage a range of hippocampal tissue, beginning in the dorsal portion. The first injection of IBO was always in the anterior-most aspect of the dorsal hippocampus (bregma $-2.4$), and additional injections moved progressively caudal and ventral. Lesions of different extent were made by injecting a total of either 0.165 $\mu$l of IBO into 6 sites within each hippocampus, 0.255 $\mu$l of IBO into 9 sites within each hippocampus, 0.34 $\mu$l of IBO into 12 sites within each hippocampus, or a total of 0.51 $\mu$l of IBO into 18 sites within each hippocampus (see Clark et al., ref. 2). In experiment 2, to produce dorsal hippocampal lesions that damage 50–75% and 75–100% of total hippocampal volume, either a total of 0.34 $\mu$l of IBO was injected into 12 sites within each hippocampus, or a total of 0.51 $\mu$l of IBO was injected into 18 sites within each hippocampus. A third set of lesion coordinates was used to produce ventral hippocampal lesions that damaged $\approx 50\%$ of total hippocampal volume. Ventral lesions were made by injecting a total of 0.355 $\mu$l of IBO into 13 sites within each hippocampus.

The procedure for the SHAM group was the same as for the lesion groups, with the exception that the dura was not punctured, the syringe needle was not lowered into the cortex, and no IBO was injected. Once awake and responsive, each rat was returned to its home cage in the colony room for a 14-day recovery period. In experiment 1, water-maze training began 5–7 weeks following surgery and after other behavioral testing (not reported here).

At completion of testing, the rats were administered an overdose of sodium pentobarbital and perfused transcardially with buffered 0.9% NaCl solution followed by 10% formaldehyde solution (in 0.1 M phosphate buffer, pH 7.4). The brains were then removed and cryoprotected in 20% glycerol/10% formaldehyde. Coronal sections (50 $\mu$m) were cut with a freezing Microtome beginning at the level of the anterior commissure and continuing caudally through the length of the hippocampus. Every fifth section was mounted and stained with thionin to assess the extent of the lesions.

To calculate the extent of damage, the damaged region (defined as tissue that was either missing or necrotic) was drawn onto the appropriate coronal section (4) by using an MZ6-series microscope (Leica, Deerfield, IL) and a computer-assisted drawing program. A software tool was then used to calculate the area of damage at each coronal level, and the sum of the damaged sections was calculated as a percent of normal area (as derived from the representative atlas sections). The total damage was the average of the damage on the left and right sides. Hippocampal damage was calculated from 11 coronal sections (bregma $-2.88$ to $-6.8$ mm in half-millimeter intervals), damage to the subiculum was calculated from five coronal sections (bregma $-4.80$ to $-6.80$ mm in half-millimeter intervals).

**Supporting Results**

**Experiment 1. Neurohistological findings.** On the basis of the size of the dorsal hippocampal lesion, rats were assigned to one of four lesion groups without knowledge of
their behavioral data; 5–30% \((n = 5)\), 30–50% \((n = 24)\), 50–75% \((n = 11)\), or 75–100% \((n = 16)\) of total hippocampal volume. All rats sustained bilateral damage to all of the cell fields of the hippocampus (CA cell fields, dentate gyrus). The extent of damage to the left and right hippocampus was similar for all groups \((P\) values >0.1). In cases where the lesion was not complete at a particular level of the dorsal hippocampus, the sparing was typically restricted to the most medial aspect of the dentate gyrus or CA1 cell field. In all rats there was minor damage to the cortex and to the fimbria overlying the dorsal hippocampus, which was associated with the placement of the Hamilton syringe during surgery. There was no evidence of damage to the amygdala or perirhinal cortex (see Fig. 6).

*Dorsal 5–30%.* Five rats had lesions that involved 5.5–28.4% of total hippocampal volume, with an average lesion size of 20.4%. One rat also had minor damage to the dorsal subiculum (<1%).

*Dorsal 30–50%.* Twenty-four rats had lesions that involved 30.2–49% of total hippocampal volume, with an average lesion size of 39.7%. In nine rats, there was minor damage to the subiculum (<5%).

*Dorsal 50–75%.* Eleven rats had lesions that involved 50.6–70.3% of total hippocampal volume, with an average lesion size of 57.3%. Nine rats also had minor damage to the subiculum (mean = 3.2%, range = 0.1–12.4%).

*Dorsal 75–100%.* Sixteen rats had lesions that involved 76.2–97.3% of total hippocampal volume, with an average lesion size of 85.5%. In all cases, the lesion extended into the subiculum (mean = 28.4%, range = 3.1–65.9%). In 11 of the rats in this group, there was also very minor bilateral damage to the entorhinal cortex (see Fig. 8).

**Experiment 2. Neurohistological findings.** All rats sustained bilateral damage to all cell fields of the hippocampus. The extent of damage to the left and right hippocampus was similar for all groups \((P\) values >0.4). There was minor damage to the cortex and to the fimbria overlying the dorsal hippocampus that was associated with the placement of the Hamilton syringe during surgery. There was no damage to either the amygdala or the perirhinal cortex.

*Dorsal hippocampal lesions.* In cases where the lesion was not complete at a particular level of the dorsal hippocampus, the sparing was restricted to the most medial aspect of the dentate gyrus or dorsal CA1 cell field.

*Dorsal 50–75%.* Eight rats had lesions that involved 51.8–70.3% of total hippocampal volume with an average lesion size of 57.2%. Additionally, all rats sustained damage to the subiculum (mean = 10.6%, range = 1.5–21%). One rat had minor unilateral encroachment of the lesion into the right entorhinal cortex (see Fig. 8).

*Dorsal 75–100%.* Eight rats had lesions that involved 76.6–85.7% of total hippocampal volume, with an average lesion size of 82.9%. All rats also sustained damage to the
subiculum (mean = 15.1%, range = 0.7–33.9%). Two rats had minor bilateral encroachment into the entorhinal cortex, and an additional rat had minor unilateral damage to the right entorhinal cortex (see Fig. 8).

**Ventral hippocampal lesions.** Ventral hippocampal lesions ranged in size from 43.9% to 53.5% of total hippocampal volume, with an average lesion size of 49.5%. In cases where the lesion was not complete at a particular level, the sparing was generally restricted to the medial dorsal hippocampus, or a portion of the ventral lateralmost CA1/2 cell field. All rats also sustained damage to the subiculum (mean = 18.3%, range = 6.7–31.7%). One rat also had minor unilateral damage to the left entorhinal cortex, and an additional rat had minor bilateral damage to the right entorhinal cortex. Last, all had minor damage to nuclei of the dorsolateral and ventrolateral geniculate, as well as the optic tract, which was associated with the placement of the Hamilton syringe (see Figs. 7 and 8).

**Experiment 1: Latency.** Repeated-measures ANOVA revealed that during the course of training, the SHAM and dorsal hippocampal lesion groups (5–30%, 30–50%, 50–75% and 75–100%) reduced the time needed to escape to the platform \[F(4, 348) = 75.9, P < 0.0001\]. There also was a main effect of group \[F(4, 87) = 14.7, P < 0.0001\] and a Group × Session interaction \[F(16, 348) = 4.0, P < 0.0001\], indicating that the groups differed in their mean latencies to find the platform and in the rate of learning across the training sessions.

Planned \(t\) tests revealed that rats with dorsal hippocampal lesions that damaged only 5–30% of total hippocampal volume performed similarly to the SHAM group [Fig. 1A; sessions 1–5: SHAM group, 16.1 ± 1.0 sec; 5–30% group, 13.2 ± 1.7 sec; \(t(39) = 1.0, P > 0.1\)]. In contrast, the three groups with large dorsal hippocampal lesions (30–50%, 50–75%, 75–100%) were slower than either the SHAM group or the 5–30% group to find the hidden platform [Fig. 1A; sessions 1–5: 30–50% group, 28.2 ± 2.5 sec; 50–75% group, 35.4 ± 4.0 sec; 75–100% group, 31.9 ± 2.7; all \(P\) values <0.05]. Last, the three groups with large dorsal lesions performed similarly [Fig. 1A; sessions 1–5, all \(P\) values >0.1].

The latency to find the visible platform ranged from 6.1 to 10.5 sec (Fig. 1A). The only difference among groups was that the 30–50% dorsal hippocampal group (mean latency = 10.5 ± 1.2 sec) was measurably slower to find the visual platform than the SHAM group [mean latency = 7.8 ± 0.5 sec; \(t(58) = 2.3, P < 0.05\)].

**Experiment 2: Latency.** Repeated-measures ANOVA revealed that during the course of training all groups (SHAM, dorsal 50–75%, dorsal 75–100%, and ventral 50%) reduced the time needed to escape to the platform \[F(4, 208) = 97.8, P < 0.0001\]. There also was a main effect of Group \[F(3, 52) = 8.2, P < 0.0001\] and a Group × Session interaction \[F(12, 208) = 2.7, P < 0.01\]. Planned \(t\) tests revealed that rats with dorsal hippocampal lesions that damaged either 50–75% or 75–100% of hippocampal volume were slower than the SHAM group to find the hidden platform (sessions 1–5: SHAM, 19.2 ± 1.5 sec; dorsal 50–75%, 31.0 ± 4.3 sec; dorsal 75–100%, 34.2 ± 3.7 sec; all \(P\) values <0.01). Further, the dorsal 75–100% group also was slower than the ventral 50% group to find the hidden platform [ventral 50% group, 22.7 ± 1.9 sec; \(t(16) = 2.9, P< 0.01\], and the
dorsal 50–75% group was marginally slower than the ventral 50% group \( t(16) = 1.9, P = 0.07 \). There was no difference between the SHAM and ventral 50% groups \( t(38) = 1.2, P > 0.2 \).

The average latency to find the visual platform ranged from 8.4 to 6.1 sec. Although the difference between group latencies was small, the dorsal 75–100% group (mean latency 9.4 ± 0.8 sec) was slower than the ventral 50% group to find the visual platform [mean latency, 6.1 ± 0.4 sec; \( t(14) = 3.83, P < 0.01 \)], and the dorsal 50–75% was marginally slower than the ventral 50% group [dorsal 50–75%, 7.4 ± 0.5 sec, \( t(16) = 2.0, P = 0.06 \)]. Finally, the dorsal 75–100% group was slower to find the platform than the dorsal 50–75% group \([t(14) = 2.2, P < 0.05] \).

**Experiment 2: NOR.** We evaluated the total amount of time spent exploring the objects during the 15-min familiarization period. The four groups averaged 106 sec of contact time with the objects (SHAM, 87.3 ± 4.5 sec; dorsal 50–75%, 70.8 ± 11.5 sec; dorsal 75–100%, 136.5 ± 21.7 sec; ventral 50%, 129.6 ± 9.7 sec). The ventral 50% and dorsal 75–100% hippocampal groups explored the objects more than the other two groups (\( P \) values <0.05).
