Rats With Hippocampal Lesions Can Learn a Place Response, but How Long Can They Retain It?

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Previous research has shown that electrolytic hippocampal lesions do not affect the acquisition of a place response if a special training procedure is used. However, 24 days later, the hippocampal rats manifest a profound deficit in the retention of the spatial information (J. M. J. Ramos, 2000). The goal of the present study was, therefore, to investigate how long the hippocampal rats can retain a place response. Results showed that, 3 days after the end of the training, lesioned rats remembered as well as the control rats, but this was no longer true 6 or 12 days after the training. This retention deficit was not observed when the spatial information was acquired by means of a guidance strategy. These results suggest that, when a special training procedure is used, the hippocampus is not necessary for the learning of a place task but is required for the formation of long-term spatial memory.

Many clinical observations in humans as well as lesion studies conducted on monkeys and rodents over the last two decades have shown the essential role played by the medial temporal lobe in the storage, retention, and formation of long-term memory (Aggleton & Brown, 1999; Eichenbaum, Dudder, Wood, Shapiro, & Tanila, 1999; Milner, Squire, & Kandel, 1996; Reed & Squire, 1998; Squire, 1992). This conclusion is based mainly on the phenomenon of temporally graded retrograde amnesia observed after hippocampal lesions. An intriguing aspect of the graded retrograde amnesia is that this phenomenon has been easily reproduced by different laboratories when nonspatial paradigms have been used (Anagnostaras, Maren, & Fanselow, 1999; Kim, Clark, & Thompson, 1995; Kim & Fanselow, 1992; Winocur, 1990; Zola-Morgan & Squire, 1990). In contrast, when spatial tasks have been used, results have been inconsistent, with some studies finding a nongraded retrograde amnesia (Bollhuis, Stewart, & Forrest, 1994; Laurent-Demir & Jaffard, 1997; Riedel et al., 1999; for review, see Nadel & Moscovitch, 1997) and others finding a temporally graded retrograde amnesia (Kubie, Sutherland, & Muller, 1999; Ramos, 1998; see also Teng & Squire, 1999). Thus, using spatial tasks, studies of retrograde amnesia have been unable to dissociate whether the hippocampus is necessary for the temporary storage and formation of long-term spatial memory or for the retrieval—performance of the spatial task.

Another problem that arises when studying the function of the hippocampal region in the storage, retention, and formation of long-term spatial memory is the fact that, when the hippocampus has been lesioned before training, it has only been possible to study the effect of the lesion on the acquisition (McDonald & White, 1993; Packard & McGaugh, 1996), but not on the retention, of spatial information (for an exception, see Vnek & Rothblat, 1996, but only when a nonspatial paradigm is used). However, several studies have shown that the deficit in the acquisition of cartographic tasks normally seen in hippocampal rats can be overcome when special training procedures are used (Day, Weisend, Sutherland, & Schallert, 1999; Whishaw, Cassel, & Jarrard, 1995; Whishaw & Jarrard, 1996). More specifically, these studies have shown, through the use of the Morris water-maze task, that hippocampal rats can learn to swim to a hidden platform if the task is initially very easy and is made progressively harder until it reaches a standard level of difficulty (see, for example, Day, Weisend, Sutherland, & Schallert, 1999). These data make it possible to study how the hippocampus contributes to the retention of spatial information when hippocampal lesions are made before training.

Working with this idea, we showed in an earlier study that, in a four-arm plus-maze apparatus, hippocampal rats can learn a place response just as well as control rats when a special training method is used (Ramos, 2000). However, 24 days later, during a retraining period, the hippocampal rats manifested a profound deficit in the retention of the spatial information. The purpose of the present study was to investigate this phenomenon further. It has recently been suggested that the hippocampus and related extrahippocampal structures of the medial temporal lobe have a time-limited role in memory storage (Alvarez & Squire, 1994; Bontempi, Laurent-Demir, Destrade, & Jaffard, 1999; Sutherland & McNaughton, 2000). That hippocampally damaged rats can learn a place response as well as control subjects, as demonstrated in a previous study (Ramos, 2000) suggests that extrahippocampal structures, presumably within the medial temporal lobe, can take responsibility for this learning and retain this information for a certain period of time. A central goal of the present study was therefore to investigate how long the hippocampus—lesioned rats can retain the spatial information that has been learned. This information would indicate how long the extrahippocampal structures can retain spatial information and the initial time-limited role that these structures play in spatial memory storage. For this purpose, in the present study, we modified the traditional training procedure to

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overcome the spatial acquisition deficit normally seen in hippocampal rats. Thus, in a retraining period 3, 6, or 12 days after acquisition, it was possible to study the retention of the spatial information acquired during the training phase.

Experiment 1

A number of studies have shown that hippocampal lesions impair the acquisition of a place response (Morris, Garrud, Rawlins, & O'Keefe, 1982; O'Keefe, Nadel, Keightly, & Kill, 1975; Sutherland, Whishaw, & Kolb, 1982). The goal of Experiment 1 was to replicate this well-established fact by using a traditional training procedure within the stimulatory context of the lab. A second goal was to investigate whether a long period of training eliminates the deficit in cartographic learning. This would make it possible to study the retention of the spatial information during a retraining phase. For this reason, the training period in this experiment was 20 consecutive days.

Method

Subjects

Twelve experimentally naive male Wistar rats from the breeding colony of the University of Granada were used. Rats were housed singly and maintained on a 12-hr light–dark cycle. Behavioral testing was performed during the light phase of the cycle.

Surgery

At the time of surgery, the rats weighed between 280 and 320 g. Rats were randomly assigned to a hippocampal (n = 6) or to a control (n = 6) group. The rats were anesthetized with sodium pentobarbital (50 mg/kg ip, Sigma Chemical, St. Louis, MO) and then placed in a stereotaxic frame with the incisor bar adjusted so that lambda and bregma were level. The dorsal hippocampus was damaged at four different anterior–posterior sites in relation to the interaural zero point (Paxinos & Watson, 1998): AP +5.9, L ±1.6, V ±6.5; AP +4.8, L ±2.5, V ±6.5; AP +3.8, L ±3.2, V ±6.5; AP +3.0, L ±4.0, V ±5.4. Bilateral electrolytic lesions were made by passing 2-mA DC cathodal current for 15 s through a monopolar electrode (insect pin, size 00) insulated with INSL-X except for 0.5 mm at the tip. Control rats received the same treatment, but no current was passed. After surgery, the rats were given 1 week to recover.

Apparatus

A four-arm plus-maze was used. Each arm of the maze measured 60 cm long × 10 cm wide and was connected to an octagonal central platform 35 cm in diameter. There were no doors separating the arms from the central platform. The walls of the central platform were made of transparent Plexiglas and were 15 cm in height. The walls of each arm were made of wood and measured 5 cm in height. The maze was 60 cm from the floor, and a 200-W light bulb was hanging from the ceiling, 1.2 m above the center of the platform. A schematic diagram of the maze and cues in the testing room has been presented elsewhere (see Ramos, 1998, Figure 1). Briefly, the maze was situated in the center of a room measuring 7 m². The main extramaze cues that the rat could use to orient itself included the following: a poster on the east wall, a radiator and a black adhesive plastic-covered window in the south wall, a cabinet and metallic shelves on the west wall, and a ceiling-height air conditioning unit and the door of the lab in the north wall. The experimenter was standing in the northeast corner of the experimental room.

Behavioral Procedure

After recovery from surgery, subjects were placed on a food-deprivation schedule to maintain them at 85–90% of their free-feeding body weight. Beginning on the same day as the deprivation program, all rats were handled on 7 successive days for 5 min each. The next day, training began. Rats received eight trials per session, one session per day, for 20 consecutive days. At the beginning of a trial, the rat was placed at the end of one of the arms used for starting (the south, north, and east arms), with its back to the central platform. The order in which the different starting arms were used was randomized in each daily session. The reward, two 45-mg food pellets (P. J. Noyes, Lancaster, NH), was placed in the food cup located at the end of the west arm. The rat was considered to have made a choice when, having entered an arm, it crossed the halfway point with its four limbs. After a choice was made and the subject reached the end of the chosen arm, a 10-cm wooden cube was placed just behind the rat. In this way, the rats remained at the end of the chosen arm for 5–6 s. The rat was then picked up and confined in a box for an intertrial interval of 30 s. Between trials, the maze was rotated 90° in a clockwise direction to prevent the rats from using olfactory signals to reach the goal arm. For this reason, the floor of the testing room was marked to ensure that the position of the maze remained constant in relation to the room cues.

Histology

When the behavioral testing was completed, the rats were deeply anesthetized with sodium pentobarbital (80 mg/kg ip) and perfused intracardially with 0.9% saline followed by a 10% Formalin solution. The brains were frozen and sliced at 50 microns. The sections were stained with cresyl violet, a Nissl stain. Histological examination to evaluate the extent of the lesion was performed with an Olympus CH-30 microscope (Técnicas Médicas, MAB, Barcelona, Spain) and the Paxinos and Watson (1998) atlas.

Results and Discussion

Histological Results

The microscopic analysis of the coronal sections taken from the hippocampal rats revealed appropriately positioned bilateral lesions (see Figure 1, Panels A and B). The lesions began in the anterior pole of the hippocampus. At this level, the CA3 field was totally (in 2 rats) or partially (in 4 rats) damaged. At more posterior levels, at the levels of the ventromedial nucleus of the hypothalamus and the mammillary nuclei, CA1 was partially damaged (between 40–80%) in all the rats. The hippocampal CA2–CA3 fields appeared partially damaged (between 40–90%) in 5 rats. As for the dentate gyrus, the most medial region appeared intact in all the rats except 1; however, the lateral stratum granulosum and hilus were affected in 4 subjects. The lesions ended at the beginning of the Sylvius aqueduct. At this level, the hippocampal CA1 field and the medial zone of the stratum granulosum and the hilus were partially damaged in 4 rats. In addition, minor bilateral or unilateral damage to the overlying retrosplenial agranular cortex was also observed. However, this damage was sustained to the same extent in control and experimental rats and therefore did not contribute to the behavioral deficit seen in the hippocampal group.

Behavioral Results

As illustrated in Figure 2, the hippocampal-lesioned group showed a profound deficit in the acquisition of the task. A two-way analysis of variance (ANOVA; Group × Day) indicated that the performance of the lesioned rats, assessed by the percentage of
correct responses recorded during each daily session, was significantly lower than that observed in the control group, $F(1, 10) = 28.20, p < .0003$. The effect of days, $F(19, 190) = 17.53, p < .000001$; and the interaction between factors, $F(19, 190) = 3.39, p < .000001$, were also significant. Despite the deficit observed in the lesioned rats, a one-way ANOVA for repeated measures revealed that the performance of the hippocampal group improved significantly as the days passed, $F(19, 95) = 4.98, p < .000001$. Even so, on the last day of training, the performance of the hippocampal group was significantly inferior to that of the control group (Day 20 of training, hippocampal = 77 ± 9.3 vs. control = 100 ± 0.0): one-way ANOVA, $F(1, 10) = 6.05, p < .03$.

Behavioral results showed that of the 6 hippocampal rats, only 3 reached a standard learning criterion (at least 14 correct trials on 2 consecutive days) during the 20 days of training. Even if we
consider only the 3 lesioned rats that succeeded in learning the
task, their performance was significantly inferior to that of the
controls. The hippocampal rats committed significantly more er-
ners before reaching the criterion than did the control rats, \( F(1, 7) = 34.54, p < .0006 \) (see Figure 3A). Also, the mean number of
days required to reach criterion was higher in the hippocampal
group, \( F(1, 7) = 63.63, p < .0001 \) (Figure 3B).

These data replicate the results obtained previously (Ramos,
2000). The main difference between this experiment and the earlier
one is that the present experiment used more training days, but,
even so, the performance of the lesioned rats remained signifi-
cantly inferior to that of the control group. These results clearly
suggest that the use of a traditional training procedure is accom-
panied by a profound deficit in the acquisition of a place response.
This corresponds with the results found by other authors using
different cartographic learning paradigms (O'Keefe & Nadel,
1978; Packard & McGaugh, 1992; Sutherland, Whishaw, & Kolb,
1982).

Experiment 2

Recent studies have shown that hippocampus-lesioned rats can
learn a place response when a special training procedure is used
(Day, Weisend, Sutherland, & Schallert, 1999; Whishaw, Cassel,
& Jarrard, 1995). It has been suggested that such special training
procedures make the acquisition of a place response possible in
hippocampal rats because they discourage the use of non-place
strategies (see, for example, Day et al., 1999). Other authors have
also suggested that the special procedures may favor the enlistment
of extrahippocampal regions in place learning, thus facilitating
acquisition (Whishaw & Jarrard, 1996). The objective of this
experiment was twofold. First, it was to overcome the spatial
acquisition deficit typically observed in hippocampal rats trained
with a traditional method. To this end, a special training procedure
(Ramos, 2000) was used. A second aim was to investigate how
long the lesioned rats can retain the learned information once the
acquisition deficit is overcome. With this in mind, a retention test
(retraining phase) was performed 3, 6, or 12 days after the acqui-
sition phase.

Method

Subjects

Subjects were 39 experimentally naive male Wistar rats (270–320 g at
the time of surgery) from the breeding colony of the University of Granada.
Rats were housed and maintained under conditions identical to those
described in Experiment 1. Rats were randomly assigned to one of six
groups (three experimental and three control groups). All of the subjects
received the same treatment except in two aspects, the first being whether
the dorsal hippocampus was lesioned or not, and the second being the time
interval between the learning (acquisition phase) and the retention (retrain-
ing phase). This interval was manipulated at three levels: 3 days (\( n = 15, \)
7 hippocampal and 8 controls), 6 days (\( n = 12, 6 \) hippocampal and 6
controls), and 12 days (\( n = 12, 6 \) hippocampal and 6 controls).

Surgery, Apparatus, and Histology

The surgical and histological procedures were identical to those de-
scribed in Experiment 1. The apparatus was the same as that described in
Experiment 1.
Behavioral Procedures

The procedure was identical to that followed in Experiment 1 except in three aspects. First, throughout the acquisition period, a piece of yellow sandpaper measuring 10 cm × 60 cm (80-grit) was positioned on the floor of the goal arm. Training ended when each rat reached a learning criterion of at least 14 correct trials (87%) on 2 consecutive days. Second, the day after reaching criterion, each rat underwent a transfer test. During this test, the sandpaper covering the goal arm (west) was removed so that the only guide available to the rats to solve the test was the configuration of the extramaze stimuli. The transfer test consisted of 8 trials. The order in which the different starting arms were used was randomized, and it was the same for all the rats. Third, when the transfer test was concluded, the subjects remained in their respective cages for 3, 6, or 12 days and were not tested in any way. Starting on Day 3, 6, or 12 the rats received retraining on the spatial task learned during the initial training phase. The procedure used during the retraining phase of testing was identical to that of training (acquisition) except that the sandpaper that had covered the goal arm in the training phase was not present. A few days before the retraining phase, the rats were food-deprived to achieve a weight of 85–90% of their free-feeding body weight.

Results and Discussion

Histological Results

Representative lesions displaying the maximum and minimum damage resulting from the lesions are represented in Figure 1B.

Behavioral Results

Acquisition. Figure 4 illustrates the results obtained during the training phase (acquisition). A two-way ANOVA (Group × Learning–Retraining Interval) did not detect significant differences in the number of incorrect trials effected before reaching criterion: group, F(1, 33) = 3.47, p = .07; learning–retraining interval, F(2, 33) = 1.50, p = .23; interaction, F(2, 33) = 0.68, p = .50 (Figure 4A). To rule out the possibility of differences in acquisition, three one-way ANOVAs were performed to compare the performance of each lesioned group with its corresponding control group. These analyses revealed that there were no significant differences in the mean number of errors to criterion between the hippocampal 3-day group and the control 3-day group, F(1, 13) = 0.03, p = .85; the hippocampal 6-day group and the control 6-day group, F(1, 10) = 1.77, p = .21; and the hippocampal 12-day group and the control 12-day group, F(1, 10) = 2.19, p = .16.

A two-way ANOVA (Group × Learning–Retraining Interval) similarly indicated that the mean number of days to criterion for the hippocampal groups did not differ significantly from that observed in the control group: group, F(1, 33) = 3.48, p = .07; learning–retraining interval, F(2, 33) = 1.48, p = .24; interaction, F(2, 33) = 0.06, p = .93 (Figure 4B). Three one-way ANOVAs did not detect significant differences when each lesioned group was compared with the corresponding control group: hippocampal 3-day versus control 3-day, F(1, 13) = 1.22, p = .28; hippocampal 6-day versus control 6-day, F(1, 10) = 0.73, p = .41; hippocampal 12-day versus control 12-day, F(1, 10) = 1.62, p = .23. It is important to note that, 24 hr after reaching criterion, when the transfer test without sandpaper was administered, both groups had similar performance, and no significant differences were detected: two-way ANOVA, group, F(1, 33) = 0.97, p = .32; learning–

Figure 4. Experiment 2. acquisition. A: Mean (± SEM) number of errors to criterion for the hippocampus-lesioned (HIP) and control (CON) groups during the training phase (acquisition) of testing. B: Mean (± SEM) number of days before criterion for the HIP and CON groups during the acquisition phase. C: Mean (± SEM) percentage correct for the HIP and CON groups during the transfer test (without intramaze cue), 1 day after reaching criterion.
retraining interval, $F(2, 33) = 2.54, p = .09$; interaction, $F(2, 33) = 0.46, p = .63$ (Figure 4C).

These results indicate that the sandpaper was necessary for the lesioned rats to learn the task at a rate similar to that of control groups. However, once the task was learned, the sandpaper did not play an important role in the expression of this learning.

Retraining. Figure 5 depicts the performance of hippocampal and control rats in the retraining phase (retention), which took place 3, 6, or 12 days after the acquisition phase. Data indicated that some hippocampal groups manifested a profound deficit in the retention of the spatial task acquired during the training phase. Thus, a two-way ANOVA showed that the number of incorrect trials before reaching criterion was again much higher in some hippocampal groups than in the control groups: group, $F(1, 33) = 21.52, p < .00005$; learning–retraining interval, $F(2, 33) = 2.41, p = .10$; interaction, $F(2, 33) = 4.54, p < .01$ (Figure 5A). Simple main effects analyses performed with one-way ANOVA revealed that there were no significant differences in the mean number of errors to criterion between the hippocampal 3-day group and the control 3-day group, $F(1, 13) = 0.50, p = .49$. However, significant differences were detected between the hippocampal 6-day group and the control 6-day group, $F(1, 10) = 6.93, p < .02$, and between the hippocampal 12-day group and the control 12-day group, $F(1, 10) = 21.34, p < .0009$. In addition, considering only the lesioned groups, the mean number of errors to criterion by the hippocampal 3-day group was significantly lower than that obtained by the hippocampal 6-day group, $F(1, 11) = 4.95, p < .04$, and by the hippocampal 12-day group, $F(1, 11) = 10.56, p < .007$. However, no significant differences were detected between the hippocampal 6-day group and the hippocampal 12-day group, $F(1, 10) = 0.22, p = .64$. Finally, considering only the control groups, no significant differences were detected in these same comparisons: control 3-day versus control 6-day, $F(1, 12) = 1.74, p = .21$; control 3-day versus control 12-day, $F(1, 12) = 2.00, p = .18$; and control 6-day versus control 12-day, $F(1, 10) = 0.05, p = .81$.

A second two-way ANOVA revealed that the number of days required to reach criterion was also significantly higher in some hippocampal groups: group, $F(1, 33) = 31.32, p < .00005$; learning–retraining interval, $F(2, 33) = 2.64, p = .086$; interaction, $F(2, 33) = 5.37, p < .009$ (Figure 5B). Simple main effects analyses revealed that there were no significant differences between the hippocampal 3-day and the control 3-day groups $F(1, 13) = 0.88, p = .36$. However, significant differences were detected between the hippocampal 6-day and the control 6-day groups, $F(1, 10) = 10.75, p < .01$, and the hippocampal 12-day and the control 12-day groups, $F(1, 10) = 22.83, p < .0007$. Considering only the hippocampal groups, the mean number of days to criterion by the 3-day group was significantly lower than that observed in the 6-day group, $F(1, 11) = 5.36, p < .04$, and that obtained by the 12-day group: 3-day versus 12-day, $F(1, 11) = 10.01, p < .01$. However, no significant differences were detected between the 6-day and the 12-day groups, $F(1, 10) = 0.01, p = .90$. Finally, when the control groups were compared, three one-way ANOVAs revealed no significant differences: 3-day versus 6-day, $F(1, 12) = 1.20, p = .23$; 3-day versus 12-day, $F(1, 12) = 1.28, p = .27$; and 6-day versus 12-day, $F(1, 10) = 0.01, p = .99$.

These findings suggest that the retention capacity of the hippocampal rat decreases progressively as the time between learning and retraining increases. Thus, hippocampus-lesioned damaged rats that have been able to learn the spatial task at the same rate as the control rats can retain this learning for only a short-to-medium length of time, but not for longer periods.

**Experiment 3**

The third experiment was a control experiment. Its purpose was to investigate the effect of hippocampal lesions on the retention of a spatial task acquired in a simple associative manner, that is, based exclusively on a single cue (guidance strategy). Several studies have suggested that the hippocampal memory system is necessary for the processing of complex relationships among allocentric–allothetic cues, but not for the acquisition of simple rules, for example, approaching a specific cue that by itself defines the location of the goal (McDonald & White, 1993; Packard, Hirsh, & White, 1989; Pearce, Roberts, & Good, 1998). Therefore, we hypothesize that the hippocampal lesion should affect neither the acquisition nor the retention of a spatial task based on a guidance strategy.

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**Figure 5.** Experiment 2, retraining. A: Mean (± SEM) number of errors to criterion for the hippocampus-lesioned (HIP) and control (CON) groups during the retraining phase (retention) of testing. B: Mean (± SEM) number of days to criterion for the HIP and CON groups during the retention phase.
Method

Subjects

Twenty-one experimentally naive male Wistar rats (280–320 g at the time of surgery) were used. Ten rats received electrolytic lesions in the dorsal hippocampus and 11 were sham-operated controls. Rats were housed and maintained under conditions identical to those described in Experiment 1. Surgery, apparatus, and histology were identical to those described in Experiment 1.

Behavioral Procedure

The procedure was identical to that described in Experiment 2 except for two aspects. First, in two of the eight daily training trials, the goal arm with the sandpaper was positioned in the west, in two trials it was in the east, in two it was in the south, and in two it was in the north. The order in which the different goal arms were used was randomized, and it was the same for all the rats. Also, the rotation between the starting arm and the goal arm was controlled to ensure that, at the end of the training period (or the retraining), the number of trials in which the goal arm was located to the right of, to the left of, or opposite the starting arm was the same. This created a situation in which the extramaze information was not relevant and in which it was necessary for the rats to use a guidance strategy versus a place or cartographic strategy to effectively resolve the spatial problem (O’Keeffe & Nadel, 1978). The second aspect that varied was that, when the rats reached the acquisition criterion, they remained in their home cages for 12 days without being tested in any way. Starting on Day 12, the rats received retraining on the spatial task learned during the acquisition phase. The procedure used during the retraining phase of testing was identical to that of the acquisition phase, that is, using a 10-cm × 60-cm piece of sandpaper to signal the position of the goal arm.

Results and Discussion

Histological Results

Representative lesions displaying the maximum and minimum damage resulting from the lesions are represented in Figure 1B.

Behavioral Results

Acquisition. Figure 6 shows the performance of the hippocampal and control groups during the acquisition phase. Two one-way ANOVAs revealed no significant differences between groups in the number of incorrect responses before reaching criterion, \( F(1, 19) = 0.08, p = .78 \) (Figure 6A), or in the mean number of days required to reach criterion, \( F(1, 19) = 0.80, p = .38 \) (Figure 6B).

Retraining. Figure 7 illustrates the data from the retraining phase. Results indicate that the hippocampus-lesioned rats remembered the information learned during the acquisition phase as well as the control rats did. Two one-way ANOVAs revealed that lesioned and control rats did not differ significantly in the number of errors before reaching criterion, \( F(1, 19) = 0.77, p = .39 \) (Figure 7A), or in the number of days of retraining before reaching criterion (\( F \) cannot be computed; Kruskal–Wallis test, \( H = 0, p = 1 \); Figure 7B).

The acquisition data agree with the findings of other authors (see, for example, Packard et al., 1989). However, to the best of our knowledge, the retention data are new, replicating results obtained in previous studies but using a different training–retraining interval (Ramos, 2000). It would appear that the hippocampal system is necessary neither for learning nor for retaining spatial information when such information is acquired through the use of a single cue (guidance strategy).

General Discussion

A considerable number of studies have shown that hippocampal lesions profoundly deteriorate the acquisition of a place response (Morris et al., 1982; Packard & McGaugh, 1992; O’Keeffe et al., 1975; Sutherland et al., 1982; Experiment 1 of this study). However, a previous study found that rats with lesions of the dorsal hippocampus were capable of learning a place response at the same rate as the control subjects when a special training procedure was used, but that a profound deficit in retention was observed 24 days later (Ramos, 2000). The aim of the present study was, therefore, to investigate how long hippocampal rats can retain the spatial information acquired by using a special training method. With this specific goal in mind, a retention test was performed 3, 6, or 12 days after acquisition (Experiment 2). The results showed a profound deficit in retention 6 and 12 days, but not 3 days, after the learning. This suggests, first, that the hippocampus is a necessary component in the long-term retention of spatial memory, given the profound retention deficit observed in the 12-day hippocampal group. It also suggests that the initial role played by extrahippocampal structures in spatial memory storage in rats with electrolytic hippocampal lesions is very limited. Specifically, start-
RETENTION IN HIPPOCAMPAL RATS

Figure 7. Experiment 3, retraining. A: Mean (± SEM) number of errors to criterion for the hippocampus-lesioned (HIP) and control (CON) groups during the retraining phase (retention) of testing. B: Mean (± SEM) number of days to criterion for the HIP and CON groups during the retention phase.

ing on the 3rd day after the end of the spatial training, the storage functions of extrahippocampal structures in electrolytically damaged rats appeared to be dramatically reduced.

The data from Experiment 2 support those obtained in other recent studies using the Morris water maze task, which show that hippocampal rats can learn a place response if special training procedures are used (Day et al., 1999; Whishaw et al., 1995; Whishaw & Jarrard, 1996). However, it is still not known exactly how these special procedures work to overcome the deficit in spatial learning typically observed when a traditional procedure is used, although some possibilities have been suggested. Different studies have shown that hippocampal rats manifest a deficit in behavioral flexibility (Bunsey & Eichenbaum, 1996; Dusek & Eichenbaum, 1997). For example, when hippocampal rats are trained on odor–odor paired associates, they show deficits in transitive and symmetrical relations when compared with controls (Bunsey & Eichenbaum, 1996). Other studies have shown that hippocampal rats perseverate on previously reinforced responses even when the reward contingencies change (Gray & McNaughton, 1983; Hirsh, 1970). As a group, these studies suggest that nonspatial deficits, particularly a deficit in behavioral flexibility, could be a contributing factor in the impaired spatial acquisition normally seen in hippocampus-lesioned rats. As has been suggested by various authors, it is possible that the use of special procedures in the training increases the behavioral flexibility of the hippocampal rats, thus helping to overcome the spatial acquisition deficit (Day et al., 1999). In our case, the sandpaper could have facilitated the development of a place strategy, probably discouraging the use of inappropriate perseverative non-place strategies and helping the lesioned rats to alter their learning strategy more easily (Day et al., 1999; Ramos, 2000).

An important question that arises from the data of Experiment 2 involves the type of strategy used by the lesioned rats when, during the training, a single cue oriented the rat in its navigation towards the goal arm. It is unlikely that the lesioned rats in Experiment 2 learned the task by using a guidance strategy. The data from Experiment 3 indicate that, when the spatial task is learned exclusively on the basis of a guidance strategy, control and lesioned rats remembered the task perfectly 12 days after the training period. For this reason, if lesioned rats from Experiment 2 had learned the spatial task by using a guidance strategy, then we would not have observed any deficit in retention during the retraining phase, yet the retention deficit in these rats was profound. This fact suggests that another probable function of the intramaze cue during the acquisition phase is to facilitate in the hippocampal rats the engagement of extrahippocampal structures that may contribute to the formation of a complex representation of the environment. Or, because the lesions to the dorsal hippocampus were small, it is possible that, during the training, the intramaze cue served to mobilize the remaining portion of the hippocampus; thus, it would appear that any learning that took place occurred in residual hippocampal tissue. Upcoming studies should look into the effect of the extension of the hippocampal lesion in the acquisition of a place response in rats trained with special training procedures.

As for the participation of extrahippocampal regions in navigation, some studies on rats have shown that lesions to the perirhinal cortex (Liu & Bilkey, 1999), the subicular complex (Oswald & Good, 2000), or the entorhinal cortex (Cho & Jaffard, 1995; Nagahara, Otto, & Gallagher, 1995; Otto, Wolf, & Walsh, 1997), among others, produce a deficit in the acquisition of spatial tasks that require the construction of a complex representation of the environment based on the learning of the relations between allocentric cues. Also, functional magnetic resonance imaging studies in humans have shown the involvement of the parahippocampal region in spatial processing (Aguirre, Detre, Alsop, & D'Esposito, 1996; Epstein, Harris, Stanley, & Kanwisher, 1999). How extrahippocampal regions may construct a complex representation of the environment based on allocentric cues is not yet known. However, a number of electrophysiological studies have identified place cells in extrahippocampal regions like the entorhinal cortex (Quirk, Muller, Kubie, & Ranck, 1992), the subicular complex (Sharp, 1999), the parasubicular (Taube, 1995), and the perirhinal cortex (Burwell, Shapiro, O'Malley, & Eichenbaum, 1998). These cells are sensitive to the position of an animal within a particular environment. Other electrophysiological studies have also found extrahippocampal head direction cells (Muller, Ranck, & Taube, 1996; Taube, Muller, & Ranck, 1990). These cells are sensitive to the head direction of an animal in a given environment, and each heading cell has only one preferred direction (Chen, Lin, Green, Barnes, & McNaughton, 1994). It has been suggested that extrahippocampal location-related cells (Sharp, 1997) and extrahippocampal direction-related cells (Golob & Taube, 1997; Kubie et al., 1999) are capable of creating a complex representation of the animal’s environmental context. It may be that the use of special
training procedures, like those used in Experiment 2 of our study, favors the development of a directional and positional extrahippocampal representation of the environment surrounding the animal. This leads to an important question: To what extent is the complex representation of the environment learned by the lesioned rats of Experiment 2 similar to the one learned by normal subjects? Alyan, Jander, and Best (2000) have hypothesized that the hippocampal place cells receive and process sophisticated place and topographic information analyzed previously in different extrahippocampal regions. They propose that the crucial function of the hippocampus is "multi-place and multi-vector topographic integration" (Alyan et al., 2000, p. 225). Future studies should better determine the ways in which hippocampal representation is more elaborate than extrahippocampal representation.

Another possible explanation for the present results is that the performance of lesioned rats in Experiment 2 may still have been mediated by an extramaze cue. Perhaps the use of the salient cue (sandpaper) during training allowed the learning of a second-order association between the salient cue and an extramaze cue that, by itself, was eventually able to support performance during the transfer test. So, an explanation for the difference in retention between the hippocampal and sham rats could be that the content of the association differed. The sham rats learned a place response, whereas the lesioned rats learned a second-order association between cues. The potential weakness of this hypothesis is that, during a second-order association, the discriminative stimulus (sandpaper) would initially be associated with reinforcement and, in a second phase of the acquisition process, would be associated with a second discriminative stimulus (an extramaze cue). This learning would presumably take longer than the acquisition of a place response. For example, in Experiment 3, the rats learned a first-order association (they associated the sandpaper with the reward), yet the hippocampal and control rats took approximately 3 days longer to learn than the rats in Experiment 2 ($p < .00007$). Also, during the acquisition phase of Experiment 3, the hippocampal and control subjects committed more errors before reaching criterion than the subjects in Experiment 2 ($p < .000003$). Thus, if the hippocampal subjects in Experiment 2 had learned through a second-order paradigm, differences in acquisition would have been observed, with the lesioned rats learning more slowly. This, however, is not the case. In a similar manner, it is likely that the representations learned by the control and lesioned rats in Experiment 2 differed in content. But this does not necessarily imply differences in retention. It must be remembered that, during acquisition, 24 hr passed between each experimental session. Therefore, if what was learned by the lesioned subjects of Experiment 2 was more poorly retained than what was learned by the controls, then, in each new session, the lesioned rats would have remembered less than the controls and thus would have taken longer to learn the task. But the two groups learned in a similar number of days. So, the most plausible explanation is that the retention differences observed in the retraining are due to the participation of the hippocampus in retention and consolidation.

An important finding of the present study is the time-limited role in spatial memory storage played by extrahippocampal structures. Experiment 2 showed that the lesioned rats could retain spatial information learned during the training phase for a period of 3 days. Beyond this period, however, when the retention test took place 6 or 12 days after acquisition, a profound deficit in information recall was observed. To better understand the role played by the hippocampus and related extrahippocampal structures in spatial retention, it would be necessary to compare the initial time-limited role in spatial storage played by the hippocampus proper with the role played by extrahippocampal structures. Previous studies revealed that, when neurologically intact rats acquire a place response, a hippocampal lesion sustained 16 days after the learning produces a profound retrograde amnesia (Ramos, 1998). Other authors have found similar results using a different methodology (Riedel et al., 1999). In this last study, reversible inactivation of the dorsal hippocampus was produced from Day 5 until Day 12 after the end of the spatial training by the chronic intrahippocampal administration of LY326325, a selective AMPA/kainate receptor antagonist. The retention test was conducted 16 days after the end of the training, with the hippocampus again working normally. The results showed poor spatial memory recall for the group that was reversibly lesioned. On the basis of the preceding data, it could be argued that 16 days after the learning (in the study by Ramos, 1998) or 12 days after the learning (in the study by Riedel et al., 1999), the dorsal hippocampus still stores spatial information relevant for the recall of the task learned before the lesion. In contrast, the data of the present study indicate that, in rats with electrolytic hippocampal lesions, the extrahippocampal structures can store spatial information only for the 3 days after the end of the learning phase. Thus, in Experiment 2, no significant differences were observed between lesioned and control rats in the retention test performed 3 days after the end of the training, but a profound deficit in retention was detected 6 or 12 days after training. These results suggest that, after learning, in the early phases of the consolidation process, the hippocampus has a more prolonged function than the extrahippocampal structures in the initial temporary storage and retention of the learned spatial information. As an alternative, it could be argued that electrolytic and partial hippocampal damage might indirectly produce an adverse effect on the functioning of other brain structures, including the entorhinal and perirhinal cortex (Murray, Bussey, Hampton, & Saksida, 2000). Future studies should further investigate this possibility, selectively lesioning extrahippocampal structures and studying the way this affects retention.

One issue that deserves attention is the retention deficit observed in the 6- and 12-day hippocampal groups. This deficit cannot be explained by a failure in retrieval, given that the lesioned rats were perfectly capable of recovering the spatial information when the retention test took place 3 days after the end of the training. It has been proposed that, after learning, the hippocampus and related extrahippocampal structures serve as a temporary memory store and that the neocortex is the repository of long-term memory (Alvarez & Squire, 1994; Bontempi et al., 1999; McClelland et al., 1995). According to this model, the consolidation of information would occur gradually in the neocortex as a result of the active participation of the hippocampus and related extrahippocampal structures. However, other research has suggested that, in rats, a period of 12 days after learning (the maximum training-retraining interval used in the present study) is still too short a time for the consolidation of the spatial information to have occurred (Kubie et al., 1999; Ramos, 1998; Riedel et al., 1999). In fact, some studies have suggested that the memory of spatial information depends substantially on the hippocampus for weeks after the training. Thus, as mentioned above, reversible inactivation of the
hippocampus between Posttraining Days 5 and 12 (Riedel et al., 1999) or dorsal hippocampal lesions 16 days after learning (Ramos, 1998) produce a profound retrograde amnesia. In contrast, when more than 2 months elapsed between the end of the spatial training and the dorsal hippocampal lesions, hippocampal damage did not produce retrograde amnesia in rats (Kubie et al., 1999; Ramos, 1998). These data suggest once again that the hippocampus works as a spatial storage region for a long period of time after learning. To conclude, the retention deficit observed in the lesioned rats when the test was administered 12 days after learning could be explained in several ways: first, by the absence in the neocortex of a strong representation of the learned spatial information (that is, no consolidation); second, because the initial temporary storage of spatial information dependent on extrahippocampal structures had already faded in rats with electrolytic hippocampal lesions; and third, because the temporary storage of the spatial information in the hippocampus proper was defective as a result of the lesion.

In summary, using a special training procedure that permits the dissociation of the processes of acquisition from those of retention, the results of the present study suggest that the hippocampus is not necessary at the time of spatial learning but is necessary for the long-term retention of spatial information. It is suggested that, after learning, the hippocampus serves as a temporary spatial memory store for a longer time than do the extrahippocampal structures. How the differential contributions of the hippocampus and extrahippocampal structures to temporary retention affect the process of consolidation should be further investigated.

References


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