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Hippocampal Lesions That Abolish Spatial Maze Performance Spare Object Recognition Memory at Delays of up to 48 Hours

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ABSTRACT: The hippocampus is widely considered to be a critical component of a medial temporal lobe memory system, necessary for normal performance on tests of declarative memory. Object recognition memory is thought to be a classic test of declarative memory function. However, previous tests of the effects of hippocampal lesions on object recognition memory have not always supported this view. One possible reason for this inconsistency is that previously reported effects of hippocampal lesions on object recognition memory tasks may have stemmed not from a deficit in object recognition memory per se, but as a result of spatial and contextual confounds in the task. Thus, in the present study, we used a spontaneous object recognition test in a modified apparatus designed to minimize spatial and contextual factors. A group of rats with complete excitotoxic lesions of the hippocampus and a group of control rats were tested on this modified spontaneous object recognition task with retention delays of up to 48 h. These rats were also tested on a spatial nonmatching-to-place task. Spatial memory performance was abolished following hippocampal lesions, whereas performance on the recognition memory task was intact at all delays tested. © **2004 Wiley-Liss, Inc.**

KEY WORDS: rat; declarative memory; medial temporal lobe

INTRODUCTION

A medial temporal lobe (MTL) memory system, including the hippocampus, the perirhinal cortex, the entorhinal cortex, and the parahippocampal cortex, is widely thought to be necessary for normal declarative memory (Eichenbaum et al., 1994; Squire and Zola-Morgan, 1991). Declarative memory in humans is defined as conscious long-term memory for facts and events. Animal models of the structures involved in declarative memory often use tests of object recognition memory, thought by some to be a "relatively pure test of declarative memory capacity" (Manns et al., 2000). The MTL memory system view predicts, therefore, that hippocampal lesions should produce impairments in tests of recognition memory. However, previous experiments in animal models of amnesia testing this prediction have yielded conflicting results. Whereas many studies have reported deficits in object recognition with complete hippocampal lesions (e.g., Beason-Held et al., 1999; Clark et al., 2000; Nemanic et al., 2004; Prusky et al., 2004; Zola et al., 2000), a comparable number report sparing (e.g., Aggleton et al., 1986; Mumby, 2001; Murray and Mishkin, 1998; Nemanic et al., 2004; Winters et al., 2004). Thus, the critical question of the role of the hippocampus in object recognition memory remains unresolved.

Two standard tests of object recognition memory used in rats are delayed non-match to sample (DNMS), and spontaneous object recognition (SOR; also referred to as the "visual paired-comparison task"; Clark et al., 2000). The SOR task has the advantage that rats are not required to learn a rule before being tested for recognition memory. Instead, the task capitalizes on the natural tendency for rats to explore a novel object in preference to a familiar object. This is important because it has been argued that intact recognition memory following combined lesions of the hippocampus and amygdala in the primate (Murray and Mishkin, 1998) could be due to extensive preoperative training to perform the nonmatching- to-sample rule, and this overtraining might have masked an impairment in subsequent tests of recognition at longer delays (e.g., Zola et al., 2000). Thus, in the present study, we assessed object recognition memory using the SOR task, which does not involve training.

Mumby (2001) recently reviewed studies in rats that used both DNMS and SOR tasks that investigated the role of the hippocampus in recognition memory. All but one of the papers reviewed involving the SOR task included rats with a fornix transection rather than lesions of the hippocampus. This type of lesion may not be functionally equivalent to a hippocampal lesion (e.g.,Clark et al., 2000; Vann et al., 2000). More to the point, the fornix is not thought to be part of the MTL memory system (Clark et al., 2000), and so fornix lesions cannot be used stringently to test the MTL system view. The present study therefore examined the object recognition memory of rats with complete excitotoxic lesions of the hippocampus.

The one study cited in Mumby's (2001) review that tested rats with excitotoxic lesions of the hippocampus on the SOR task reported an impairment at delays of 10 min and 1 h (Clark et al., 2000). It remains unclear, however, whether standard SOR tasks test "pure" object recognition memory, or whether they test memory for objects set in the context in which the object is presented. Indeed several authors have suggested that the hippocampus may be important for object recognition when spatial or contextual factors become important (Aggleton and Brown, 1999; Bussey and Aggleton, 2003; Gaffan, 1994; Nadel, 1995; Zola et al., 2000).

Therefore, if one is interested in the neural structures involved in "pure" object recognition memory, the presence

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of such spatial or contextual factors could be an unwanted confound. This may be particularly important in the standard version of the SOR task. This task is normally carried out in a large open arena, within which the rat can explore at will. Because the rat can often be up to 1 m from the farthest wall, it will have a clear view over the wall of the contextual cues that make up the room. It is difficult to know what cues normal rats use when directing their spontaneous behavior, and so it is harder still to determine whether abnormal patterns of spontaneous object exploration within the arena are influenced by these spatial or contextual confounds. The possibility of spatial and contextual confounds being present in this task may be particularly important when studying the role of the hippocampus in recognition memory, as it is known that hippocampal lesions can lead to impairments in spatial or contextual memory (Holland and Bouton, 1999; Jarrard, 1993; Morris, 1991). Moreover, such lesions can lead to hyperactivity and impulsivity (Whishaw and Jarrard, 1995), which could confound the study of SOR when tested in an open field.

In the present study, we attempted to minimize such spatial and contextual confounds by using a specially designed apparatus. This Y-shaped apparatus has high, white walls and a ceiling to occlude the rat's view of the room. This reduces the context surrounding the objects by reducing presence of any cues other than the objects themselves and making the apparatus itself homogeneous. In addition, the arms in which the objects were placed were short and narrow. This minimizes locomotor exploration of the environment, and allows placement of the objects close together and close to the rat, to maximize the amount of exploration of the objects relative to the surrounding context.

Rats with excitotoxic lesions of the hippocampus were tested in this apparatus to assess their recognition memory performance at retention delays of 15 min, 1 h, 24 h, and 48 h (Experiment 2). To test the functional efficacy of the lesions, and to control for possible order confounds, the rats were also tested on a spatial nonmatching-to-place task both before (Experiment 1) and after (Experiment 3) the object recognition testing.

EXPERIMENT 1: NONMATCHING-TO-PLACE IN A CROSS-MAZE

The aim of this first experiment was to test the functional efficacy of the hippocampal lesions by testing the rats on a task on which subjects with hippocampal damage would be expected to be impaired. The task was carried out in a cross-shaped maze in a room with many distinct cues. The rats learned to remember the arm of the maze that was rewarded in a sample phase, and on choice to enter the other arm for a reward. The task was run with two possible starting locations, and therefore could not be solved using only egocentric strategies, e.g., "turn left, then turn right."

Materials and Methods

Subjects

Eighteen naive, male rats of the Lister Hooded strain (Harlan, UK) were used for this study. All subjects were housed in groups of four under reversed diurnal conditions (12 h light/12 h dark), and all testing occurred at a regular time during the dark period. The animals were maintained at >85% of normal body weight by restricting their daily food. Prior to surgery animals weighed 270–330 g. Animals had ad libitum access to water throughout the testing period.

Surgery

Prior to surgery, rats were randomly allocated to one of two experimental groups; 8 rats were assigned to receive hippocampal lesions, and 10 rats received sham control surgeries. The rats were anesthetized with tribromoethanol (Avertin, 10 ml/kg) by intraperitoneal injection and placed in a Kopf stereotaxic head-holder. The scalp was cut and retracted to expose the skull. Craniotomies were then made directly above the target regions using a dental drill. Following surgery, all animals had the wound sutured, received 10 ml of 10% glucose/saline solution subcutaneously and were allowed to recover. Behavioral testing began 2–3 weeks after surgery.

The hippocampal lesions were made with ibotenic acid (IBO; Biotechnology, USA) dissolved in phosphate buffer (pH 7.4) following the protocol of Jarrard (1989). Several injections of ibotenic acid (10 mg/ml) were made at different rostrocaudal and dorsoventral levels via a 1-μl beveled Hamilton syringe. After the needle was slowly lowered, 0.05, 0.08, or 0.1 μl was injected over 2 min. The needle was removed very slowly, 4 min after the injection. A total of 0.6 μl per hemisphere was injected. The stereotaxic coordinates were modified from those used by Jarrard (1989) as follows (bregma is used as the zero point for the A-P and M-L coordinates, while the D-V measure is taken from the surface of the cortex, the incisor bar was set to −3.3 mm): A-P, −2.4; M-L, 1.0; D-V, −3.4; A-P, −3.0; M-L, 1.4; D-V, −2.6; −3.4; A-P, −3.0; M-L, 3.0; D-V, −3.0; A-P, −4.0; M-L, 2.6; D-V, −2.3; −3.3; A-P, −4.0; M-L, 3.7; D-V, −3.0; A-P, −4.9; M-L, 3.9; D-V, −3.5; −7.0; A-P, −5.7; M-L, 4.1; D-V, −3.8; A-P, −5.7; M-L, 5.1; D-V, −4.0; −4.9, −5.8. Rats in the sham control lesion group were subjected to exactly the same initial surgery, but no injections were made.

Histology

After completion of the experiment, the animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (Euthatal TM, Rhône Merieux, Essex, UK) and perfused transcardially with saline followed by 4% formol-saline. The brain was removed and postfixed in formol-saline before being transferred into 20% sucrose in 0.1 M phosphate buffer and left overnight. Coronal sections were cut at 60 μm on a freezing microtome and stained with cresyl violet Nissl stain.

Apparatus

The cross-maze used in this experiment (see Fig. 1) was made of wood, with four identical arms 14 cm wide and 1.65 m long each with a wooden edge 2 cm high extending from a central platform. At the end of each arm was a recessed metal food well. The maze

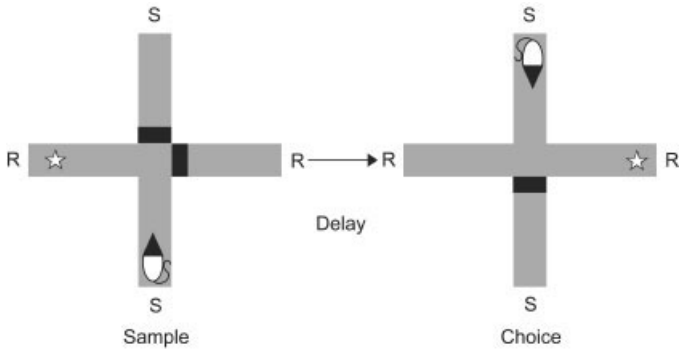

FIGURE 1. Example of a trial in the cross-maze. R and S indicate "reward" and "start" arms, respectively (see text for explanation), a star indicates a reward is present in the arm, a black line indicates the arm is blocked. Left is the sample phase, with the rat placed in the near start arm and the left reward arm baited. The rat must choose the baited arm; the other two arms are blocked. Right is the choice phase; with the rat placed in the far start arm and the right arm baited. The rat can choose either reward arm, a correct response is rewarded.

was elevated 50 cm off the floor. The rat had a clear view of the surrounding room from all positions on the maze. Wooden blocks were used to block selected arms during a trial. The maze was placed approximately in the center of a room that was ~2 m × 3 m. The room was filled with a diverse arrangement of items, such as a black water maze on its side, a rack of empty cages, a sink, and some full shelves. Below the central platform of the maze was a radio, which provided a constant noise to mask sounds from neighboring rooms. The experimenter stood in the room during testing. Correct arm choices were reinforced during the trials with a piece of breakfast cereal (Honey Nut Cheerios, Nestle, UK).

Behavioral procedure

The method used was a modification of a nonmatching-to-place task that was used by Warburton et al. (1997). The task was run in a cross-shaped maze. Two arms were designated "start" arms and two were designated "reward" arms (Fig. 1).

Rats were trained on the task with a minimum delay (~5 s) between the sample and choice stages. Each session of 8 trials was run over two days. All rats were initially run for 4 sessions to obtain acquisition data for all animals. Thereafter, they were run until they reached a criterion of 75% correct in one session.

Habituation. Rats were placed in one of the start arms, and allowed to explore the maze freely for 5 min. On day 1, food rewards were scattered over all the arms; on days 2 and 3, food was in the wells of only the reward arms.

Testing. Each trial consisted of two phases, sample and choice, separated by a delay:

Sample Phase: The rat was placed in the end of a start arm and allowed to explore. One reward arm and the unused start arm were both blocked. At the end of the open reward arm was a food reinforcer. Once the animal had eaten the reward it was removed from the maze. Both possible start arms and reward arms were used during testing on different trials; the two arms used on a given trial were determined by a pseudo-random (Gellerman) sequence.

Delay: During acquisition the delay was as short as possible (~5 s) and the experimenter held the animal during the delay.

Choice Phase: The rat was placed in a start arm; for half the trials this was the same as the sample start arm and for the other half this was different from the sample start arm. The unused start arm was blocked. On choice the rat was allowed to choose either of the reward arms; to obtain a food reward the rat had to choose the reward arm not visited in the sample phase. Once the animal had made a choice, and after eating the reward if it made the correct choice, the arm chosen was recorded and the animal was removed from the maze.

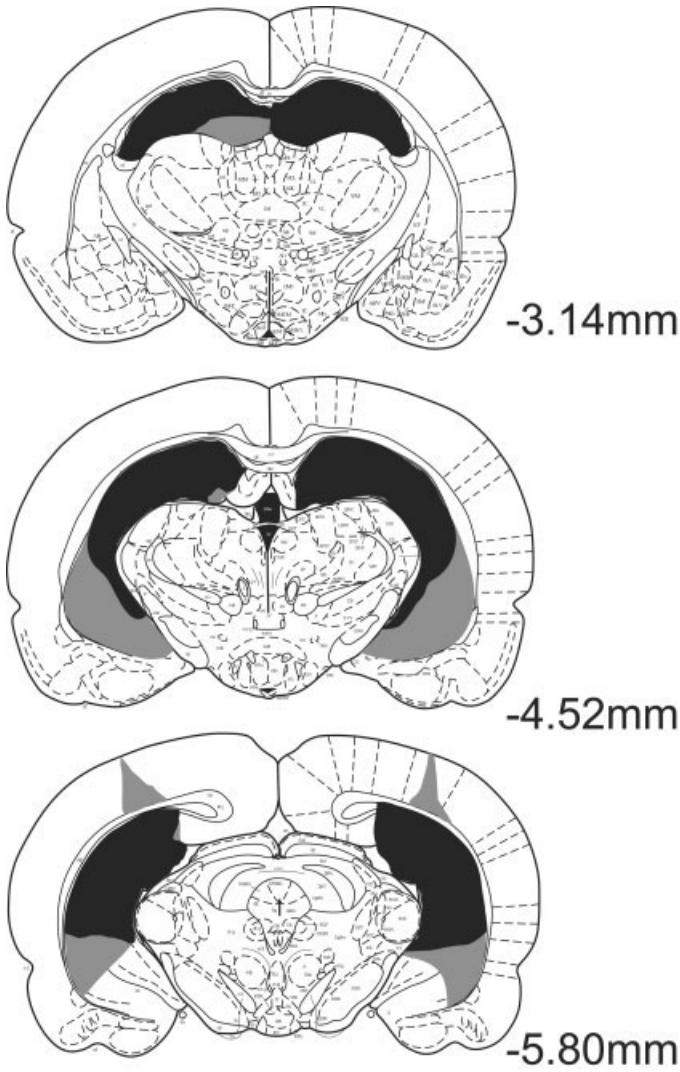

FIGURE 2. Coronal sections illustrating the extent of the largest (gray) and smallest (black) lesions of the hippocampus at −3.14 mm, −4.52 mm, and 5.8 mm from bregma according to the atlas of Paxinos and Watson (1997).

Results

Histology

All but one animal in the hippocampal lesioned group had bilateral lesions of the hippocampus. The exception had minimal damage to the right hippocampus and so was eliminated from the study. Of the remaining animals, two had the smallest extent of damage (Fig. 2, black), and had sparing of the posterior ventral hippocampal structures. Two animals had the largest extent of damage (Fig. 2, gray), and in addition to the hippocampal damage they also had minor damage to the dorsal visual areas V2L (Paxinos and Watson, 1997).

Behavioral testing

Each animal was given four sessions of training (29 trials), and the fourth and any subsequent sessions were then assessed to see if the rat had reached the criterion of 75% correct (to a maximum of 8 sessions, 61 trials). Owing to time constraints, the full complement of trials could not be run for the first two sessions. Instead session 1 consisted of 6 trials and session 2 consisted of 7 trials; thereafter all sessions consisted of 8 trials. All rats were given the same number of trials per session.

The control rats took an average of 29.4 trials to reach criterion; nine of the ten control animals reaching it in the fourth session when they were first assessed for reaching criterion. Although this is a low criterion, six of the seven hippocampal-lesioned animals failed to reach this criterion in the eight sessions tested.

The percentage correct performance for the two groups of animals in the first four sessions of training is given in Figure 3. An analysis of variance revealed a significant main effect of lesion ($F(1,16) = 25.01$, $P < 0.001$), with no other significant effects or interactions (all $P > 0.05$).

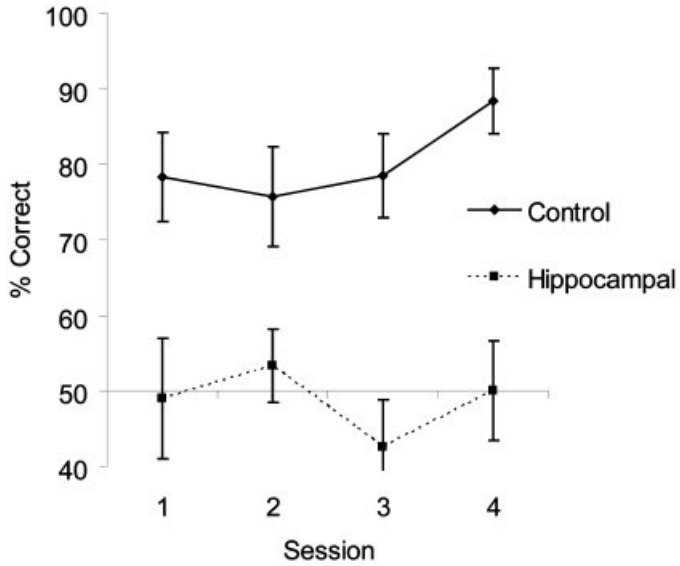

FIGURE 3. Performance of control and hippocampal lesioned animals on the first four sessions of the cross-maze task at minimal delays (~5 s). Error bars indicate SEM.

To test whether the hippocampal rats performed significantly differently from chance, their performance over the four sessions was averaged and a t-test was used to compare this value with a score of 50% (chance performance). This analysis showed that the performance of the hippocampal lesioned rats was not significantly different from chance ($t = 0.177$, $P > 0.05$).

EXPERIMENT 2: OBJECT RECOGNITION

This experiment tested whether the hippocampus is necessary for object recognition memory. The two groups of animals were run on the SOR task with delays of 15 min, 1 h, 24 h and 48 h. The apparatus used was specially designed to minimize spatial and contextual confounds (see Introduction).

Materials and Methods

Subjects

The same subjects were used as for Experiment 1, having already undergone the surgical procedures described in Experiment 1. Testing began six weeks after the completion of Experiment 1.

Apparatus

The task was carried out using a Y-shaped apparatus made of white Foamalux (a foam PVC sheet material, Brett Martin, UK). The three arms of the apparatus were separated by 120-degree angles. The objects were placed in two of the three arms, the "object" arms; they were both 23 cm long and 10 cm wide and in every respect identical to each other (see Fig. 4). The third arm was the start arm; it was 10 cm wide with a guillotine door 10 cm from the center of the apparatus. During exploration time this door remained shut. Behind the door was the start box (10 cm × 20 cm), which was covered with an opaque plastic lid.

A white shelf 40 cm from the top of the apparatus effectively formed a ceiling, preventing the rat from viewing any part of the room. The walls of the apparatus were all 40 cm high, so when inside it the rats could neither see out nor jump up the walls to climb out. The apparatus was lit by a fluorescent lamp placed centrally above it. The floor was not washed between rats so that it became saturated with smells, but was wiped between trials with a dry cloth when necessary. A video camera was attached to the underside of the shelf above the apparatus looking down on it to film the rats in the apparatus. This was connected to a Phillips TV/VCR monitor, which showed the rat in the apparatus during the experiment. The experimenter stayed in the test room during testing and watched the rat on the monitor while scoring its exploration behavior with the help of a computer program written by SEF in Visual Basic (Microsoft, USA).

Stimuli

Objects used were made of metal, ceramic, and plastic. They ranged in size from 10 cm wide by 10 cm deep × 2 cm high, to 5 cm wide × 5 cm deep × 15 cm high; when placed in the appa-

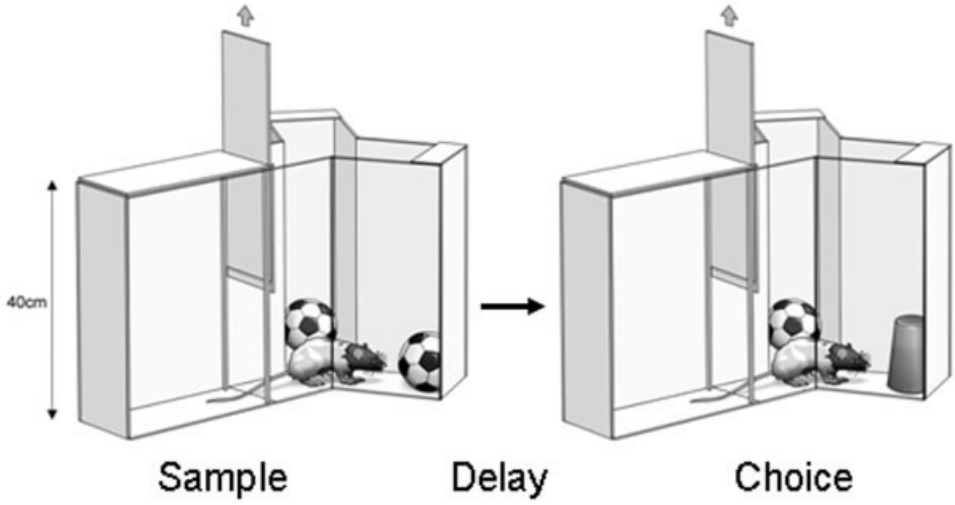

FIGURE 4. Apparatus used in for the spontaneous object recognition (SOR) task showing examples of the Sample and Choice phases. The near wall appears transparent for illustrative purposes only. The guillotine door is shown raised. (Illustrations by Charles Henn).

ratus, the object was ~15–20 cm from the center of the apparatus. All objects were secured to the floor of the apparatus with Blu-Tack (Bostik, UK), and were always placed in the apparatus in the same orientation. The objects all varied in shape, color, and patterning, and rats could explore the objects using touch, taste, and smell, as well as vision. They could explore the face of the object and could rear up and explore the top surface. Objects were chosen that the rats could not chew, or leave scratch marks on, and triplicate copies of each object were used within one testing session. Objects were wiped down with 50% ethanol solution between each trial to discourage discrimination using olfactory cues. As far as could be ascertained, the objects had no natural significance for the rats and they had never been associated with a reinforcer. The rats had never encountered the objects before.

Procedure

Each rat was given one trial a day for four consecutive days at each delay. Four trials were given per week. A different delay was tested on each of four consecutive weeks. To control for possible order effects, the delays were not tested sequentially, but rather in the following order: 15 min, 24 h, 48 h, and then 1 h.

Four pairs of objects were used to test each delay. No rat was tested on the same pair of objects more than once. The order in which the objects were presented was counterbalanced between rats and delays, such that each pair was used for all the rats and in all the delays. Within the pair, the specific object presented in the sample phase was also counterbalanced so half the rats were shown one object and half the other. The side of the apparatus in which the novel object was placed was counter-balanced in a pseudo-random order, such that it was equally as likely to occur on the left as on the right.

Habituation. Rats were placed in the empty apparatus for 5 min on the two days before testing began to familiarize them with the apparatus. At the start of a trial, a rat was placed in the start box and was released into the apparatus when the door was opened. When the rat moved into the main part of the apparatus of its own accord the guillotine door was shut behind it.

Testing. Each trial consisted of two phases: sample phase, and choice phase, separated by a delay (see Fig. 4):

Sample Phase: The rat was placed in the start box and released into the apparatus with two identical copies of the sample object at the end of each object arm. The experimenter watched the rat on the monitor and scored the rat's exploration of each object using a Visual Basic computer program. The experimenter scored the rat as exploring an object when its nose was within 2 cm of the object. Exploration was not scored if the rat looked at the object from a distance, looked over the object to the back wall, looked behind the object, or climbed onto the object. When the rat had explored for a total of 25 s, or when the rat had been in the box for 3 min, whichever happened first, the rat was removed.

Delay: During the delay the rats were kept in a transport box in the testing room for the shortest delay, and returned to their home cage for the other three delays.

Choice Phase: The rat was placed in the start box and released into the apparatus with an identical third copy of the object seen on sample and its paired object, which was novel. As on sample, the experimenter watched the rat and scored the rat's exploration of each object using a Visual Basic computer program. The rat remained in the apparatus for 3 min, and was then removed and returned to its home cage.

Data Analysis

Performance was measured by calculating a discrimination ratio from the exploration that took place in the first minute of the choice phase (Dix and Aggleton, 1999). The discrimination ratio is the difference in exploration between the novel and familiar objects as a ratio of the total object exploration.

Results

Behavioral testing

The rats' exploration during the sample phase was analyzed by analysis of variance (ANOVA). This analysis revealed no main effect of delay or of lesion (both $F < 1$), demonstrating that the two groups of rats did not differ significantly in the amount explored during the sample phase (means of 21.1 s for controls and 21.3 s for hippocampal lesioned animals), and that at each delay the amount explored during the sample phase did not differ (means of 21.1 s at 15 min, 21.1 s at 1 h, 20.8 s at 24 h and 21.8 s at 48 h).

The amount of exploration that took place during the choice phase was also analyzed. Again there was no main effect of delay or of lesion (both $F < 1$), demonstrating that the two groups of rats did not differ significantly in the amount explored during the choice phase (means of 25.1 s for controls and 26.4 s for hippocampal lesioned animals) and that at each delay the amount explored during the choice phase did not differ (means of 24.1 s at 15 min, 25.9 s at 1 h, 27.5 s at 24 h, and 25.2 s at 48 h). The recognition memory performance, as measured by the discrimination ratio, was calculated. An average score for each group at each delay is plotted in Figure 5. On this scale, a score of zero indicates no preference, a positive score indicates a preference for exploring the novel object, and a negative score indicates a preference for exploration of the familiar object. ANOVA revealed a significant main effect of delay ($F(1,16) = 13.68$, $P < 0.001$), no significant effect of lesion and no interaction (both $F < 1$).

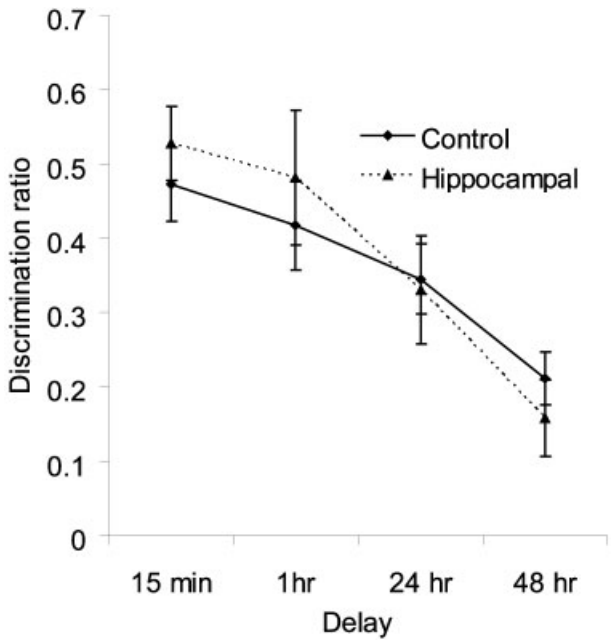

FIGURE 5. Performance of control and hippocampal rats on the spontaneous object recognition (SOR) test at increasing delays. Performance is shown as a discrimination ratio; a score of zero indicates no side preference and a positive score indicates a preference for exploring the novel object Error bars indicate SEM.

EXPERIMENT 3: CROSS-MAZE TASK REPEATED

The aim of this experiment was to test whether the lack of impairment following hippocampal lesions on the recognition memory task was due to recovery of function. Thus, rats were retested on the spatial cross-maze task used in Experiment 1 at 5-s delay for two sessions.

Materials and Methods

Subjects

The same subjects were used as for Experiment 1, having already undergone the surgical procedures described in Experiment 1. Testing began four weeks after the completion of testing for Experiment 2.

Apparatus

The same apparatus was used as for Experiment 1, placed in the same testing room. The same reinforcer (Nestle Honey Nut Cheerios) was used.

Procedure

Before testing, the rats received two days of rehabituation to the cross-maze apparatus.

The same procedure was used as in Experiment 1 for training, i.e., rats were run on a minimal delay (~5 s). Rats were tested for 2 sessions.

Data Analysis

The same performance measures were used as in Experiment 1.

Results

Behavioral testing

The percentage correct performance score for the two groups of animals in the two sessions of testing is given in Figure 6. ANOVA revealed a significant main effect of lesion ($F(1,16) = 32.4$, $P < 0.001$), with no other significant effects or interactions.

To test whether the hippocampal rats performed significantly differently from chance, their performance over the two retest sessions was averaged and a *t*-test was used to compare this value with 50% (chance). This analysis showed that the performance of the hippocampal lesioned rats on the spatial task retest was not significantly different from chance ($t = 0.541$, $P > 0.05$).

DISCUSSION

The present study sought to test whether the hippocampus is critical for object recognition, using an apparatus designed to minimize spatial and contextual confounds. It was found that rats with

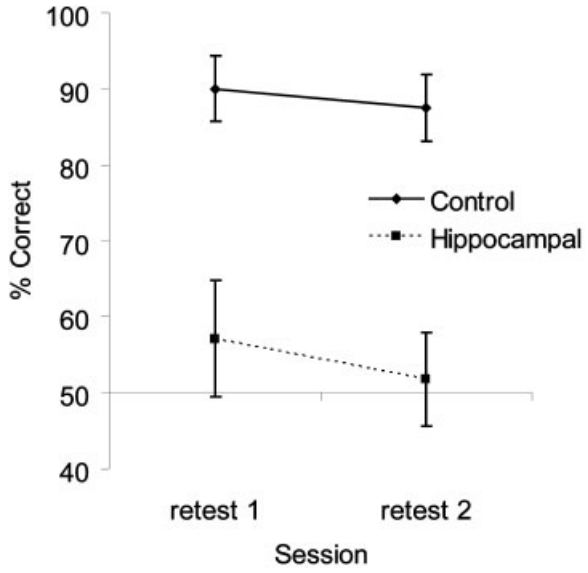

FIGURE 6. Performance of control and hippocampal lesioned animals on two retest sessions of the cross-maze task. Error bars indicate SEM.

complete excitotoxic lesions of the hippocampus were clearly unimpaired on the SOR task, even at delays of 48 h. This is to our knowledge the longest delay tested in rats using this task. The increasing delay significantly reduced performance in all the animals tested, indicating normal forgetting in the control group. This is important because the presence of normal forgetting in controls brings performance down from a ceiling, so that any impairment in the lesioned animals would have become apparent at the longer delays. This was not the case.

In contrast, the same hippocampus-lesioned rats showed a profound impairment on a spatial nonmatching-to-place task. This spatial test was given both before and after the object recognition test, and rats with hippocampal lesions were severely impaired on both tests; indeed, the performance of hippocampus-lesioned rats never rose above chance level. This persistent deficit shows that the lack of impairment on object recognition was not due to functional inefficacy of the lesions, or to recovery of function as the experiment progressed. Together these results demonstrate that the hippocampus is not necessary for object recognition memory with delays as long as 48 h.

It might be argued that the testing method used in the present study is not sufficiently sensitive to be able to detect impairments following brain damage. A companion to the present study (Winters et al., 2004), however, suggests otherwise. Although excitotoxic hippocampal lesions did not impair object recognition memory in that study (thus replicating the findings of the present study), combined lesions of the peri- and postrhinal cortex did impair object recognition memory, using the identical method to that used in the present study. Furthermore, these peri and postrhinal lesions did not impair all types of memory; performance in a radial arm maze task was unimpaired (see also Bussey et al., 1999). Performance following hippocampal lesions was, however, severely impaired in this spatial task. This companion study thus demonstrates the efficacy of the method in detecting lesion-induced impairments on object recognition memory. It also indicates a double dissociation between the effects of hippocampal and perirhinal cortex lesions.

The question of whether the hippocampus is critical for object recognition memory has been a central issue in the quest to understand the anatomical basis of memory. Whereas some laboratories consistently report impairments following hippocampal lesions (e.g., Clark et al., 2000; Nemanic et al., 2004; Zola et al., 2000; Zola-Morgan and Squire, 1986; Zola-Morgan et al., 1989), others do not (e.g., Aggleton et al., 1986; Mumby, 2001; Murray and Mishkin, 1998; Nemanic et al., 2004; Winters et al., 2004). Murray and Mishkin (1998), for example, found no impairment following combined lesions of the hippocampus and amygdala on the DNMS recognition task. It has been argued, however, that the extensive pretraining used in the experiment of Murray and Mishkin (1998) might have masked an impairment in the subsequent tests of recognition at longer delays (Zola et al., 2000). Such explanations cannot be applied to the lack of effect in the present study because the behavior was spontaneous; there was no pretraining at all. It therefore seems unlikely that the lack of effect reported by Murray and Mishkin (1998) was a spurious result, due to extended pretraining.

These data contrast with those reported by Clark et al. (2000), who reported a significant impairment following excitotoxic hippocampal lesions on the SOR task at delays of 10 min and 1 h. In many respects, the behavioral testing methods of the present study and that of Clark et al. (2000) were identical. The most noticeable difference between the methods in these two studies was the apparatus used. The apparatus used in the present study was explicitly designed to minimize possible spatial or contextual confounds. The lack of impairment in such an apparatus is consistent with the hypothesis that previously reported effects of hippocampal lesions on object recognition tasks may have been due to spatial or contextual factors. In line with this hypothesis, a recent study by Prusky et al. (2004) has shown that rats with hippocampal damage were impaired on a new version of the DNMS task in a 1.4-m $\times$ 80-cm water tank using visual stimuli of $\sim$25 cm $\times$ 34 cm, where the size of the apparatus and the stimuli could feasibly contribute spatial or contextual confounds to the task. Several authors have already suggested that the hippocampus may be important for object recognition when spatial or contextual factors become important (Aggleton and Brown, 1999; Bussey and Aggleton, 2003; Gaffan, 1994; Nadel, 1995; Zola et al., 2000).

It should be emphasized, however, that the present findings by no means prove that previously reported impairments in object recognition following hippocampal lesions were entirely due to spatial or contextual confounds. While the present data are consistent with this idea, the present study did not explicitly test it. In order to do this, one must show that hippocampal impairments on an object recognition task can be both present and absent when some spatial or contextual variable is manipulated. The significance of these data is that they highlight the need to understand better what these variables are. What is it that makes the Y-shaped apparatus used in this study different from the conventional rectangular arena? This may include variables such as the distance from

which the rat views the object, the distance covered by the rat to reach the object or the complexity of the context or background around the object, all of which were designed to be minimized in the Y-shaped apparatus. Testing the importance of such variables on the presence of hippocampal impairments on an object recognition task is a natural follow-up experiment to the present study. This would give a valuable insight into the role of the hippocampus in long term memory. Such an experiment would also evaluate the suggestion made by Winters et al. (2004) and Bussey and Aggleton (2003) that real-world event memories lie in the middle of a continuum between object and spatial memory, and that the hippocampus becomes involved to the extent that there is a spatial component to the memory task.

CONCLUSIONS

Many recent behavioral studies investigating the lower-level mechanisms of memory in the hippocampus, using pharmacological, genetic and other methods, have assumed that the SOR task taps hippocampal function. We suggest that under certain conditions it may do so, not by design from the demands on object recognition memory per se, but rather as a result of the presence of spatial or contextual factors. In contrast, the hippocampus appears to be critically important in tests of spatial and contextual memory. This leads us to suggest that at present the best task for studying the low-level mechanisms of memory in the hippocampus may not be object recognition, but rather tasks that tax hippocampal function directly such as spatial tasks like the Morris swim task. Further investigation is needed to identify the precise conditions in which performance on the SOR task does require an intact hippocampus.

If the hippocampus does not play a role in "pure" object recognition memory, as the present results suggest, one of at least two possible conclusions can be drawn. Either the hippocampus does underpin declarative memory, but the SOR task is not an appropriate test of declarative memory, or, alternatively, the task may be an appropriate test of declarative memory, but the hippocampus does not underpin all types of declarative memory. Most likely, as suggested by Zola et al. (2000), the role of the hippocampus is to provide place or contextual information that can be bound with object representations from the perirhinal cortex to form complex, multimodal memories.

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