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Retrograde amnesia in rats with lesions to the hippocampus on a test of spatial memory

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Abstract

The present study examined remote spatial memory in a test that spans several months to determine whether remote memories are spared relative to more recent ones, as predicted by models of memory consolidation. At 3, 6 or 12 months of age, groups of rats received forced-choice training as to the location of food reward in a cross maze. At 12.5 months, rats received bilateral neurotoxic lesions to the hippocampus or a control surgical procedure and 2 weeks later their memory for the spatial location was tested. Their performance was compared to that of rats with hippocampal or control lesions with no prior training on several measures of savings. The hippocampal group with no pre-training, as expected, was severely impaired in learning the location of the food reward. Compared to this group, rats with hippocampal lesions that were pre-trained consistently performed better at the shortest training–surgery interval but not at the longer ones. That is, rats with hippocampal lesions exhibited retrograde amnesia at all training–surgery intervals and a forgetting curve that paralleled that of the control groups. The results were interpreted within a framework that distinguishes between relational and associative context, and as providing evidence that the hippocampus is necessary for the retention and retrieval of memories that are bound to relational context, regardless of the age of the memory.

Keywords: Hippocampus; Retrograde amnesia; Spatial memory; Rats; Brain-damage; Cross-maze; Forgetting

1. Introduction

Research into remote memory following medial temporal lesions (MTL) has yielded two patterns of retrograde amnesia (RA) in rats, monkeys and humans. One pattern is characterized by a temporal gradient in which remote pre-morbid memories are retained better than recent memories (e.g., Kim & Fanselow, 1992; Winocur, 1990; Zola-Morgan & Squire, 1990; for review, see Squire, Clark, & Knowlton, 2001). Indeed, retention of remote memory may be normal. Traditionally, this pattern has been interpreted in terms of consolidation theory (Squire, 1992), which states that a period of time is required to form enduring representations. The hippocampus and related MTL structures are said to be necessary for memory retention (and retrieval) only until the consolidation process is complete, after which memories can be recovered directly from extra-hippocampal structures.

A second general pattern of RA has been observed in which memory loss is severe and extensive for the entire period that is tested (Sutherland et al., 2001; Warrington & Sanders, 1971). In some cases, RA parallels the normal forgetting curve, with superior memory for recently acquired information (Viskontas, McAndrews, & Moscovitch, 2000). In other cases, there is no gradient to speak of, with all memories being equally inaccessible (for review, see Fujii, Moscovitch, & Nadel, 2001). Contrary to consolidation theory, these

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results indicate that the hippocampus and related structures can contribute indefinitely to memory retention and retrieval. This view is broadly consistent with multiple trace theory (MTT), which posits that the hippocampal complex is needed for maintaining and recovering detailed representations of some kinds of memories, regardless of their age (Moscovitch, 2002; Nadel & Moscovitch, 1997).

In attempting to resolve the discrepancy between the two patterns of RA, Rosenbaum, Winocur, and Moscovitch (2001) proposed that the respective patterns were linked to context-dependent and context-free memories in animals, which may correspond to episodic and semantic memory in humans. Context-dependent memories are those in which the complex of cues that defines the target event are linked relationally to each other in spatial (e.g., allocentric cues in water, radial arm and cross mazes) or non-spatial (e.g., configural learning) ways, or both. By contrast, context-free memories exist separately from the relational context, but may be supported by associations formed with elements of the context independently of each other (associative context; e.g., food smell in the food-preference task (Winocur, 1990) and conditional or background stimuli in avoidance conditioning (Winocur, Rawlins, & Gray, 1987). Our view, in line with a growing body of evidence that links hippocampal function to the process of forming contextual associations (Anagnostaras, Gale, & Fanselow, 2001; Nadel & Willner, 1980; Winocur, 1997; Winocur et al., 1987), is that hippocampal lesions affect only those remote memories that are dependent on relational context. The distinction between relational and associative context is similar to the distinctions made by Sutherland and Rudy (1989) between configural and simple associations and by Anagnostaras, Gale, and Fanselow (2001) between unified representations and elemental associations.

It follows from our position that if a memory is always dependent on relational context, the hippocampus will always be implicated and that damage to the structure will disrupt performance regardless of when the memory was formed. Spatial memory prototypically relies on relational context and, consequently, performance on tests of this type of memory should be impaired following hippocampal lesions at all delays after acquisition. The following experiment provides a direct test of this prediction by assessing the effects of hippocampal lesions on remote spatial memory.

It is well known that rats with hippocampal lesions are reliably impaired in learning spatial locations in various tasks, where successful performance depends on effective use of relational cues in the environment (McDonald & Hong, 2000; Morris, Garrud, Rawlins, & O'Keefe, 1982; O'Keefe & Nadel, 1978, 1979; Olton, Becker, & Handelmann, 1979; Winocur, 1982). By comparison, relatively few studies have examined remote spatial memory in rats with hippocampal lesions, and most of them used variations of the Morris Water Maze with retention intervals from 2 to 15 weeks (Bolhuis, Stewart, & Forrest, 1994; Mumby, Astur, Weisend, & Sutherland, 1999; Sutherland et al., 2001; see also Kubie, Sutherland, & Muller, 1999 who used a dry-land version of the water maze). For the most part, hippocampal rats appeared to show extensive memory loss without a temporal gradient (but see Ramos, 1998). However, these studies are inconclusive as to whether the hippocampal contribution is time dependent. In at least two cases (Mumby et al., 1999; Sutherland et al., 2001), control performance deteriorated markedly over the testing interval, reducing the difference between hippocampal and control groups and giving the impression that the memory deficit had diminished over time. The difficulty is further compounded because even the controls performed at near floor levels at the longer intervals (see Squire et al., 2001, for a similar critique).

What is needed is a test of spatial memory in which control performance is above chance even at long intervals. As well, it is important that the intervals be sufficiently long to allow for the possibility of long-term or prolonged consolidation, if indeed it occurs. To this end, we selected a cross-maze task, on which normal rats showed savings over retention intervals spanning several months. Different groups of rats were trained on the cross-maze at 3, 6 or 12 months of age, then subjected to hippocampal or control surgery at about 12.5 months of age and tested 2 weeks later for 3 days. In addition, separate groups of rats with hippocampal or control lesions, with no prior training, were administered the cross-maze task as a test of spatial learning. This condition was included to assess the effects of previous training, over and above any new learning during the test phase, and to confirm the effects of hippocampal lesions on spatial learning. If there were savings, previously trained rats with control lesions should perform better than their untrained counterparts at some or all of the delays; at issue, is whether this holds true for rats with hippocampal lesions.

Consolidation theory posits that the contribution of the hippocampus is time-dependent, even for memories of relational context. As a result, a temporal gradient should be observed following hippocampal lesions, with impairment at short delays before consolidation is complete, but normal performance at long delays when memory has been fully consolidated. By contrast, our view and that of MTT predict that hippocampal lesions will lead to impaired performance at all delays because the spatial nature of the task ensures that the memories will always be dependent on relational context and, necessarily, will rely on the hippocampus.

To test these predictions, we examined performance on the first trial of day 1 testing, which would be the purest measure of memory for the pre-operatively learned spatial location. In addition, we examined the rate of re-learning over 10 trials of day 1, the assumption being that pre-operatively acquired spatial memory would facilitate re-learning. As a final measure of savings, we assessed total performance over 3 days of testing. In all cases, controls should show savings at long training-test intervals. According to consolidation theory, rats with hippocampal lesions should show greater savings at the longer intervals, whereas MTT predicts that impairment at the longer intervals should be at least comparable to that at the shorter intervals.

2. Method

2.1. Subjects

Subjects were experimentally naïve, male Long Evans rats obtained from the Trent University Breeding Centre. For the most part, rats were housed in shoebox cages in groups of three, during which time food and water were available on an ad lib basis. At various times depending on experimental conditions, rats were transferred to individual wire cages and placed on a restricted diet (see below for details).

2.2. Surgery and histology

Rats received 20 mg/kg (i.p.) diazepam (valium) and 5 mg/kg (i.p.) atropine and 10 min later were anesthetized with 65 mg/kg sodium pentobarbital (i.p.). All lesions were stereotaxically placed with coordinates, based on the Paxinos and Watson (1997) atlas, measured in relation to bregma and the horizontal skull surface. The procedure for making hippocampal lesions was slightly modified from the technique developed by Jarrard and Meldrum (1993). Using a small dental burr, eight holes were drilled through the skull directly above the hippocampus in each hemisphere. For hippocampal (HPC) groups, hippocampal damage was produced by 10 intra-cranial micro-injections of a solution containing the cellular neurotoxin, NMDA (5 mg/μl phosphate buffer per site) into each hemisphere. The coordinates were: anterior/posterior: 3.1, 3.1, 4.1, 4.1, 5, 5, 5, 5.8, 5.8, 5.8. Lateral: ±1, 2.2, 2.2, 3.5, 3, 5.2, 5.2, 4.4, 5.1, 5.1. Ventral: 3.6, 3.6, 4.4, 4.4, 4.1, 5, 7.3, 4.4, 6.2, 7.5. The solution was infused through 30-gauge stainless steel needles for 38 s, using a 10-μl syringe attached to a motorized infusion pump. The last two ventral hippocampal sites were injected for 2 min each. The needles were removed 2 min after each injection. In the procedure for operated control (OC) groups, incisions and burr holes were identical to the lesioned animals with the exception that there was no penetration of brain tissue. Those rats that exhibited signs of seizure activity during surgical recovery were given injections of diazepam (10 mg/kg, i.p.).

Following behavioural testing, rats with hippocampal lesions were deeply anesthetized with sodium pentobarbital and perfused with 0.9% saline followed by 10% formalin. The fixed brains were removed from the skull and stored in 10% formalin. Brains were transferred to a 20% buffered sucrose solution 36 h prior to sectioning. The brains were then frozen and sliced at 40 μm. Every fifth section was mounted on gelled glass slides and stained with formal-thionin.

Tissue damage in the lesioned brains was assessed with the aid of the Paxinos and Watson (1997) stereotaxic atlas of the rat brain. Figures of coronal sections from the atlas were scanned into a computer and the lesions were measured using an image processing and analysis program (Scion Image). The amount of damage in each coronal section containing a lesion was outlined and the section was mapped on to the corresponding figure from the atlas. Percentage of hippocampal tissue destroyed in each section was calculated by dividing the area of damaged tissue by the total area of the hippocampus in that section.

2.3. Apparatus

The apparatus was a four-arm open cross-maze constructed of wood and painted flat gray. The arms were of equal size (56 cm long × 15 cm wide with 2.5 cm sides) and radiated horizontally from a central area (15 cm × 15 cm). A recessed food-cup was placed 2.5 cm from the end of each arm. The maze, which was elevated several feet above the floor, was positioned so that each arm faced due North, South, East or West. A guillotine door separated the central area from each of the arms.

2.4. Procedure

The design of the study, including the timing of pre-operative training, surgery and post-operative testing in the various conditions, is depicted in Fig. 1.

2.4.1. Remote spatial memory

As can be seen in Fig. 1, rats in the remote memory conditions received pre-operative training at 3, 6 or 12 months of age and surgery was performed at approximately 12.5 months. (Those rats that were trained at 12 months received surgery within a few days of completing training.) Thus, the training–surgery intervals for the various conditions were 9, 6 and 0 months, respectively.

Behavioural training and testing procedures were identical for all groups regardless of the time of pre-operative training. One week before the beginning of training, rats were transferred to individual cages and placed on a 23.5 h food-deprivation schedule during which they received about 20 g of lab chow each day. Rats then received three daily familiarization sessions in the cross-maze. For each session, rats were placed individually in the maze and allowed to explore and eat pieces of Froot Loop cereal that were scattered throughout each arm. Each session ended when all the food was eaten or when 15 min had elapsed.

The procedure for days 4 and 5 was similar except that Froot Loop pieces were available only in the food cups. On days 6–9, the food cups were again baited and the rats received a number of simulated trials. On these trials, each rat was placed in one of the arms and allowed to eat from any one of the food cups. When the cereal was eaten, the rat was removed and placed in a holding cage to await the next simulated trial. Five such trials, each beginning with the rat being placed in a different arm, constituted a daily session. Forced-choice training began on day 10. For these trials, the East arm was always open, as was another randomly selected arm, which served as the starting arm. The other two arms were blocked by their guillotine doors. Only the East arm was baited. At the beginning of each trial, the rat was placed at the beginning of the starting arm, allowed to enter the East arm,

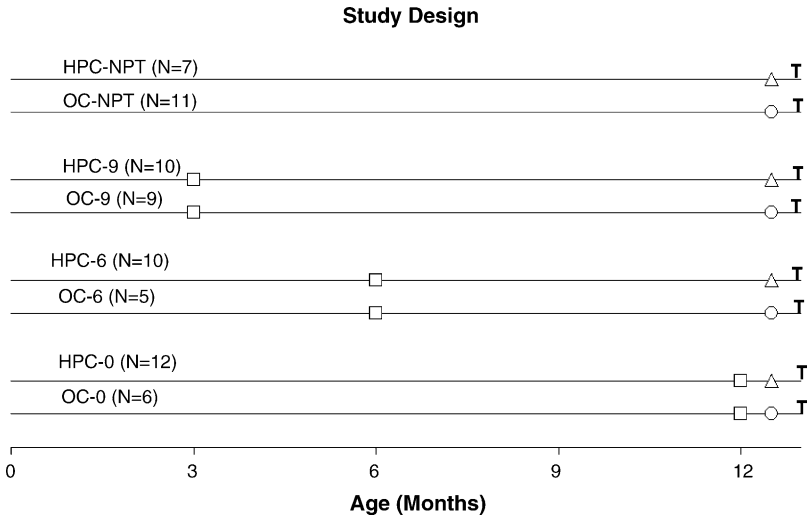

Fig. 1. Study design denoting training, surgery and testing schedules for hippocampal and operated control groups in the various conditions. Legend: HPC, hippocampal; OC, operated control; *N*, number of rats/group; NPT, no pre-training condition; 0, 6, 9, remote memory conditions (numbers denote the interval, in months, between pre-training and surgery); (□), pre-training; (Δ), hippocampal surgery; (◯), operated control surgery; **(T)**, post-operative testing.

and eat the Froot Loop cereal in the food-cup. The rat was then transferred to a holding cage for approximately 2 min to await the next trial. The rats received 10 such trials daily on 10 consecutive days. At the end of training, rats trained at 3 and 6 months of age were returned to their group cages where they remained on ad libitum food and water to await surgery. Rats trained at 12 months of age remained in individual cages on a restricted diet until surgery was performed 1–2 days later.

Food deprivation was reinstated 1 week after surgery and follow-up testing was initiated a week later. For the test trials, all arms were open but only the East arm was baited with Froot Loop cereal. The starting arm was determined on a random basis with the qualifier that it could not be the East arm. For each test trial, the rat was placed in the starting arm and allowed to move freely throughout the maze. The rat was removed after it had eaten the Froot Loop cereal in the East arm or if it entered with all four paws any of the unbaited arms. It was then returned to a holding cage for the approximately 2 min inter-trial interval. Each rat received 10 such trials daily on three consecutive days.

A total of 52 rats served as subjects in the remote memory test. The number of lesioned and control rats in each remote memory condition is indicated in Fig. 1.

2.4.2. Spatial learning (no pre-operative training)

Eighteen rats, aged 12–13 months, were tested in this condition—seven with bilateral hippocampal lesions and eleven operated controls (see Fig. 1), following the surgical procedures described above. These rats received no pre-operative training. Two weeks after surgery, rats were placed on a 23.5 h food deprivation schedule for several days. They then received the same familiarization and simulated training sessions in the cross-maze as rats in the remote memory conditions. Following the completion of these sessions, spatial learning trials were initiated and administered according to the procedures followed during post-operative, remote memory testing.

3. Results

3.1. Anatomical

Analysis of lesion-induced damage revealed that six HPC rats did not sustain any noticeable hippocampal damage and, as a result, their data were excluded from the study. In all of the 39 rats with acceptable hippocampal lesions, damage extended to dorsal and ventral regions of the structure. Twenty-six of the lesioned rats sustained damage to 50–75% of the hippocampus proper. Nine had very large lesions that affected 75–95% of the hippocampus, including extensive damage to all the subfields (CA1-CA3, dentate gyrus). Four rats with relatively small lesions that affected 20–30% of the hippocampus were included as their performance fell within the range of their group. Overall, the median value for hippocampal destruction was 72%, with the extent and pattern of damage similar in all conditions. In all cases, extra-hippocampal damage was minor or non-existent.

Fig. 2 shows reconstructions of the maximal and minimal extents of acceptable hippocampal lesions across the various conditions.

3.2. Behavioural

There was no relationship between performance by rats with hippocampal lesions in any of the conditions and the extent of their lesions. As a result, all rats with hippocampal lesions were identified as HPC rats for purposes of analysis.

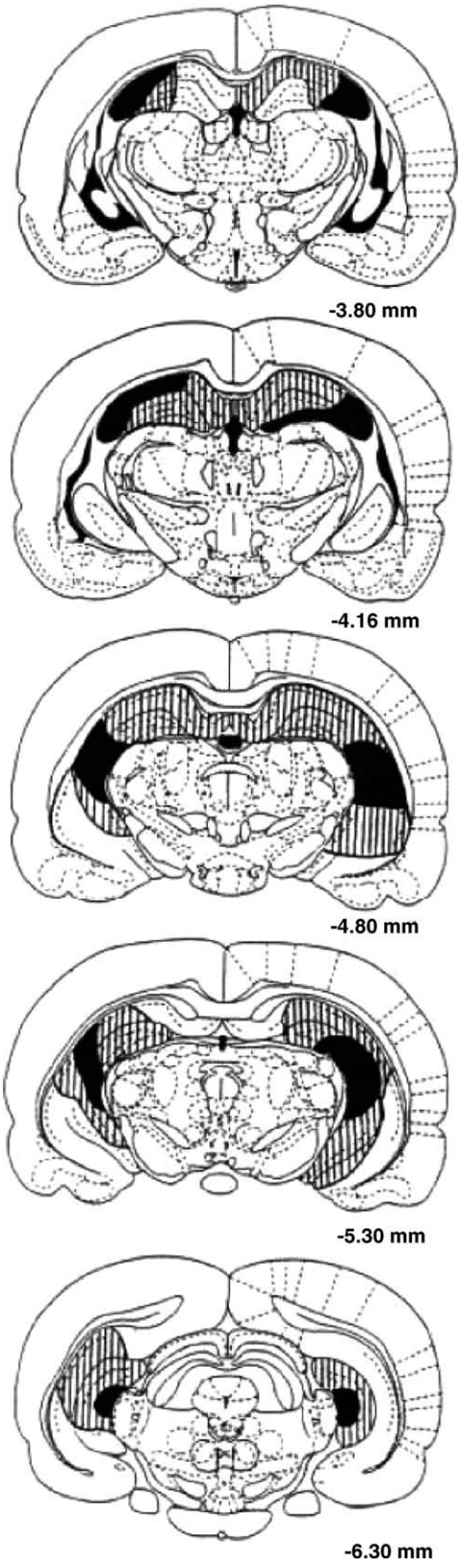

Fig. 2. Coronal sections illustrating minimal (solid) and maximal (cross-thatched) extents of acceptable hippocampal lesions across the four conditions. The numbers represent the distance in millimetres from bregma.

Table 1
The number of rats in each group that chose the correct arm on trial 1 of day 1 of post-operative testing in the various conditions

	0	6	9	NPT
OC	5/6 (.83)	2/5 (.40)	5/9 (.55)	3/11 (.27)
HPC	5/12 (.42)	4/10 (.40)	4/10 (.40)	2/7 (.29)

Values in parenthesis are probabilities. OC, operated control; HPC, hippocampal; NPT, no pre-training; 0, 6, 9, denote remote memory conditions (numbers refer to interval, in months between pre-training and surgery).

As stated in Section 1, we examined performance using three different measures of savings: (1) first trial of day 1 testing; (2) rate of re-learning over 10 trials of day 1 testing; (3) savings over 3 days of testing as measured by total accuracy. We report the results of each in turn.

3.2.1. Post-operative day 1—first test trial

Post-operatively, the purest measure of rats' memory for the pre-operatively learned spatial location is their response on the first trial of testing. Table 1 indicates the number of rats in each group that correctly chose the baited East arm on trial 1 of day 1 testing (probabilities in parentheses). The probability of rats selecting the correct arm by chance is assumed to be .25. [1] A binomial test of the observed probabilities revealed that OC-0 and OC-9 rats performed significantly above chance on trial 1 ($p = .005$ and .05, respectively). This indicates that control rats that received training as much as 9 months before surgery benefited from that training at the very beginning of testing. Though OC-6 did not show significant evidence of savings on this test ($p = .37$), they did show it on other measures. There was no evidence of savings in any of the HPC groups on trial 1, but the HPC-0 group did show savings on other measures (see below).

3.2.2. Post-operative day 1—performance change over 10 test trials

To assess further the savings that resulted from prior training, we examined performance patterns of all rats on the first day of post-operative testing where contamination effects of new learning were minimal. This analysis was based on whether or not the rats selected the baited East arm on each of the 10 trials. Thus, for each rat there were 10 sequential binary values, with the hypothesis that the probability of entering the baited East arm changes across the 10 trials. To assess how this probability changed, we fit a logistic regression model for each rat and extracted the parameter estimate

[1] We have selected .25 as the true mathematical level of chance performance on a given trial. On the grounds that it is unlikely that any rat would re-enter the start arm, it could be argued that for practical purposes chance performance is .33. At that level, only OC-0 rats performed significantly above chance on the first trial ($p < .02$). By day three of testing (see below), OC rats in all conditions and HPC-0 and HPC-6 rats performed above chance even at the .33 level. The important exceptions were the HPC-9 rats and hippocampal rats in the NPT condition, whose confidence intervals covered chance performance. Adoption of the higher chance level does not alter the main conclusion that control rats generally retain pre-morbidly acquired spatial memories better than do rats with HPC lesions.

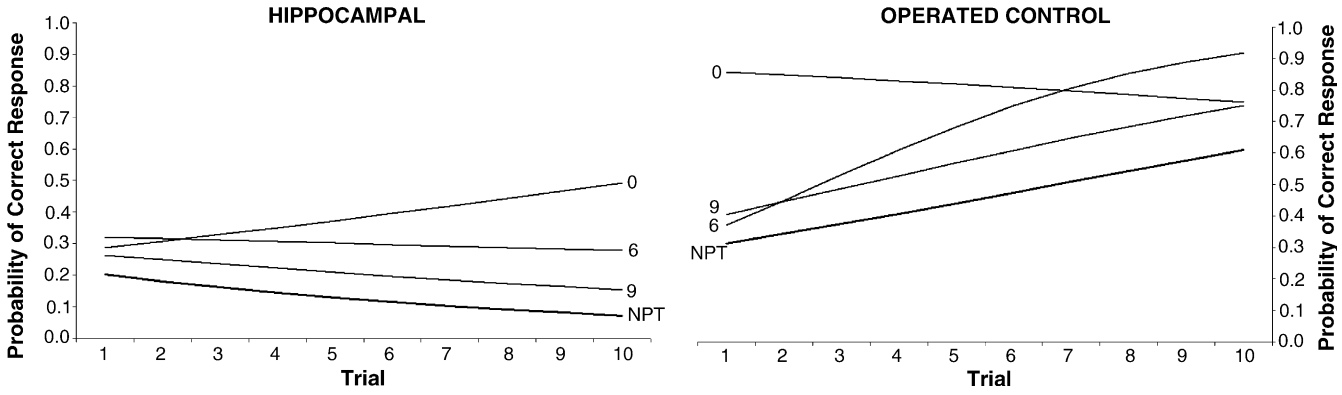

Fig. 3. Logistic functions representing the probability of rats selecting the correct arm on each of the 10 trials of day 1 post-operative testing.

(β). This parameter estimate, reflecting the rate of change (of the log odds) with respect to trial, is a measure of each rat's improvement, or learning, over the 10 trials. The rationale was that rate of learning (slope over 10 trials) will be influenced by the pre-operative memory that is retained.

Fig. 3 shows logistic functions based on the average slope and intercept estimates for each group during day 1 testing. As can be seen in the figure, rats in the OC-0 group performed at or near ceiling from the beginning of testing, indicating excellent savings that were maintained throughout. (This restricted the slope estimates for rats in the OC-0 group and qualifies comparisons between these rats and the other control rats.) Confidence intervals (CI) were used to determine whether the slope estimates, β, included the zero slope, thereby indicating no improvement. Examination of average slope estimates in the remaining control groups showed that the OC-6 group had the largest average improvement (95% CI for β extends from .29 to .36; mean = .33). The OC-9 group had the next largest average improvement (95% CI for β from .08 to .25; mean = .16) followed by the HPC-0 group (95% CI for β from .05 to .15; mean = .10). CIs for average slope estimate of the other groups covered the zero slope value, including the OC rats in the NPT condition, which had nominally positive rates of change, but displayed considerable variability.

A 2 × 4 ANOVA investigated the factorial effects of group (HPC versus OC) and length of interval between training and surgery (0, 6 or 9 months, or no pre-training—NPT) on the logistic regression slope estimates, β. This analysis showed an interaction between Group and Interval ($F_{3,62} = 4.24$, $p = .009$). As noted in the previous paragraph, there was a ceiling effect in the OC-0 group and improved but highly variable performance, in the OC-NPT group. These features make analyses of the slopes of OC rats less informative and, as a result, we focused on the HPC rats. Contrasts among the HPC rats showed that slope estimates were significantly lower in the NPT group than in the 0-interval group ($t_{35} = 2.65$, $p = .01$), confirming savings in the HPC-0 rats. Comparisons of slope estimates between HPC-NPT and HPC-6, and between HPC-NPT and HPC-9 groups yielded no significant differences.

Taken together, these analyses show that control rats with 6 or 9-month intervals between training and surgery re-established the spatial memory at a fairly rapid rate over the first 10 test trials. The OC-0 rats were already performing well on the first trial and maintained that level throughout. By comparison, of the HPC groups, only rats in the HPC-0 group showed savings during day 1 testing.

3.2.3. *Post-operative day 1—total correct responses*

As a further measure of savings, we examined overall accuracy on day 1. As Fig. 4 shows, rats with hippocampal lesions were consistently impaired on day 1 relative to operated controls. Both groups, however, showed effects of prior training when compared to the NPT group, but only when training–surgery intervals were relatively short. These impressions were confirmed by ANOVA. The total number of times each rat first selected the baited East arm on each trial of the first day (range from 0 to 10) was entered into a 2 × 4 ANOVA investigating the factorial effects of group (HPC versus OC) and length of interval between training and surgery (0, 6 or 9 months, or NPT). There was no Group × Delay interaction ($F_{3,62} = .26$, $p = .86$). There was a main effect of Group ($F_{1,62} = 59.65$, $p < .001$) with OC rats performing better than HPC rats. There was also a main effect of interval ($F_{3,62} = 8.83$, $p < .001$) with contrasts showing that NPT rats performed significantly worse than 0-interval rats ($t_{62} = 4.94$, $p < .001$) and 6 months interval rats ($t_{62} = 2.91$, $p = .005$) and nominally worse than 9 months interval rats ($t_{62} = 1.66$, $p = .10$).

To underscore the point that the gradients are parallel in both groups and that the Interval main effect was not driven exclusively by the OC groups, we note that in the NPT condition, correct responses were lower on average by 2.8 responses (37%) in the OC group and by 2.4 responses (51%) in the HPC group, relative to corresponding groups in the 0-interval condition.

3.2.4. *Post-operative day 3—total correct responses*

The same analysis was conducted on the day 3 data to determine if the benefits following prior training that were

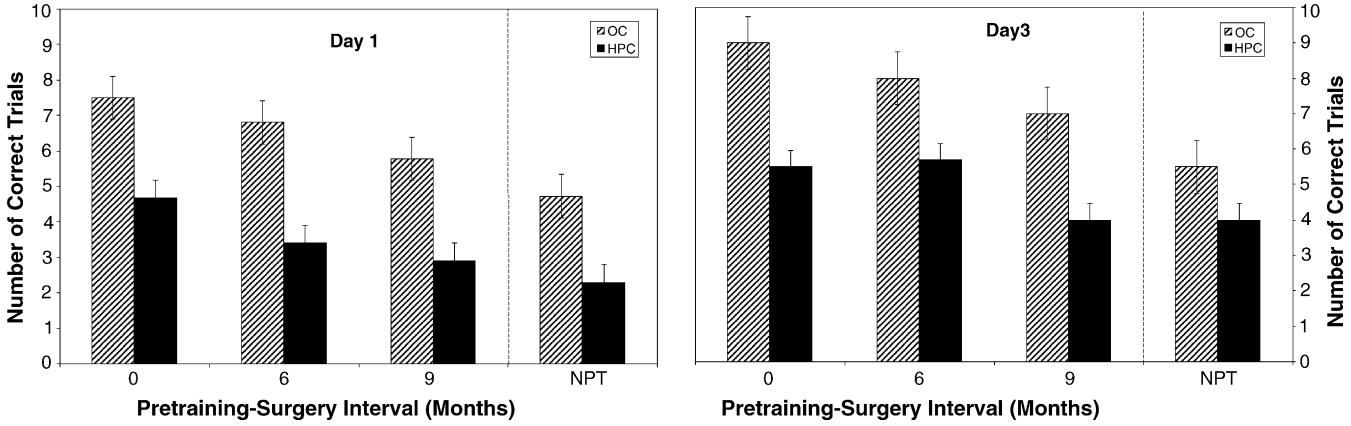

Fig. 4. Mean number of correct responses by hippocampal and operated control groups on days 1 and 3 of post-operative testing in the various conditions.

observed in both groups on day 1 continued to influence performance on day 3 (see Fig. 4). For the most part, the results paralleled those of day 1. Once again, there was no Group × Interval interaction ($F_{3,62} = .77$, $p = .51$). There was a main effect of Group ($F_{1,62} = 27.58$, $p < .001$) with OC rats continuing to perform better than HPC rats.

There was also a main effect of interval ($F_{3,62} = 5.57$, $p = .002$) with contrasts showing that NPT rats performed significantly worse than 0-interval rats ($t_{62} = 3.61$, $p = .001$) and 6 months interval rats ($t_{62} = 2.88$, $p = .005$).

On the third post-operative test day, the HPC and OC rats continued to show declines from 0-interval to the NPT condition. Relative to the 0-interval condition, in the NPT condition, correct responses were lower by 3.5 (38%) in the OC rats and by 1.5 (27%) in the HPC rats. In line with the day 1 pattern, OC rats showed a steady decline in the number of correct responses across the four conditions. The shape of the HPC gradient altered in that the HPC rats showed little difference in savings between the 0 and 6 months interval, but an abrupt decline at the longest (9 months) interval and in the NPT condition.

It is also worth noting that, after 3 days of testing, confidence intervals for performance by OC rats in all conditions and HPC-0 and HPC-6 rats were well above the 25% chance level. The important exceptions were the HPC-9 rats and hippocampal rats in the NPT condition, whose confidence intervals covered chance performance.

These results indicate that, on day 3, rats with hippocampal lesions were still impaired relative to control rats, but that both groups continued to benefit from prior training. All pre-trained groups exhibited savings, relative to the NPT condition but, in the HPC rats, the savings were confined to relatively short training–surgery intervals.

4. Discussion

In this study, we investigated the effects of hippocampal lesions on spatial memory acquired immediately before surgery or 6 and 9 months earlier. The major finding was that rats with hippocampal lesions were severely impaired at remembering a pre-operatively learned spatial location at all intervals, with evidence of savings only at the shortest delay. On three measures of savings—trial 1 performance on day 1 post-operative testing, rate of re-learning over 10 trials on day 1 testing and total accuracy on post-operative days 1 and 3—rats with hippocampal lesions consistently performed worse than operated controls regardless of interval. Since the latter two measures may have been contaminated by learning effects over testing, performance in all groups was compared on these measures to that of rats with hippocampal or control lesions with no prior training. As expected, the hippocampal group with no pre-training was severely impaired in learning the location of the food reward. Compared to this group, rats with hippocampal lesions that were pre-trained consistently performed better at the shortest training–surgery interval (0-interval) but not at the longer ones, as indicated by rate of learning on day 1, and savings over 10 trials on days 1 and 3. In other words, rats with hippocampal lesions exhibited RA at all training–surgery intervals and a forgetting curve that paralleled that of the control groups. These findings demonstrate that the longer the delay between training and surgery, the greater the loss of spatial memory in both HPC and OC rats. These data provide no evidence that the most remote spatial memories are spared relative to recent memories in rats with hippocampal lesions; indeed the opposite pattern was observed.

The forgetting curve observed in rats with hippocampal lesions contrasts with the type of temporally graded RA that would be predicted by consolidation theory, which is that the more remote memories should be retained best. Advocates of consolidation theory, however, have suggested that successful performance on spatial navigation tests, such as the cross maze, requires on-line processing and retention of relational cues in the environment (Knowlton & Fanselow, 1998). According to this view, these on-line mnemonic processes are disrupted by hippocampal lesions. As Knowlton and Fanselow note, “it should not matter when the

hippocampus was lesioned with respect to initial training, because the hippocampus is essential for [current] performance" (p. 295). This view maintains that memory for learned spatial tasks should be impaired equally across all time periods, as was found to be the case in some studies (Bolhuis et al., 1994; Mumby et al., 1999; Sutherland et al., 2001). Although on-line mnemonic processing may be a hippocampally dependent component of spatial navigation, our results clearly show that both lesioned animals and controls exhibited time-dependent forgetting in which recent memories were preserved better than remote memories. Consequently, the pattern of RA observed in the present study, which is the reverse of that predicted by consolidation theory, cannot be explained fully by Knowlton and Fanselow's on-line processing account.

The same argument rules out the possibility that the remote memory losses of HPC rats were due to an inability to process information needed to perform the spatial task. Had deficits in processing spatial information been the source of the impairment, HPC rats would have performed as poorly across all intervals, which they did not.

Because the cross-maze task entailed repeated testing, it could be argued that the poor performance of the HPC rats in the remote memory tests represents a learning deficit, rather than a failure to remember the pre-operative training. While there is no doubt that hippocampal lesions disrupt spatial learning in rats, this interpretation is undermined by the analysis of performance during day 1 testing. As expected, this analysis showed that OC and HPC rats with training immediately prior to surgery exhibited improved reacquisition rates during testing, relative to corresponding groups in the non-pretrained spatial learning condition. The OC rats in the long interval conditions, despite making relatively few correct responses in the first few trials of testing, rapidly reacquired the spatial response thereafter. By comparison, HPC rats in the long interval conditions did not show this rapid reacquisition of the spatial response. Taken together, these results indicate that both HPC and OC rats exhibited considerable savings during testing in the 0-interval condition, whereas, at longer delays, the OC rats revealed greater memory for pre-operative training than the HPC rats. The results support the conclusion that the impaired performance of rats with hippocampal lesions in the remote memory tests was related to a time-dependent memory loss that was over and above the effects of the lesion on spatial learning.

Related to the issue of relearning versus remembering the spatial response, it is interesting to note a study by Ramos (1998) that also tested the effects of hippocampal lesions on remote spatial memory in a cross-maze. In that study, the longest training–surgery interval was 64 days and, at that interval, rats with hippocampal lesions exhibited normal memory for the learned response. In examining the last five trials of an 18-trial test session, a measure that, as Ramos acknowledged, confounds the effects of savings and learning, rats with hippocampal lesions exhibited a temporally graded RA. When he examined only the first five trials, a measure of memory that was less contaminated by learning effects, Ramos reported that "a significant retrograde amnesia became apparent, but it was not temporally graded" (p. 1466). In that critical respect, Ramos' findings are consistent with the present results.

There is no apparent explanation as to why, on some measures, Ramos observed a temporally graded RA, whereas we did not. There are lesion-related differences between Ramos' study and ours. In particular, Ramos' lesions were restricted to the dorsal hippocampus. By comparison, our lesions were typically larger and extended to the dorsal and ventral regions of the structure. Perhaps the larger amount of remaining hippocampal tissue in Ramos' rats allowed for memory recovery at 2 months. The fact remains, however, that if temporally graded RA is determined by the length of the interval, we should have observed similar savings in the HPC and OC rats tested at the 6 month training–surgery interval, but we did not. As well, Ramos' lesions were electrolytic whereas ours were neurotoxic, produced by NMDA infusion. It is conceivable that seizure activity associated with NMDA lesions prevented HPC rats from demonstrating preservation of old memories. However, this is unlikely because HPC rats showed savings and better memory in the 0-interval condition than at the longest interval. There is no apparent reason why seizures should selectively affect remote memories, while sparing recent memories.[2]

It should be noted that there was some variability in the size of the hippocampal lesions in our study. Recently, Broadbent, Squire, and Clark (2004) found that rats with damage to 30–50 or 50–100% of the dorsal hippocampus were similarly impaired on a test anterograde spatial memory. In that respect, our finding that lesion size did not affect performance at any interval is consistent with Broadbent et al. (2004) results. Nevertheless, in light of several reports that spared spatial memory is inversely related to amount of hippocampal damage (Martin, de Hoz, & Morris, in press; de Hoz, Knox, & Morris, 2003; Moser, Moser, Forrest, Andersen, & Morris, 1995) we cannot rule out the possibility that the savings observed in the HPC group at the shortest delay is attributable to residual hippocampal tissue, and can account for the normal pattern of forgetting exhibited overall by HPC groups. Even if this were the case, consolidation theory would have difficulty accounting for the poor memory in hippocampally lesioned rats at the longer intervals. Assuming that the residual tissue is needed for on-line processing, according to consolidation theory, it should be the preserved remote memories represented in non-hippocampal regions that should benefit most, not the more vulnerable recent ones. A similar argument applies to the possible disruptive effects of NMDA-induced seizures during hippocampal surgery. The most parsimonious interpretation of our findings is that spatial memory on the

[2] It is noteworthy that since submitting this paper, two other studies have reported results consistent with our own, namely, that hippocampal lesions lead to extended retrograde amnesia for spatial memory with little or no sparing (Martin et al., in press; Clark et al., in press).

cross-maze remains dependent on the hippocampus throughout, with recent memories being most strongly represented before forgetting occurs.

In the cross-maze task, as in most tests of spatial learning and memory, rats could find a particular location on the basis of local cues that serve as a reliable marker. Thus, in the present study, upon reaching the center of the maze, it is conceivable that rats responded to specific internal or external cues that could be associated with food, rather than to a configuration of spatial cues in the environment. While this possibility cannot be categorically ruled out, it is extremely unlikely. The most compelling argument against this interpretation comes from numerous reports that rats with hippocampal lesions are extremely efficient at using landmark cues, when they are available, to find reward in a variety of spatial tasks, including variations of the cross-maze test (e.g., Winocur, 1982; Morris et al., 1982; Ellen & Deloache, 1968; Ramos, 2000). On such tasks, hippocampal rats typically perform as well as normal controls. If such cues were used by rats with hippocampal lesions in our study, their performance would resemble that of controls at least in the no-training condition, and possibly in the prior training conditions (Gaskin, Tremblay, & Mumby, 2003). The present results, however, clearly show that rats with hippocampal lesions were severely impaired in all learning and memory conditions, and exhibited signs of improved performance only when they had received prior training on the task shortly before surgery.

Our study, using different methods, is consistent with that of Martin et al. (in press) in showing a normal forgetting curve in rats with partial hippocampal lesions on a test of remote spatial memory, which parallels that observed in humans with MTL damage on some tests of autobiographical memory (Viskontas et al., 2000). The typical finding in rats is extensive memory loss without a gradient (Bolhuis et al., 1994; Clark, Broadbent, & Squire, in press; Mumby et al., 1999; Sutherland et al., 2001); for similar findings in mice, see Cho, Kesner, & Brodale, 1995). There may be a number of reasons that some studies report more extensive loss, including differences in tasks and lesion size. For example, Martin et al. (in press) reported that rats with complete hippocampal lesions, unlike those with partial lesions, exhibited no gradient of forgetting, but virtually total loss of memory at all delay intervals. However, the important point for the hypothesis being tested here is that none of these studies reported a temporally graded RA, in which remote memory was spared relative to recent memory. This point is underscored by the absence of such a gradient in our study where, by extending the training–surgery interval to 9 months, which is more than twice as long as in other studies, we provided ample opportunity for memory consolidation.

The present results are in accord with an interpretation that emphasizes the importance of contextual factors in recent and remote memories (Nadel & Moscovitch, 1997; Rosenbaum et al., 2001). That view is based on the premise that experiences are associated with a complex of contextual cues that are relationally linked to each other and, for a period of time at least, guide appropriate behaviour. These associations are represented in the hippocampus and, as long as memories retain their dependency on relational context, the hippocampus is necessary for recovering them. For tasks, such as the cross-maze, that involve spatial memory, dependence on relational context is invariant over time. In such cases, hippocampal lesions should produce equivalent deficits across all intervals tested, which was found to be the case in the present study.

This interpretation can also account for the temporally graded pattern of RA that has been associated with hippocampal lesions on non-spatial tests (Bunsey & Eichenbaum, 1995; Clark et al., 2002; Winocur, 1990; Winocur, McDonald, & Moscovitch, 2001 contextual fear conditioning—Anagnostaras et al., 1999; Kim & Fanselow, 1992; Maren, Aharonov, & Fanselow, 1997). We believe that performance on such tasks can be supported by both relational and associative context. With the passage of time, memories for the responses become more generic and increasingly independent of the relational context in which they were learned; they become reliant more on associative context or become context-free. As that happens, the hippocampus participates less in recovery of the learned behaviour, which now can be mediated exclusively by extra-hippocampal structures. Hence, the pattern of temporally graded RA following hippocampal lesions that is seen on such tasks. What has been interpreted by some as an increase in consolidation (e.g., Kim & Fanselow, 1992), is regarded instead as a shift from dependency on relational context shortly after learning, to associative context or context independence at longer intervals.

A recently completed study in our lab (in preparation), utilizing the socially acquired food preference task, designed originally by Galef and Wigmore (1983) has provided direct support for our position. In this task, a rat must remember a preference for a particular diet, which was acquired through interaction with another rat that had recently sampled the diet. As indicated above, rats with hippocampal lesions reliably display a temporally graded RA, in which preferences acquired earlier are remembered better than those acquired more recently. Our test of the context-dependent hypothesis also was conducted on anterograde memory function. Normal rats and rats with hippocampal lesions acquired a food preference in Context A and were retested for that preference, at short or long delays, in the same Context or Context B, where environmental cues were very different. The results in normal animals showed that changing the context disrupted memory for the acquired food preference at short delays, confirming that the memory was dependent on relational context, but not at long delays, indicating that the memory had become context-free. By contrast, rats with hippocampal lesions were unaffected by context change even at short delays, indicating that they were not using relational contextual cues to remember the food preference.

This evidence supports our view that recent memories are tied to relational context and it is precisely when memory is dependent in this way that hippocampal lesions produce RA. The approach of varying contextual factors has general

application and can be used to determine whether loss of contextual associations with time underlies the temporally graded RA observed in other tests, such as contextually dependent fear conditioning.

5. Conclusion

The present results clearly demonstrate that hippocampal lesions in rats produce impairment in remote spatial memory extending for up to 9 months. The memory loss, which was over and above the effects of the lesion on spatial learning, paralleled the forgetting curve of normal animals, with recently acquired memories better preserved than remote ones. This gradient was opposite to that predicted by consolidation theory but broadly consistent with MTT. The results were interpreted within a framework that distinguishes between relational and associative context, and provide evidence that the hippocampus is necessary for the retention and retrieval of memories that are bound to relational context regardless of their age.

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