

Research report

Perirhinal cortex lesions produce variable patterns of retrograde amnesia in rats

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Abstract

Two experiments examined the contribution of the perirhinal cortex (PRh) to retrograde memory for the location of a platform in a water maze. In a previous study, we found that electrolytic lesions of the PRh produced retrograde amnesia, without a temporal gradient, for water-maze problems acquired 4 weeks and 2 days before surgery [Behav. Brain. Res. 114 (2000) 119]. In Experiment 1, we used the same mixed design as in our previous report (time of learning was a within-subjects factor), but PRh lesions were made by aspiration. Contrary to our earlier report, these PRh rats displayed good retention of both platform locations. Combined, these findings indicate that the lesion method may contribute importantly to the pattern of deficits observed. Experiment 2 was conducted similar to Experiment 1, except that a completely between-subjects design was used (time of learning was a between-subjects factor). Rats that received PRh lesions approximately 2 days after the last training session displayed impaired retention of the platform's location, whereas rats that received PRh lesions 4 weeks after training did not. This finding of a temporally graded retrograde amnesia is consistent with our earlier report, and further suggests that the involvement of the PRh in the retention of water-maze problems is time-limited. However, also consistent with our earlier report, the PRh-lesioned rats in Experiment 2 that displayed a retention deficit rapidly reacquired the task. This finding, combined with the negative findings in Experiment 1, suggests that the contribution of the PRh to retrograde memory for platform locations is subtle and may not be due to impaired spatial memory abilities. Additionally, the conflicting results of Experiments 1 and 2 underscore the importance of the design employed in studies of retrograde amnesia in animals.

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1. Introduction

Damage to medial-temporal-lobe structures characteristically produces retrograde amnesia in which information acquired prior to the damage is lost. In some cases, the memory loss is temporally graded, such that information acquired nearer to the time of damage is lost but information acquired long before is intact. This pattern is thought to occur due to a disruption in a consolidation process, wherein memories become permanent over time. It is thought that structures in the medial temporal lobe, particularly the hippocampus, are initially essential to the storage and retrieval of memories, but, in time, those memories become permanently stored elsewhere and the medial-temporal-lobe structures are no longer required. Thus, newer memories rely on the integrity of this region, whereas older memories do not [37].

The perirhinal cortex (PRh) is a medial-temporal-lobe structure that is a site of convergence for highly processed, uni- and poly modal information from sensory association cortices, including piriform, cingulate, insular, temporal, parietal, and occipital [4,11,40]. Its primary efferent is the entorhinal cortex [2,5,38], which in turn has a major projection to the hippocampus through the perforant path [43]. There are also reciprocal connections between the PRh and the postrhinal cortex (parahippocampal gyrus in primates; [2]), subiculum [10,16], CA1 subfield of the hippocampus [39], and amygdala [33,36].

Investigations into the mnemonic consequences of PRh damage indicate an integral role for this structure in both anterograde and retrograde object-recognition memory ([14, 22,27,28]; see [24,30] for reviews). The role of the PRh in anterograde and retrograde memory for place information is less clear. We previously reported that PRh lesions in rats do not produce anterograde deficits in allocentric spatial working memory [14,15] or reference memory ([15,26];

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also see [9]) in a water maze. These data are consistent with other evidence that PRh rats display intact anterograde performance on other spatial tasks, including a T-maze [8,13], and a radial-arm maze [7,9,12]. By contrast, there are several reports that PRh lesions produced mild anterograde deficits in allocentric spatial reference memory [17,18,21,41], and marked deficits in allocentric spatial working memory [18–21,42]. These equivocal results suggest that some aspect of spatial learning and memory may be reliant on the integrity of the PRh, and it is likely that its contribution is sensitive to differences in methodology.

Much less is known about the involvement of the PRh in retrograde spatial memory. We recently reported that PRh lesions produced retrograde amnesia, without a temporal gradient, for locations of a platform in a water maze learned 4 weeks and 2 days before surgery [26]. This finding was intriguing, as we had not previously observed anterograde spatial memory deficits in rats with PRh lesions [15,26]. It also provides further support for the notion that under specific circumstances the PRh is recruited to support some, as yet unidentified, aspect of spatial memory. Since there is little data available on the effects of PRh lesions on retrograde spatial memory, we sought to further investigate this finding.

One methodological difference between our report of retrograde amnesia [26] and our reports of intact anterograde memory for water-maze problems [14,15,26] is the lesion technique used to ablate PRh tissue. In the anterograde studies the PRh lesions were made using aspiration, whereas in the retrograde study we made the PRh lesions electrolytically. Also, some key studies discussed above, which reported anterograde spatial memory deficits, made electrolytic PRh lesions [17,18,20,41,42]. Thus, it was of interest to us to determine whether the lesion method could be a critical factor in the retrograde amnesia that we observed. While both techniques produce widespread damage to both cell bodies and fibers in the target region, it is possible that electrolytic, but not aspiration lesions, disrupt processes elsewhere in the medial temporal lobe, perhaps in the entorhinal cortex or the hippocampus. This might occur because the strong current used during the electrolytic surgery (1.5 mA for 10 s at five sites per hemisphere) produces an abnormal cascade of activation that may disturb normal function in structures efferent to the PRh. In Experiment 1, we used the same within-subjects design, and training and testing procedures as in our previous study [26] to assess performance on the water-maze problems learned 4 weeks prior to and the week of surgery. However, unlike in the previous study, the present PRh lesions were made using aspiration.

This study also focused on our previous use of a mixed design in which the time of learning (REMOTE and RECENT) was a within-subjects factor [26]. This type of design is typically favored in studies assessing retrograde amnesia as it is thought to more closely reflect the syndrome as it occurs in human patients. There are, however,

disadvantages to using this type of design in animal studies (see [29] for a review). For example, animals may acquire a learning set that will affect the rate of learning or the manner in which they learn subsequent problems. Therefore, it is difficult to ensure that the same amount and type of learning is occurring when subjects are trained on ostensibly equivalent problems. In Experiment 2, we sought to explore the possible impact of this by conducting the same study as in Experiment 1 using a completely between-subjects design.

2. Experiment 1: PRh lesions and retrograde memory for water-maze problems with time of learning as a within-subjects factor

The main purpose of Experiment 1 was to reproduce our previous finding of retrograde amnesia for places in rats with PRh lesions, but to do so in rats with aspiration lesions of the PRh. Several recent studies have attempted to determine whether certain aspects of the training and testing procedures can account for the inconsistent results discussed above, but no critical procedural features have been identified so far (Glenn and Mumby, unpublished data, also see [19,21]). To our knowledge, only electrolytic lesions of the PRh have been used to assess retrograde spatial memory [26]. Thus, it was important to determine whether the retrograde amnesia we observed occurs following PRh lesions that are made in other ways. We decided to use aspiration because deficits in the acquisition of spatial information were not observed when this technique was used [9,14,15].

2.1. Method

2.1.1. Subjects

Seventeen experimentally naïve, male, Long–Evans rats (Charles Rivers, St. Constant, Que., Canada) weighing between 300–350 g at the start of the experiment served as subjects. Rats were singly housed in opaque cages and had free access to food and water throughout the experiment. The colony was maintained at 21 °C with a 12:12 h light-dark cycle (lights on at 8 a.m.). All procedures were conducted during the light phase of the cycle.

2.1.2. Apparatus

The water maze was a circular pool (137 cm diameter, 46 cm deep). A plexiglas platform (10 cm × 10 cm × 28 cm) was submerged approximately 2 cm below the surface of the water which was made opaque through the addition of skim milk powder. Water temperature was 23 ± 1 °C. Distal, extramaze cues (including laboratory equipment, posters on the wall and sounds from a radio) were available. Rats could use these cues to locate the hidden platform in the maze. A VP118 super tracker (HVS Image, Hampton, UK), and a Panasonic video camera (WV-BP120), configured to an IBM-compatible computer with HVS Water software were used to record swim data.

2.1.3. Procedure

2.1.3.1. Presurgery training. All rats were trained on two place problems prior to surgery. Two different rooms with distinct distal cues were used to distinguish the two problems. The use of the rooms was counterbalanced across training time points. The first problem was learned 4 weeks before surgery (REMOTE) and the second problem was learned during the week before surgery (RECENT). Training on each problem was the same and consisted of one 8-trial session per day for 3 consecutive days in which the platform remained in the same place within the pool. On each trial a rat was placed in the pool at one of four release positions corresponding arbitrarily to geometric N, S, W, and E. They had 60 s to find and escape onto the platform. If they did not do so within that time they were placed on the platform by the experimenter. All rats spent 10 s on the platform before being removed from the pool. The intertrial interval was approximately 5 min.

2.1.3.2. Surgery. Approximately 48 h after the final day of training on the RECENT problem, rats received either bilateral PRh lesions ($n = 9$) or sham surgery ($n = 8$). PRh rats were anesthetized with pentobarbital (65 mg/kg). A scalp incision was made and the muscle overlying the temporal skull was displaced. A portion of skull overlying the PRh was removed using a hand-held dental drill. Tissue was aspirated using a glass pipette attached to a vacuum pump. Sterile gelfoam (Upjohn Company, Don Mills, Ont., Canada) was placed in the cavity, the muscle was replaced, and the incision was sutured with wound clips. Sham rats were anesthetized, and a scalp incision was made and sutured. Scalp wounds of all rats were treated with a topical antibiotic. Rats were permitted to recover for 2 weeks.

2.1.3.3. Postsurgery testing. Retention tests were conducted on 2 consecutive days, 1 day for each problem, with the sequence counterbalanced within groups. Each test session consisted of 16 trials. Trials 2 and 14 were probe trials (EARLY and LATE probe respectively) in which the platform was removed from the pool and the search patterns of rats were collected for a 30-s period. The latency to the first platform crossing was used as a measure of escape latency on probe trials.

2.1.3.4. Histology. After the completion of behavioral testing, rats were administered an overdose of pentobarbital and were transcardially perfused with 0.09% saline followed by 10% formalin. Brains were extracted and stored in a 30% sucrose–formalin solution for a minimum of 72 h prior to sectioning. Using a cryostat, brains were sectioned at a width of 30 μm and every 5th section through the lesion was retained and mounted on gel-coated slides for analysis. The slides were Nissl stained and examined microscopically to determine the extent of perirhinal and extra-perirhinal damage.

We quantified the lesions by estimating the percentage of tissue loss to the PRh and surrounding structures in the rats with the largest and smallest lesions; all other lesions fell within the reported ranges. Percentages of tissue damage were calculated based on the approximate total area of the target region and the area of the damage within that region. The boundaries of the PRh were based on those described by Burwell [3]; the rostral border was placed at -2.80 mm relative to Bregma, and the caudal border was placed at -7.8 mm relative to Bregma. Estimates of PRh damage were made by examining the lateral reconstruction of the lesion and the coronal reconstruction at each of the three planes shown in Fig. 1 (-3.8 , -5.3 and -6.8 mm relative to Bregma). In each of these four views, an estimate of the percentage of PRh damaged was made for both hemispheres, which were then averaged. Estimates of damage to the lateral entorhinal cortex were also made from both the lateral and coronal reconstructions of the lesions, whereas estimates of damage to the postrhinal cortex were made from the lateral reconstruction only. All other extra-PRh damage was evaluated by examining the coronal reconstruction of the lesion.

2.2. Results

2.2.1. Histological results

Fig. 1 shows the location and extent of the PRh lesions. There was substantial, and nearly complete, bilateral damage to the PRh in each lesioned rat. The PRh was 90% destroyed in the rat with the largest lesion, and 70% destroyed in the rat with the smallest lesion. All PRh rats also had bilateral damage to the lateral entorhinal cortex; this damage was primarily in the posterior extent of the lesions, with less, and frequently unilateral, damage evident in the anterior extent of the lesions. The rat with the largest lesion had approximately 40% damage to the lateral entorhinal cortex, whereas the rat with the smallest lesion sustained about 25% damage to this region.

Damage to the anterior portion of the postrhinal cortex occurred in seven of the nine PRh rats. In four of these rats this damage was bilateral and was estimated to include approximately 10% of the postrhinal cortex. In the other three rats, the postrhinal damage was unilateral and was estimated to include approximately 10–15% of the postrhinal cortex.

Four of the nine PRh rats also had unilateral damage to the ventral portions of the temporal association cortex. This region was estimated to be about 10% damaged in both the rat with the largest lesion and the rat with the smallest lesion. The CA1 subfield of the hippocampus sustained minor damage, unilaterally, in three rats. One rat had unilateral damage to the piriform cortex, and another rat had unilateral damage to the dorsolateral amygdala.

2.2.2. Behavioral results

The presurgery performance of the PRh and Sham groups was well matched in terms of their latencies to escape onto

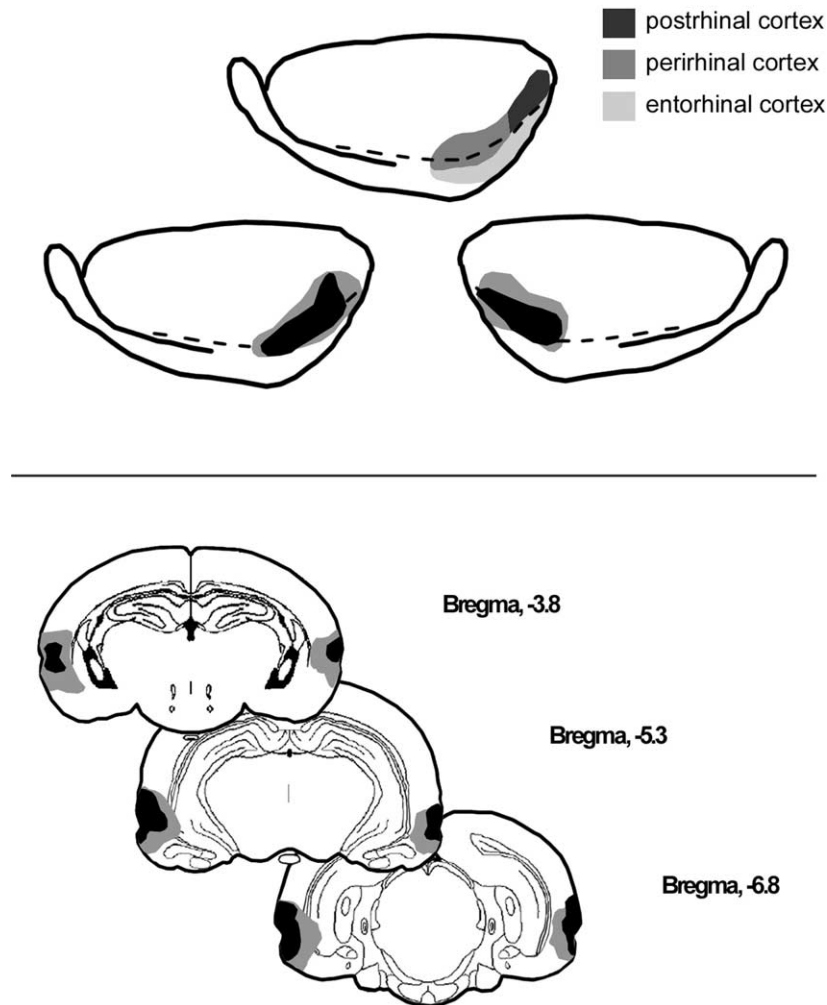


Fig. 1. The top panel shows the approximate boundaries of the perirhinal, postrhinal, and entorhinal cortex on the lateral surface of the brain [6], and the location and extent of the largest (grey) and smallest (black) PRh lesions. The bottom panel shows the coronal view of the largest (grey) and smallest (black) PRh lesions at three planes [32].

the platform on the final day of training for both the REMOTE (PRh: $M = 6.91$ s, S.E.M. = 0.55; Sham: $M = 7.90$ s, S.E.M. = 0.86) and RECENT problem (PRh: $M = 5.74$ s, S.E.M. = 0.32; Sham: $M = 5.38$ s, S.E.M. = 0.37).

Fig. 2A shows the percentage of swim time that PRh and Sham rats spent in the target quadrant (the quadrant that contained the platform during training) on the EARLY probe (trial 2) of retention testing. A 2×2 ANOVA revealed a significant main effect of Time ($F(9, 15) = 9.50$, $P = 0.008$), indicating that, overall, rats spent more time in the quadrant that contained the platform for the RECENT problem than the REMOTE problem. The main effect of Lesion and the interaction between Lesion and Time were not significant ($F_s < 1$). Fig. 2B shows the percentage of swim time that PRh and Sham rats spent in the target quadrant during the LATE probe (trial 14). A 2×2 ANOVA was conducted and the main effect of Lesion approached statistical significance ($F(1, 15) = 4.26$, $P = 0.057$), indicating a tendency for PRh rats to spend more time in the target quad-

rant relative to Sham rats. The main effect of Time and the interaction between Lesion and Time were not significant ($F_s < 1$).

Fig. 3A shows the latencies of rats to escape onto the hidden platform during the retention test for the REMOTE problem. A 2×16 ANOVA revealed a significant main effect of Trials ($F(13, 195) = 7.60$, $P = 0.001$); rats tended to have longer latencies to find the hidden platform on initial trials. The main effect of Lesion, and the interaction between Lesion and Trials were not significant ($F_s < 1$). Fig. 3B shows the escape latencies during the retention test for the RECENT problem. As for the REMOTE problem, a 2×16 ANOVA revealed a significant main effect of Trials ($F(13, 195) = 1.76$, $P = 0.052$), and nonsignificant effects of Lesion and Lesion \times Trials ($F_s < 1$).

An interesting finding was that PRh-lesioned rats tended to have longer escape latencies on trials that followed probes (see Fig. 3A, trial 1, and Fig. 3B, trials 3 and 15). However, the large variance on those trials obscured any

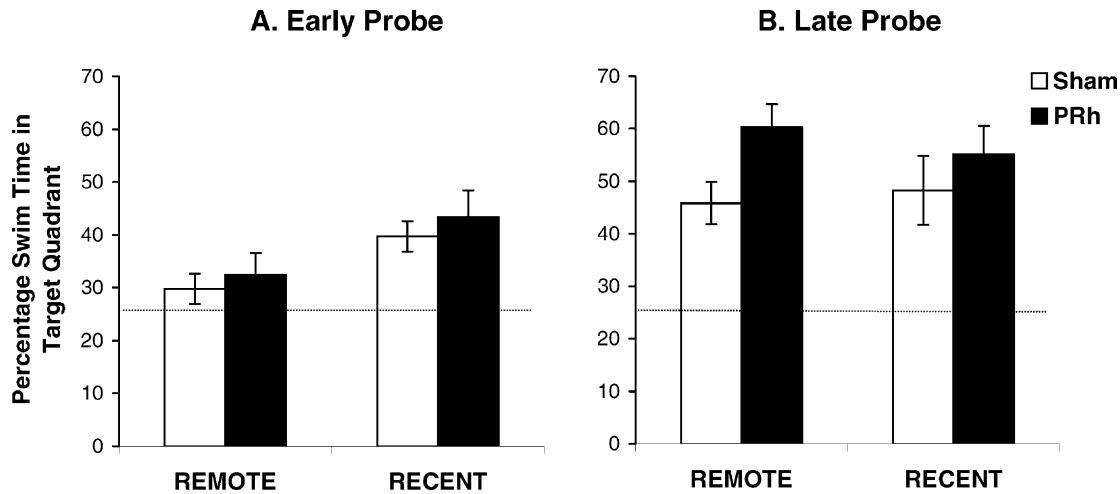


Fig. 2. Mean percentage of total swim time spent in the target quadrant (the quadrant that normally contained the platform) on probe trials conducting during retention testing for the REMOTE and RECENT problems. Panel A shows the EARLY probe data (trial 2) and panel B shows the LATE probe data (trial 14). The dashed lines indicate chance performance (25%). Error bars represent S.E.M.s.

statistically significant group effects when analyzed separately ($P_s > 0.05$).

2.3. Discussion

The PRh lesions in this experiment did not produce retrograde amnesia for the water-maze problems learned 4 weeks and 2 days prior to surgery. PRh rats spent as much time as Sham rats searching for the platform in the target quadrant on the EARLY probe for both the REMOTE and RECENT problems. This finding is inconsistent with our previous finding that PRh rats did not search the pool preferentially on EARLY probe trials for either the REMOTE or RECENT problem [26]. We also previously observed that PRh rats had significantly longer latencies to escape onto the hidden platform on the first trial compared to Sham rats during the retention test for the RECENT problem, but not the REMOTE problem. In the present experiment, PRh and Sham

rats' latencies did not differ significantly on initial trials of retention testing.

This experiment utilized the same design and training/testing procedures as in our previous study [26]. However, a major difference between the two experiments is that, in our previous study, PRh lesions were made electrolytically, whereas in the current experiment they were made by aspiration. Both techniques produced widespread damage to the PRh; in our previous study the largest lesion included approximately 95% PRh tissue, and the smallest lesion included approximately 75% PRh tissue, which is not substantially different from the present lesions. Thus, the discrepant findings cannot be attributed to variations in PRh sparing. However, the aspiration lesions in the present experiment tended to include more tissue in adjacent regions. In our previous experiment, both the largest and smallest lesions included damage to approximately 25% of the lateral entorhinal cortex and less than 5% damage to the posttrihinal

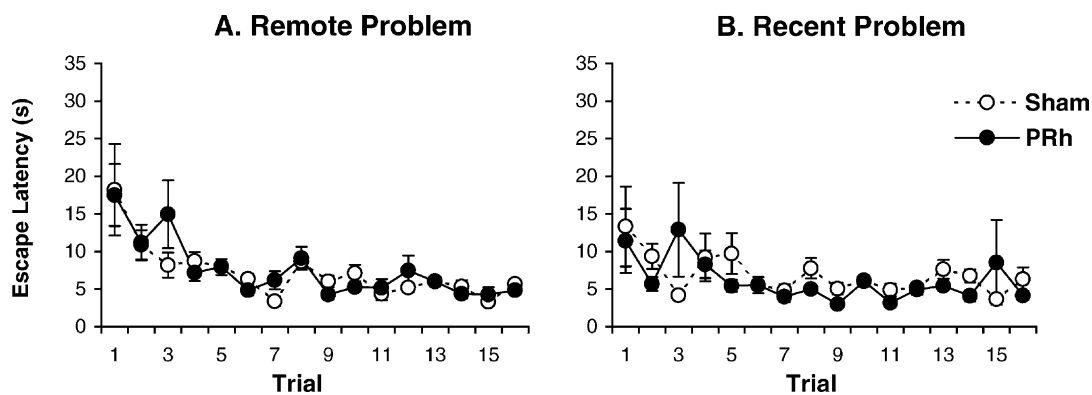


Fig. 3. Mean latency to escape onto the hidden platform during retention testing. Panel A shows the escape latencies for the REMOTE problem, and panel B shows the escape latencies for the RECENT problem. Trials 2 and 14 were probe trials in which the platform was removed from the pool, consequently, the latency to the first platform crossing was used as a measure of escape latency in both figures. Error bars represent S.E.M.s.

cortex. In the present experiment the lesions were estimated to include between 25 and 40% damage to the lateral entorhinal cortex and between 10 and 15% damage to the postrhinal cortex. It seems unlikely that the larger, less specific lesions of this experiment would not produce deficits, and the more discrete lesions in our earlier experiment would. It is possible that subtle differences resulting from conducting the present experiment several months after the original experiment may have contributed to the disparate findings.

Despite the lack of retrograde amnesia in the PRh rats in the current study, there was other evidence that they were not performing the task in the same way as the Sham rats. We observed that PRh rats took longer to locate the hidden platform on normal trials (in which the platform was present) that immediately followed probe trials (in which the platform was absent). While the differences were not statistically significant, they were observed following three of the four probe trials administered during the retention tests. This finding suggests that PRh damage may disturb some aspect of task performance that may be of use in solving the task, but is not critical to successful retention. Thus, this transient contribution may be subtle and difficult to detect. Alternatively, it is possible that the PRh rats learned more from the probe trials than Sham rats (i.e. the platform is no longer located in its usual position) and, therefore, they displayed a tendency to avoid the platform in its usual location on the next, normal trial.

3. Experiment 2: PRh lesions and retrograde memory for water-maze problems with time of learning as a between-groups factor

In Experiment 1, we found that aspiration lesions of the PRh did not produce retrograde amnesia for the platform locations learned prior to surgery. Combined with our previous finding [26], this suggests that the lesion method may be a critical factor in the expression of retrograde amnesia for place information. In this experiment, we addressed the possibility that the design used may also play an important role in whether retrograde amnesia is observed. We did this by conducting an experiment similar to the one above and the one in our previous study [26] but with time of learning (REMOTE versus RECENT) as a between-groups factor.

3.1. Method

3.1.1. Subjects

Twenty-two experimentally naïve, male, Long–Evans rats weighing between 300–350 g at the start of the experiment served as subjects. Housing and colony conditions were the same as in Experiment 1.

3.1.2. Apparatus and materials

The water maze and the testing rooms were the same as in Experiment 1.

3.1.3. Procedure

3.1.3.1. Presurgery training. Four weeks prior to surgery (REMOTE time point), half the rats were taught the location of a stationary, hidden platform in a water maze. During the week before surgery (RECENT time point) the other half of the rats were taught the problem. The same two, distinct testing rooms used in Experiment 1 were used in this experiment. The rooms were counterbalanced at each time point. Each rat received the same training as in Experiment 1; three 8-trial sessions on consecutive days.

3.1.3.2. Surgery. Approximately 48 h after the final day of training on the RECENT problems, all rats underwent either bilateral aspiration lesions of the PRh (REMOTE, $n = 6$; RECENT, $n = 6$) or sham surgery (REMOTE, $n = 5$; RECENT, $n = 5$). Surgical procedures were the same as in Experiment 1. Rats recovered for 2 weeks prior to the commencement of retention testing.

3.1.3.3. Postsurgery testing. The retention test consisted of a single, 15-trial session, and trials 2 and 13 were probe trials (EARLY and LATE probe, respectively) in which the platform was removed from the pool and the search patterns of rats were collected for a 30-s period.

3.1.3.4. Histology. All procedures were as in Experiment 1.

3.2. Results

3.2.1. Histological results

Fig. 4 shows the location and extent of the PRh lesions. As in Experiment 1, there was nearly complete, bilateral destruction of the PRh in each lesioned rat. The PRh was 100% damaged in the rat with the largest lesion, and approximately 80% damaged in the rat with the smallest lesion. All PRh rats in this experiment also had bilateral damage to the lateral entorhinal cortex. This structure was approximately 50% damaged in the rat with the largest lesion, and 15% damaged in the rat with the smallest lesion. This damage also tended to be worse in the posterior extent of the lesions, with more bilateral sparing of the lateral entorhinal cortex in the anterior extent of the lesions.

There was also bilateral damage to the anterior region of the postrhinal cortex in eight of the 12 rats. This damage was estimated to encompass approximately 10–30% of the postrhinal cortex. The other five rats had unilateral damage to this area, estimated to include approximately 5–10% of the postrhinal cortex. Five of the eight rats with bilateral postrhinal cortical damage were in the REMOTE group, and the other three were in the RECENT group.

Four of the 12 PRh rats had bilateral damage to the ventral portions of the temporal association cortex, and two had unilateral damage in this region. The rat with the largest lesion sustained approximately 25% damage to this area,

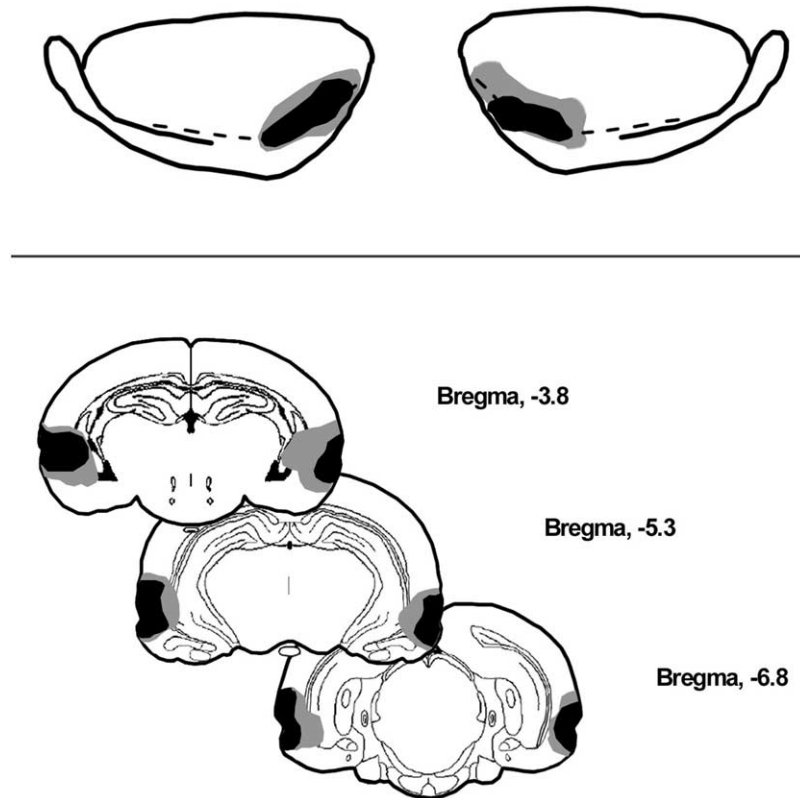


Fig. 4. The top panel shows the location and extent of the largest (grey) and smallest (black) PRh lesions. The bottom panel shows the coronal view of the largest (grey) and smallest (black) PRh lesions at three planes.

whereas in the rat with smallest lesion less than 5% was damaged. One rat had a small amount of bilateral damage to the piriform cortex. There was also slight, unilateral damage to the amygdala in two rats, and bilateral damage in one rat.

In two PRh rats there was unilateral damage to the CA1 subfield of the hippocampus, in one rat there was minor, but bilateral damage to this region, and another rat had unilateral damage to the subiculum. One of the PRh rats with unilateral damage to the hippocampus, the rat with bilateral damage to the hippocampus, and the rat with unilateral damage to the subiculum were in the REMOTE group. The other rat with unilateral damage to the hippocampus was in the RECENT group.

Overall, the location and extent of the PRh damage in Experiments 1 and 2 was comparable. The main difference was the greater extra-PRh damage in the lesions from Experiment 2. In particular, the entorhinal and postrhinal cortices sustained more damage in this experiment than in Experiment 1.

3.2.2. Behavioral results

The presurgery performance of the four groups of rats was well matched in terms of their latencies to escape onto the hidden platform on the final day of training (PRh-REMOTE: $M = 7.37$ s, S.E.M. = 0.78; Sham-REMOTE: $M = 10.83$ s, S.E.M. = 1.72; PRh-RECENT: $M = 8.14$ s,

S.E.M. = 1.34; Sham-RECENT: $M = 7.58$ s, S.E.M. = 1.57).

Fig. 5A shows the percentage of swim time that PRh- and Sham-lesioned rats spent in the target quadrant on the EARLY probe (trial 2) of the retention test. A 2×2 ANOVA revealed a significant main effect of Lesion ($F(1, 17) = 6.51$, $P = 0.021$), indicating that, overall, Sham rats spent more time in the target quadrant than PRh rats. There was no significant main effect of Time ($F(1, 17) = 1.40$, $P = 0.253$). A significant interaction between Lesion and Time was also observed ($F(1, 17) = 9.32$, $P = 0.007$). Posthoc tests revealed that, for the REMOTE problem, PRh and Sham rats did not differ significantly ($P = 0.441$). However, for the RECENT problem, Sham rats spent significantly more time in the target quadrant relative to PRh rats ($t(9) = 3.63$, $P = 0.003$).

One-sample t -tests revealed that both REMOTE and RECENT Sham-lesioned rats spent more time in the target quadrant on the EARLY probe than would be expected by chance ($t(3) = 3.09$, $P = 0.027$ and $t(4) = 7.87$, $P = 0.001$, respectively). Neither REMOTE nor RECENT PRh rats spent more time in the quadrant than would be expected by chance ($t(5) = 1.79$, $P = 0.067$ and $t(5) = 0.79$, $P = 0.234$, respectively).

Fig. 5B shows the percentage of swim time that PRh and Sham rats spent in that quadrant during the LATE probe

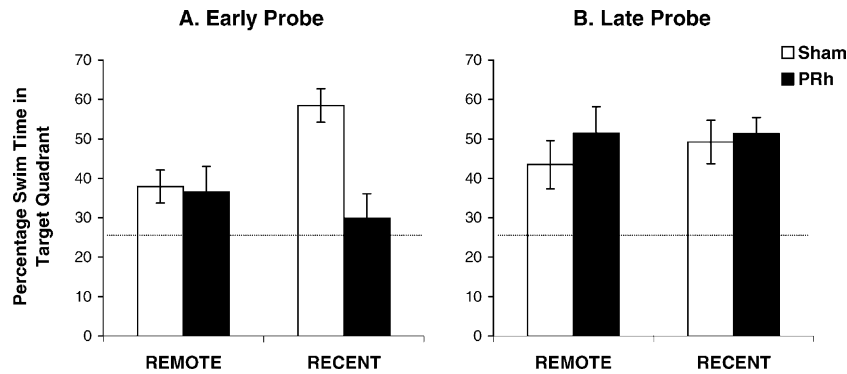


Fig. 5. Mean percentage of total swim time spent in the target quadrant (the quadrant that normally contained the platform) on probe trials conducted during retention testing for the REMOTE and RECENT problems. Panel A shows the EARLY probe data (trial 2) and panel B shows the LATE probe data (trial 13). The dashed lines indicate chance performance (25%). Error bars represent S.E.M.s.

(trial 13) of retention testing. A 2×2 ANOVA revealed no significant main effects of Lesion or Time, and no significant interaction between Lesion and Time (all $F_s < 1$). One-sample t -tests did show that all four groups of rats spent significantly more time in the target quadrant than would be expected by chance (REMOTE: Sham— $t(3) = 3.00$, $P = 0.029$, PRh— $t(5) = 3.90$, $P = 0.006$; RECENT: Sham— $t(4) = 6.34$, $P = 0.002$, PRh— $t(5) = 4.41$, $P = 0.004$).

Fig. 6 depicts the latencies to escape onto the hidden platform for each trial during the REMOTE and RECENT retention tests. A 2×15 (Lesion \times Trials) repeated-measures ANOVA for the REMOTE problem revealed no significant main effect of Lesion, nor was there a significant interaction between Lesion and Trials ($F_s < 1$). There was a significant main effect of Trials ($F(14, 112) = 1.93$, $P = 0.030$, Fig. 6A)—overall, rats tended to have longer latencies on initial trials. A similar analysis of escape latencies during the retention test for the RECENT problem revealed a significant main effect of Trials ($F(14, 126) = 2.71$, $P = 0.009$), but no significant main effect of Lesion ($F < 1$). There was a significant interaction between Lesion and Trials ($F(14, 126) = 2.26$, $P = 0.002$, Fig. 6B). Posthoc

analyses revealed that, on trial 1 of the RECENT problem, PRh rats displayed significantly longer escape latencies relative to Sham rats ($t(9) = 2.236$, $P = 0.026$). The PRh and Sham rats that learned the REMOTE problem did not differ significantly on trial 1 ($P = 0.421$).

3.3. General discussion

The main finding of Experiment 2 was a temporally graded retrograde amnesia for the location of a hidden platform in a water maze. PRh rats that were trained during the week of surgery (RECENT) displayed impaired retention of the platform's location, whereas PRh rats trained 4 weeks prior to surgery (REMOTE) did not. The impairment on the RECENT problem was indexed by a longer latency to find the hidden platform on trial 1 of the retention test, relative to Sham rats. Additionally, PRh rats failed to show a preference for the quadrant that contained the platform on the EARLY probe trial of the retention test, whereas Sham rats showed a strong preference for this quadrant. There were no significant differences in escape latency or probe performance between the PRh and Sham rats that learned the REMOTE problem.

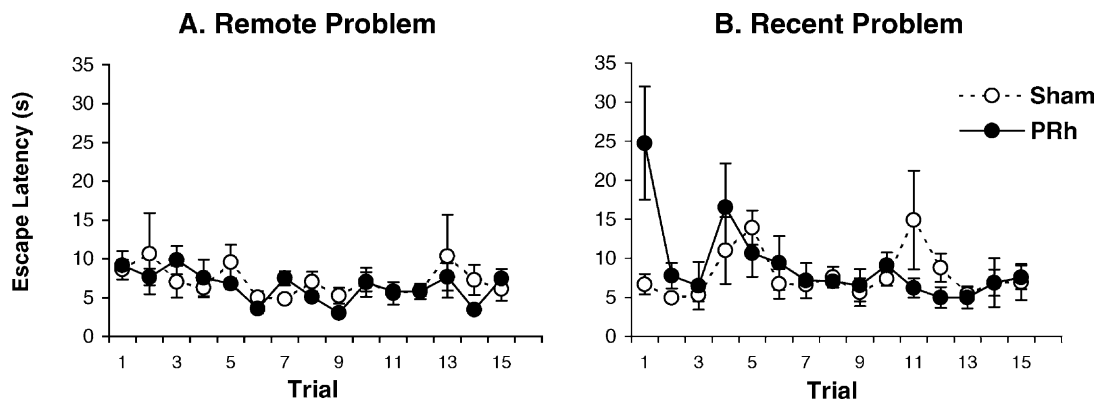


Fig. 6. Mean latency to escape onto the hidden platform during retention testing. Panel A shows the escape latencies for the REMOTE problem, and panel B shows the escape latencies for the RECENT problem. Trials 2 and 13 were probe trials in which the platform was removed from the pool, therefore, the latency to the first platform crossing was again used as a measure of escape latency in both figures. Error bars represent S.E.M.s.

These findings are consistent with our previous report of retrograde amnesia for a water-maze task in rats with PRh lesions [26]. Though we did not previously observe evidence of a temporally graded retrograde amnesia on the EARLY probe in that experiment, we did observe that PRh rats had longer escape latencies than Sham rats on the first trial of the RECENT problem, but not the REMOTE problem. Additionally, though PRh rats that learned the REMOTE problem in Experiment 2 did not differ significantly from Sham rats on the EARLY probe, unlike Sham rats, the time PRh rats spent in the target quadrant was not significantly different from chance, nor was it different from the amount of time the PRh rats that learned the RECENT problem spent in the target quadrant. Thus, the pattern of deficits in Experiment 2 could be viewed as a flat gradient of retrograde amnesia. It is, however, difficult to directly compare these two experiments, as both the lesion method and the design were different, but when considered together they suggest that the PRh contributes to the retention of water-maze problems. The precise nature of this contribution is not clear. As in our previous study, the PRh rats in Experiment 2 of the present study that displayed retrograde amnesia were able to rapidly relearn the problem during the retention test, as evidenced by their performance on the LATE probe trial and their escape latencies throughout the session. Thus, it seems unlikely that the retrograde amnesia we observed was due to a spatial navigation deficit.

Due to the transient and subtle nature of retrograde amnesia for water-maze problems following PRh lesions, it seems likely that the PRh is essential to some element of task performance that is not required to solve the problem, but may be used to aid performance. For example, it is possible that the PRh temporarily interferes with the ability of rats to remember rules that guide performance (e.g. locate the hidden platform). Another possibility is that the PRh is important for the retention of information about context in which training occurred. Therefore, during the retention test, there is a minor, but recoverable, impairment in performance. Consistent with this interpretation, Bucci et al. [1] reported that PRh lesions produced both anterograde and retrograde deficits in fear conditioning to the training context, but not to a discrete stimulus. Additionally, Bussey et al. [7] found that PRh lesions produced an impairment in the ability of rats to acquire a conditional task in which the identity of a rewarded object depended on the context. Thus, PRh rats that show deficits on the initial portions of the retention test may be unable to recognize the context, or certain features of the context, while retaining the ability to form and flexibly use spatial representations of the environment. A related possibility is that PRh damage limits the extent to which rats can identify or recognize extramaze features of the environment. The PRh is thought to be important for performance on tasks that require the processing of object information [30]. Thus, the use of extramaze cues as landmarks to aid navigation is one strategy that may be compromised in PRh rats. This possibility is also consistent with a minor, but recoverable deficit,

as these rats may be able to locate the hidden platform by adopting other strategies that rely less on landmark use.

In Experiment 1, we did not observe retrograde amnesia for the platform locations learned before surgery in rats with aspiration lesions of the PRh. This finding was in contrast with our previous observation of retrograde amnesia in rats with electrolytic lesions of the PRh in an experiment that used the same mixed design, with time of learning as a within-subjects factor. Thus, the behavioral consequences of electrolytic lesions of the PRh appear to differ from those following aspiration lesions. The application of repetitive, high currents in the PRh region during surgery might initiate an abnormal and possibly detrimental pattern of activation in regions efferent to the PRh, particularly the entorhinal cortex or hippocampus. Thus, it is possible that this could result in an impairment in the consolidation of the place information. The absence of a temporal gradient in that case is consistent with the several reports that hippocampal lesions impair retrograde memory for places, even problems learned as much as 14 weeks before surgery [25]. Additionally, any such disturbance in hippocampal functioning may not be permanent, thus, enabling the rapid reacquisition that was observed during retention testing. This hypothesis warrants further investigation.

In Experiment 2, we did observe retrograde amnesia in PRh rats that learned a single water-maze problem approximately 2 days before surgery, but not in PRh rats that learned the same problem 4 weeks before surgery. The main difference between Experiments 1 and 2 was the design; specifically, time of learning was a within-subjects factor in Experiment 1 and a between-subjects factor in Experiment 2. A comparison of the findings of Experiments 1 and 2 underscore the importance of considering the design of retrograde amnesia studies. In Experiment 1 we did not observe retrograde amnesia, whereas in Experiment 2 we did. It should be noted that there were differences in the extent of the extra-PRh damage between the two studies: The PRh lesions in Experiment 2, unlike those in Experiment 1, tended to encroach upon the anterior portion of the postrhinal cortex. We cannot rule out the possibility that this contributed to the differences in the results of the two experiments. However, we did not detect any substantial degree of postrhinal cortical damage to account for the observed deficits in our original report [26]. Also, Bussey et al. [8] and Bussey et al. [9] assessed spatial memory abilities in rats with combined lesions of the PRh and postrhinal cortex and did not observe any deficits in performance using a water-maze task and the radial-arm maze, and a T-maze, respectively.

Within-subjects designs are typically selected because it is thought that they most accurately reflect the human syndrome. As previously mentioned, there are disadvantages to using this type of design [29]. The rate at which animals acquire the first problem will usually be much slower than the rate at which they acquire subsequent problems. In Experiment 1, when time of learning was a within-subjects factor, the average escape latency on the first training

session of problem 1 was 35.96 s, whereas on the first training session of problem 2 the average escape latency was 16.19 s. Accordingly, the researcher must decide whether to equate exposure to each problem, or whether to establish a learning criterion. In the former situation, subsequent problems may be ‘over-learned’, whereas in the latter situation, subsequent problems will be less familiar. Another concern is equating how each problem is learned. Animals in retrograde memory experiments are still quite dissimilar from human subjects even if they are taught several problems prior to brain damage. It seems unlikely that the animal will not recall prior learning events and attempt to coordinate them with the learning of subsequent problems. Thus, the first problem may be learned in one way, whereas the incorporation of that information when learning other problems may alter the details of what is learned, thus, engaging different brain regions than those utilized during the learning of the first problem. Finally, there is evidence that animals that are given ‘reminders’ prior to amnesic treatments will display flat gradients of retrograde amnesia, when compared to animals not given reminders, which will display temporally graded retrograde amnesia [31,34]. In a within-subjects study, the subsequent problems may remind animals of previous problems, resulting in a more labile memory for those earlier problems. It has been argued that the activity state of a memory, rather than its age, best predicts its vulnerability [23,34,35]. Thus, memories which are in an ‘active state’ when a trauma occurs will be lost, whereas ‘inactive’ memories will be spared.

The investigation of putative consolidation processes and retrograde amnesia in human subjects is frequently plagued by interpretational difficulties. For example, it is often impossible to determine exactly when certain memories were acquired, or how well certain information was learned. It may even prove impossible to confirm the reliability or accuracy of certain memories. In cases where the human subject has sustained a brain injury, it is extremely rare for the damage to be circumscribed to a specific brain region. Therefore, many researchers have adopted the use of animal models to investigate retrograde amnesia. The findings from the present study with rats, combined with our previous work [26], indicate that animal models may also be beset with similar interpretational problems [29].

Between-groups designs may not accurately reflect the human syndrome. However, the aim of animal studies of retrograde amnesia is not solely to reproduce patterns of memory loss observed in human patients. They also serve to provide clues about the organization of memory, and the brain regions critical for memory consolidation. We provide evidence in the current study that the two types of designs can also yield different results when most other variables are held constant. The apparatus, rooms, and surgical and behavioral training and testing procedures were the same in both experiments. The lesion technique was also the same and the extent of the PRh damage was comparable. As previously discussed, there were differences in the amount

of extra-PRh damage that may account for the differing results, however, it appears that the design may also be an important factor. Furthermore, the escape latencies of Sham rats during the retention tests of our previous experiment ([26], Fig. 3) and Experiment 1 was comparable; between 15 and 20 s on initial trials for the REMOTE problem and between 10 and 15 s for the RECENT problem. These scores are different from those of the Sham rats in Experiment 2; between 5 and 10 s on initial trials for the REMOTE and RECENT problems. This further supports our interpretation that the lesion method led to the differences between our original experiment and Experiment 1, whereas the design led to the differences between Experiments 1 and 2 in the present study.

These two experiments not only provided more information about the contribution of the PRh to retrograde spatial memory, they also yielded insight into how certain methodological factors, namely lesion method and design, deserve careful attention in future studies. These factors may bear importantly on the pattern of behavioral deficits that are observed, and could have consequences for future animal studies of retrograde amnesia. As many researchers are currently using excitotoxins to lesion the PRh, and because there is evidence that both excitotoxic and electrolytic lesions of the PRh produce anterograde spatial deficits, it would be of particular interest to assess retrograde place memory in rats with excitotoxic lesions of the PRh.

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