

Research report

Dissociation in retrograde memory for object discriminations and object recognition in rats with perirhinal cortex damage

Dave G. Mumby *, Melissa J. Glenn, Catherine Nesbitt, Diana A. Kyriazis

Center for Studies in Behavioral Neurobiology, Department of Psychology, DS-413, Concordia University, 7141 Sherbrooke St. W., Montreal, Que., Canada H4B 1R6

Received 13 August 2001; received in revised form 9 October 2001; accepted 9 October 2001

Abstract

This experiment examined the effects of perirhinal cortex (PeRh) lesions on rats' retrograde memory for object-discriminations and retrograde object recognition. Rats learned one discrimination problem or five concurrent discrimination problems 4 weeks before surgery, and a new problem or five new problems during the week preceding surgery. Each rat was also familiarized with a sample object in an open field, 5, 3, or 1 week before surgery. PeRh-lesioned rats displayed normal retention of the object discrimination problems, but on a test of novelty preference they showed evidence of impaired recognition of the sample objects. A similar dissociation was observed on anterograde tests of object-discrimination learning and object recognition. The findings suggest the perirhinal cortex plays an essential role in rats' ability to discriminate the familiarity of objects previously encountered either before or after surgery, but this ability may not be essential for accurate performance of a simple object-discrimination task. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Perirhinal cortex; Object recognition; Object discrimination; Retrograde amnesia; Anterograde amnesia

1. Introduction

Damage to the medial–temporal-lobes can produce retrograde amnesia in which recently formed memories are more severely affected than remote memories formed long before the injury. It is widely believed that temporal gradients occur in retrograde amnesia because damage to the hippocampal formation disrupts processes involved in the consolidation of long-term memories. However, several other structures are typically damaged along with the hippocampal formation in patients with temporal-lobe amnesia, including the entorhinal and perirhinal cortices, the parahippocampal gyrus, and various white matter. Findings from studies in monkeys and rats suggest that damage in these areas may underlie some of the anterograde memory deficits

in patients with temporal lobe amnesia. For example, perirhinal cortex lesions impair anterograde object-recognition memory in rats [2,15,24] and monkeys [14,19,35]. It is reasonable to suspect that perirhinal damage may also contribute to some aspects of retrograde amnesia following large temporal-lobe lesions. Accordingly, the present study examined the effects of perirhinal cortex damage on rats' retrograde memory for objects using two tasks—object discrimination and object recognition.

The nonrecurring-items delayed nonmatching-to-sample (DNMS) task is often used to assess anterograde object-recognition abilities in brain-damaged monkeys and rats [27]. However, the DNMS task is not suited for modelling retrograde amnesia because normal rats and monkeys perform poorly when the retention delay is longer than a few minutes. Retention lasting at least several days in control rats is necessary if a test of memory is to be useful in modelling brain-damage produced retrograde amnesia. For this reason,

* Corresponding author. Tel.: +1-514-848-2233; fax: +1-514-848-4545.

E-mail address: mumby@vax2.concordia.ca (D.G. Mumby).

several investigators have used simple object-discrimination tasks to study retrograde memory in rats and monkeys. Normal animals are capable of learning object-discrimination problems quickly and remembering them for weeks, which makes it possible to compare memory for problems learned at different time points spanning several weeks before surgery.

Monkeys with large surgical lesions of the medial temporal lobes have displayed both temporally-graded [34] and nongraded [26] retrograde amnesia for object discriminations learned between 2 and 16 weeks before surgery. The lesions in those studies included most of the hippocampal formation (dentate gyrus, cornu Ammonis, and the subiculum) the entorhinal cortex and parahippocampal gyrus, the temporal stem and other white matter, and in the Salmon et al. [26] study, the perirhinal cortex and amygdala. The noncircumscribed lesions make it impossible to know what features of the brain damage were responsible for the impairments. More recently, rats with large excitotoxic lesions restricted to the hippocampal formation displayed normal retention of object-discrimination problems learned between 13 weeks and 2 h before surgery [20]. These findings suggest that the hippocampal formation is not required for long-term consolidation of information underlying accurate performance of an object-discrimination task, and that the deficits observed in monkeys with large temporal-lobe lesions were due to damage outside the hippocampal formation, or a combination of hippocampal and extrahippocampal damage.

A few studies have assessed retrograde amnesia for object discriminations following perirhinal cortex damage, but the findings are inconclusive. Monkeys with rhinal cortex lesions (combined damage to perirhinal and entorhinal cortices) displayed retrograde amnesia with a flat gradient for object discriminations learned up to 16 weeks before surgery [14,28]. There are reports of temporally-graded retrograde amnesia [16], and no retrograde amnesia [4], in rats with perirhinal cortex lesions that also included much of the lateral entorhinal cortex. Rats with perirhinal cortex lesions that included only slight damage to the entorhinal cortex have displayed both graded [32] and nongraded [21] retrograde amnesia for object-discriminations, but in both cases the deficits were very mild and did not resemble the severe retrograde amnesia in monkeys with large temporal lobe lesions [26,34]. Direct comparison of data from rats and monkeys must be made with caution, especially when trying to equate the severity of a lesion-induced deficit. However, in those studies that reported severe retrograde amnesia in rats, damage to the entorhinal cortex was extensive and may have been the cause of the impairment, either by itself or in combination with perirhinal cortex damage.

Another possibility is that the conditions under which object-discrimination problems are learned can

affect the extent to which performance depends upon the perirhinal cortex. Performance of visual-discrimination tasks involving either real objects or graphic images on a computer touch-screen may be more sensitive to perirhinal cortex damage when numerous problems are learned concurrently than when only one or a few are learned concurrently. Some support for this hypothesis comes from previous studies reporting severe retrograde amnesia following perirhinal cortex damage, in which the subjects had to remember many object-discrimination problems, and others that found no retrograde amnesia or only mild retrograde amnesia when the subjects had to remember only a few discrimination problems [4,21]. We tested this hypothesis directly by training rats on either one discrimination problem or five discrimination problems concurrently, at remote and recent presurgery time periods, and testing their retention of those problems after perirhinal cortex lesions.

Despite its practical advantages, the object-discrimination task may not be entirely appropriate for modelling the types of retrograde memory deficits displayed by human amnesic patients. This is because discrimination problems are learned over several trials and selection of the S+ is repeatedly paired with food reward. It is therefore possible that accurate performance is established and maintained by processes similar to those underlying certain types of learning that are spared in human amnesia. For example, animals could reliably select the correct object on the basis of an object–reward or object–response association. Similar types of simple associative learning are often spared in amnesic patients [17].

A model of retrograde amnesia that used an object-recognition test would have more psychological validity, because amnesic patients with temporal-lobe damage typically have impairments in visual recognition memory, at least when their damage extends beyond the hippocampal formation [3]. As already mentioned, the DNMS test of object recognition is not suited for studies of retrograde amnesia, but another paradigm that is sometimes used to assess anterograde object recognition might be—namely, the spontaneous novelty-preference test of object recognition [13]. This task is based on rats' natural propensity to explore novelty. On the standard version, a rat is placed in an open-field arena and allowed to explore two identical sample objects for a few minutes. After a retention delay, the rat is allowed to explore two new objects—one is identical to the sample and the other is novel. Normal rats spend more time exploring the novel object during the first few minutes of the retention test, and when this bias is observed it is inferred that the rat remembers the sample object. With conventional procedures that utilize a single sample-exposure phase, which usually lasts 2–5 min, rats typically are able to discrim-

inate the sample and novel objects after retention delays lasting several hours. We have found that if rats are given repeated brief exposures to the sample object over a period of a few days, they can discriminate the sample from a novel object after delays of several weeks [22]. In the present experiment, we used this modification of the standard novelty-preference paradigm to assess the effects of perirhinal cortex lesions on retrograde object recognition.

2. Method

2.1. Subjects

The subjects were 32 experimentally naive, male, Long-Evans rats (Charles River, St. Constant, Que.) between ≈ 10 and 12 weeks old at the beginning of the experiment. They were housed individually with continuous access to water under a 12:12 light–dark cycle, with light onset at 08:00 h. Their body weights were initially reduced to $\approx 90\%$ of presurgery levels by giving them daily rations of rat chow. Thereafter, they received ≈ 25 g of chow per day throughout the remainder of the experiment, except for the 1st week of postsurgery recovery, during which they received food ad libitum.

2.2. Materials

The apparatus for the object-discrimination task has been described in detail elsewhere [25]. Briefly, it consisted of an elevated runway, separated from identical goal areas at each end by opaque guillotine doors. Each goal area contained two food wells into which food pellets (45 mg Bio-Serv, Inc., Frenchtown, NJ) could be delivered by hand through plastic tubes that were mounted on the outside of the apparatus. A short divider wall protruded from the centre of the end wall to separate the two food wells.

The stimuli for the object-discrimination problems were objects of various shapes, sizes, textures, and colours, each made of a similar plastic material. Each object was large enough to cover a food well but small enough and light enough to be easily displaced by a rat. The objects were washed after every session with a solution of diluted chlorine bleach to remove any extraneous scents they might have acquired during displacement by the rats or handling by the experimenter.

The object-recognition task was conducted in a open-field arena ($60 \times 70 \times 70$ cm³) constructed of grey PVC plastic. A stainless-steel tray served as the floor and was covered with wood shavings. The floor could be removed through a slot at the bottom of one wall to facilitate changing the shavings between each trial. A videocamera was positioned over the arena and the

rats' behaviour during sample and test phases was videotaped for later analysis.

The objects for the recognition task were made of metal, glass, porcelain, or glazed ceramic. There were four copies of each object, which were used interchangeably. The two objects used for the retrograde object-recognition task were a black porcelain statuette (15 cm high), and a round stainless steel cup (9 cm top diameter, 4.5 cm high). The two objects used for the anterograde object-recognition test were a decorative drinking glass (6.5 cm top diameter, 10 cm high), and a blue glass vase (7 cm diameter, 11.5 cm high). Attached with epoxy to the bottom of each object was a small glass jar (6 cm high), and attached to the floor of the arena were two inverted jar lids, each positioned 10 cm from opposing corners of the arena. Objects were fixed in place by screwing the jars into the lids.

3. Procedure

3.1. General design

Rats learned object discrimination problems during the 4th week before surgery (the REMOTE time period) and during the week immediately preceding surgery (the RECENT time period). Half of the rats learned a single discrimination problem at each time period (the 1OD rats) and the other half learned five discrimination problems concurrently at each time period (the 5OD rats). Each rat later received either bilateral perirhinal cortex lesions or sham surgery, yielding four groups: PeRh-1OD ($n = 6$), SHAM-1OD ($n = 10$), PeRh-5OD ($n = 8$) and SHAM-5OD ($n = 8$).

Each rat was also familiarized with a sample object in the open field during either the 5th week before surgery (SHAM, $n = 5$; PeRh, $n = 4$), the 3rd week before surgery (SHAM, $n = 8$; PeRh $n = 5$), or the 1st week before surgery (SHAM, $n = 5$; PeRh, $n = 5$).

After surgery, we trained the rats on a new discrimination problem, then tested their retention of the presurgery discrimination problems. Next we assessed their ability to recognize the presurgery sample object, and lastly, we administered an anterograde object-recognition test.

3.2. Presurgery training: object discrimination

The rats were habituated to the apparatus and shaped to retrieve food pellets from the food wells [25]. Twenty objects were divided into ten pairs, each pair serving as the discriminanda for an object-discrimination problem. One of the objects in each pair was designated S+ (rewarded) and the other one was designated S− (not rewarded), counterbalanced within groups.

To begin a training session, the rat was placed into the apparatus and allowed to explore for ≈ 1 min. To begin the first trial, one of the guillotine doors was closed, and the experimenter positioned S+ and S– over the food wells on the other side of it. The experimenter opened the door, and the rat approached and displaced one of the objects. If it displaced S+, a food pellet was delivered to that food well; if it displaced S–, no food was delivered. A rat was considered to have made a choice if the object was displaced enough to expose the food well. The experimenter then closed the far door and positioned S+ and S– over the food wells on the other side of it, in preparation for the next trial. The intertrial interval was ≈ 15 s. The location of S+ (i.e. left or right well) varied pseudorandomly across trials.

Four weeks before surgery, the rats in groups SHAM-5OD and PeRh-5OD learned five of the problems (the REMOTE set), and the rats in groups SHAM-1OD and PeRh-1OD learned a single problem from that set. During the week immediately preceding surgery, the rats in groups SHAM-5OD and PeRh-5OD learned the other five problems (the RECENT set), and the rats in groups SHAM-1OD and PeRh-1OD learned a single problem from that set.

Training occurred over a 5-day period. For rats in the 5OD groups, each session consisted of 10 trials on each of the five discrimination problems; thus, there were a total of 50 trials per problem spread over the five training days. The trials were administered in blocks of five, each block comprising a different discrimination problem. The five problems were presented in a different sequence on each session, and the sequence was repeated twice per session. The rats spent the 30 s interval between successive trial blocks enclosed in the central area of the apparatus.

For rats in the 1OD groups, each session comprised ten trials of a single discrimination problem, administered in two 5-trial blocks, separated by a 12 min interval; thus, there were a total of 50 trials on this problem over the five training days. The rat remained in the apparatus during the interval between trial blocks.

3.3. Presurgery training: object recognition

Each rat was familiarized with a sample object in the open field arena during either the 5th, 3rd, or 1st week before surgery. The rats received one sample-exposure session per day for five consecutive days. On these sessions a rat was placed into the arena with two copies of the sample object and left to explore for 5 min before it was returned to its home cage. The black porcelain statuette was the sample for half of the SHAM rats and half of the PeRh rats. For the other half, the sample object was the round stainless steel cup.

3.4. Surgery

Surgery was performed under pentobarbitol anaesthesia (65 mg/kg), between 24 and 48 h following each rat's final presurgery object-discrimination session. In preparation for making perirhinal cortex lesions, a coronal scalp incision was made and the skull overlying the perirhinal cortex was exposed. A hole was cut into the skull with a dental drill, the dura overlying the rhinal fissure was incised, and portions of the perirhinal cortex were aspirated with a vacuum pump and a glass Pasteur pipette. The cavity was filled with Gelfoam (Upjohn Company, Don Mills, Ont., Canada), and the incision was closed with wound clips. Rats in the SHAM groups received the same anaesthetic dose and scalp incision, but their skulls were not damaged. The rats were allowed to recover for 14 days before post-surgery testing commenced.

3.5. Postsurgery testing: object discrimination

Postsurgery testing began with assessment of the rats' ability to learn a new object discrimination problem (the NEW problem). Training on this problem consisted of a single session for each rat, and the main learning measure was the number of trials required to reach a criterion of ten consecutive correct trials, at which point the training session ended; however, each rat received a minimum of 40 trials.

One reason for training the rats on a new discrimination problem before conducting the retrograde memory test was to mitigate concern that the lesions might produce transient performance effects that had nothing to do with memory, such as positional biases (e.g. always choosing the object on the right) or a tendency to make hasty responses. Such effects could obscure the retention of presurgically acquired discriminations and could be mistaken for retrograde memory deficits. We reasoned that the rats would have to overcome any disruptive performance effects of the lesions in order to learn the NEW problem. Having done so, their subsequent performance on the REMOTE and RECENT problems during the retention test would be easier to interpret.

Retention testing began the day after rats received their single training session on the NEW discrimination problem. For the rats in groups PeRh-1OD and SHAM-1OD, there was a single test session on which each of the three previously learned discrimination problems were administered in blocks of five trials—the first block of trials involved the NEW discrimination problem, and was followed by blocks of trials on the REMOTE problem and on the RECENT problem, with the order balanced within groups. The cycle was repeated three times within the session, so there were a total of 15 trials of each problem.

For the rats in groups PeRh-5OD and SHAM-5OD, there were five consecutive daily retention sessions, each structured in a similar manner to the single retention session for the 1OD rats. Each session included three different problems—the NEW problem, one problem from the REMOTE set, and one problem from the RECENT set. Trials of each problem were administered in blocks of five, in the order NEW, REMOTE, RECENT, with the cycle repeated three times for a total of 15 trials of each problem.

An extinction procedure was employed on trials for the REMOTE and RECENT discrimination problems. No food reward was given for responses on those trials; however, food reward was still delivered for selecting S+ on the NEW discrimination trials. This extinction procedure was used to circumvent the possibility that anterograde strengthening of object–reward associations would obscure the status of retrograde memory for the problems that had been learned before surgery. In a previous experiment, rats tested with this procedure continued to readily displace objects throughout the extinction trials [21].

3.6. Postsurgery testing: object recognition

The retrograde object-recognition test occurred between 15 and 20 days after surgery. In order to increase the amount of object exploration during the retention test, rats were first rehabilitated to the open-field arena for 5 min with no objects, after which they were returned to their home cages. The retention test was conducted ≈ 1 h later. The rat was placed back into the arena, which now contained a copy of the sample object and a novel object, and was left to explore for 5 min. For the rats that had the black porcelain statuette as the sample, the stainless steel cup served as the novel object; for the rats that had the stainless steel cup as the sample, the black statuette served as the novel object.

The main dependent measure was time spent exploring the sample and novel objects during the first 2 min of the test phase. Previous reports indicated that rats' tendency to spend more time exploring the novel object diminishes after the first 2 min of the test trial, presumably because the difference in familiarity between the two objects diminishes as the trial progresses [10]; pilot experiments in our lab confirmed this finding. A rat was considered to be engaged in object exploration when its head was oriented within 45° of an object and within 4 cm of it. The main index of retention was the exploration ratio—the proportion of total object-exploration time during the test phase that was spent exploring the novel object; $t_{\text{novel}}/(t_{\text{novel}} + t_{\text{sample}})$.

A test of anterograde object recognition was conducted for each rat ≈ 5 days following the retrograde recognition test. A rat was placed into the arena with two identical copies of a sample object, and left to

explore for 5 min. It was then returned to its home cage for a 15 min retention delay. For the test phase, the rat was returned to the arena, which now contained a copy of the sample object and a novel object, and left to explore for 5 min. The same pair of objects was used for the anterograde object-recognition test for each rat—a decorative drinking glass, and a blue glass vase. For approximately half of the rats in each group, the drinking glass was the sample and the vase was the novel object, and the opposite was true for the remaining rats.

4. Results

4.1. Histological findings

Fig. 1 shows the location and extent of the lesions. The perirhinal cortex was nearly completely destroyed bilaterally in each PeRh rat; however, in some of them a small anterior portion of perirhinal cortex was spared in at least one hemisphere. Each PeRh rat also had minor bilateral damage to portions of lateral entorhinal cortex. Most of the lesions included slight to moderate damage to ventro-posterior portions of area Te2. Each PeRh rat also sustained minor bilateral damage to anterior portions of the postrhinal cortex. Precise quantification of the postrhinal damage was not possible for all rats, so the mean percentage of postrhinal cortex removed could not be determined. However, in the rat with the most extensive postrhinal damage, the lesion included roughly the anterior 35% of the postrhinal cortex in one hemisphere and $< 20\%$ in the other. In most of the remaining rats the postrhinal damage was estimated to be less than 10% in one or both hemispheres. In four PeRh rats (three from the 5OD group, and one from the 1OD group) there was minor unilateral damage to the ventral subiculum and temporal CA1 field.

4.2. Behavioural results

4.2.1. Presurgery object discrimination

The PeRh and SHAM groups were well-matched on performance during presurgery acquisition of the object-discrimination problems. Fig. 2 shows the mean percent correct for each group on the final presurgery training session with the REMOTE and RECENT problems. Scores for the 5OD groups reflect the mean of the rats' average scores across the five problems in each set. Analysis of variance (ANOVA), $2 \times 2 \times 2$ with Lesion and Problem Set Size as between factors and Time of Training as a within-subjects factor, revealed no significant main effects or interactions. Scores for the 5OD rats at the end of training were significantly better on the RECENT problem set than on the RE-

MOTE problem set ($F[1, 14] = 25.66, P < 0.001$), but PeRh-5OD and SHAM-5OD rats did not differ in this respect ($F[1, 14] < 1$). Scores for the PeRh-1OD rats and the SHAM-1OD rats did not differ significantly on either the REMOTE or RECENT problems (both the P values < 0.10).

4.2.2. Anterograde object discrimination: acquisition

Postsurgery testing began with a single training session on the NEW discrimination problem. Fig. 3 shows the mean number of trials each group required to reach the criterion of 10 consecutive correct trials on the NEW problem. There were nonsignificant main effects of Lesion, $F[1, 27] < 1$, and Problem Set Size, $F[1, 27] < 1$, but the interaction was significant ($F[1, 27] = 6.22, P < 0.02$). Follow-up analyses revealed only nonsignificant main effects of Lesion—there were no significant differences between the SHAM-1OD and

PeRh-1OD groups, or between the PeRh-5OD and SHAM-5OD groups (P values > 0.05). The simple main effect of Problem Set Size was significant for the PeRh rats ($F[1, 27] = 5.06, P < 0.05$), indicating that the PeRh-5OD rats required fewer trials to reach criterion on the NEW problem than the PeRh-1OD rats; however, this effect of set size was not seen for the SHAM rats ($P < 0.05$).

4.2.3. Anterograde object-discrimination: 24 h retention

Retention testing began the day after the single training session on the NEW discrimination problem. The rats with perirhinal cortex lesions displayed normal 24 h retention of the NEW problem. Fig. 4 shows the group scores on the retention tests. The 5OD groups received five daily retention sessions, each of which included trials with the NEW problem, but the data for the NEW problem shown in Fig. 4 are from only the

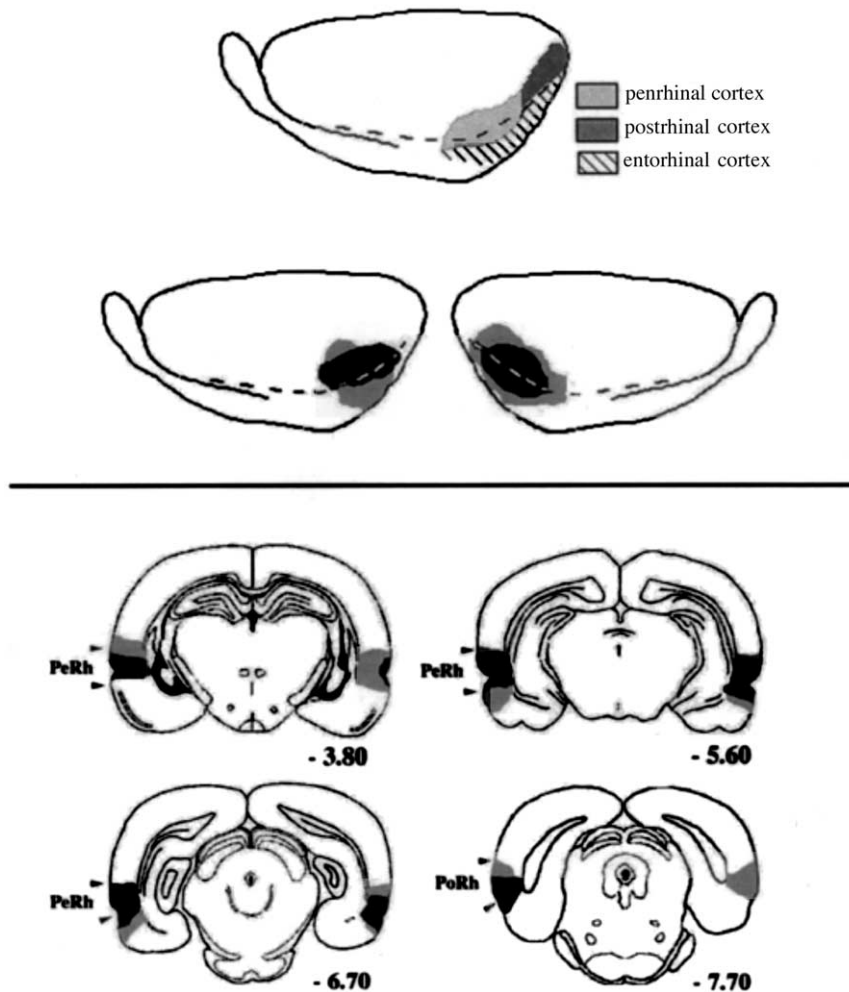


Fig. 1. *Top* shows the approximate boundaries of perirhinal, postrhinal, and entorhinal cortex on lateral surface of rat brain, according to Burwell, Witter, and Amaral [8], and the location and extent of the largest (grey) and smallest (black) PeRh lesions. The largest and smallest lesions were both from rats in the PeRh-5OD group. *Bottom* shows the depth of the largest (grey) and smallest (black) lesions at four coronal planes (distance in mm, relative to bregma). The anterior region of the postrhinal cortex is shown in the most caudal section (-7.7 mm). Arrows indicate the approximate dorsal and ventral boundaries of the perirhinal and postrhinal cortex, based on Burwell et al. [8].

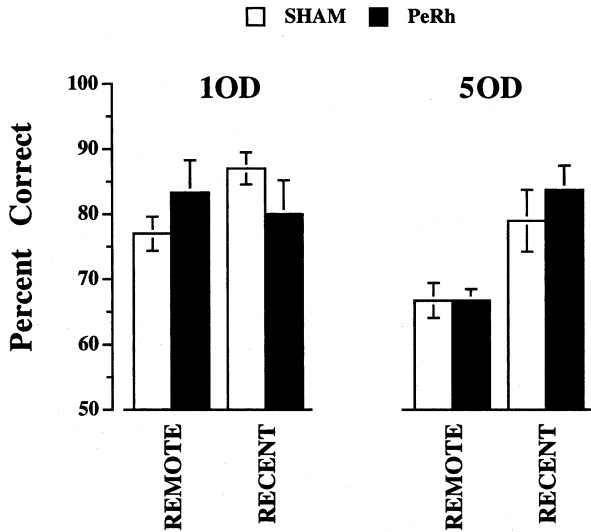


Fig. 2. Mean percent correct on the final presurgery training session with the REMOTE and RECENT object-discrimination problems. The PeRh and SHAM groups did not differ significantly on the final training session before surgery in any of the conditions. Scores for the 50D groups are the means of the rats' average scores across the five problems in each set.

first retention test day, in order to facilitate comparison with the 10D groups, which received only a single retention session. Performance on the NEW problem was stable across the five sessions in both 50D groups (PeRh-50D range = 90.8–98.3%; SHAM-50D range = 90.8–96.7%; data not shown).

There were no significant differences between PeRh-10D and SHAM-10D rats, or between PeRh-50D and SHAM-50D rats, on retention trials for the NEW problem (both the *P* values > 0.40). The PeRh rats' accurate performance was apparent even on the first five-trial block of that session, as they selected the correct object on a mean of 4.88 out of five trials.

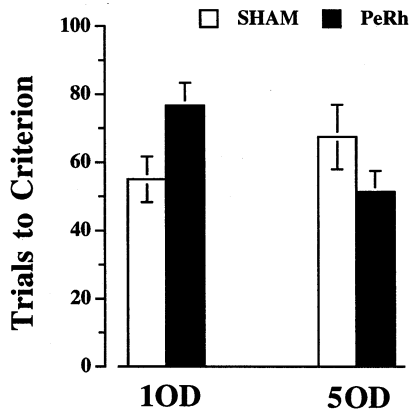


Fig. 3. Mean number of trials each group required to reach the criterion of 10 consecutive correct trials on the NEW problem. Error bars represent SEMs.

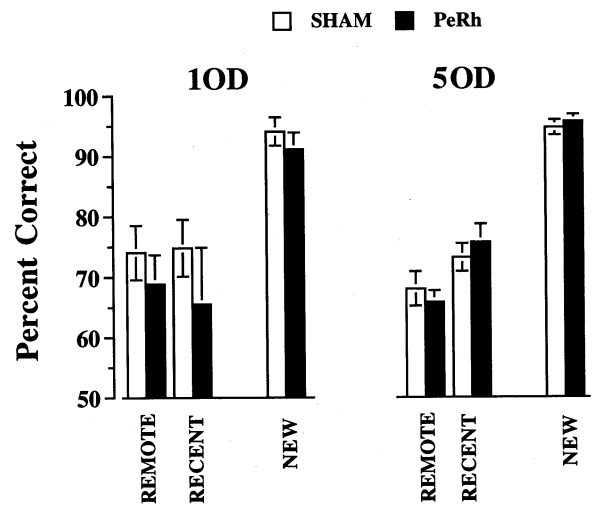


Fig. 4. Mean percentage of retention trials on which S+ was selected for REMOTE, RECENT, and NEW problems. For the 10D groups, data for the NEW problem are from the single retention session; for the 50D groups, the corresponding data are from the first retention test day. Error bars represent SEMs.

4.2.4. Retrograde object discrimination

The rats with perirhinal cortex lesions displayed no evidence of retrograde amnesia for the object-discrimination problems in either the 10D or 50D condition. All rats persisted in readily displacing objects throughout retention testing for REMOTE and RECENT problems despite the extinction schedule that was in effect on those trials, and all showed a strong bias for selecting the previously rewarded object (S+) on all problems. Selection of the previously rewarded object was considered a correct choice.

Fig. 4 shows the results of retention testing for the REMOTE and RECENT problems. Scores for rats in the 50D group represent the rat's mean percent correct across the five problems of each set. A repeated measures ANOVA, with lesion-type (PeRh versus SHAM) and discrimination condition (10D versus 50D) as between-subjects factors, and time of learning (REMOTE versus RECENT) as a within-subjects factor, yielded no significant main effects or interactions.

4.2.5. Retrograde object recognition

The rats with perirhinal cortex lesions displayed evidence of retrograde amnesia on the object-recognition task, but without a temporally-graded effect. Fig. 5 shows the mean exploration ratios on the retrograde recognition test. SHAM rats had exploration ratios that were significantly above chance in the -1 week condition and -3 week condition (*P* values < 0.05) but not the -5 week condition, indicating good retention of the sample object for the two most recent time periods, with significant forgetting over the full range of learning-surgery intervals. In contrast, PeRh rats' exploration ratios were not significantly different from

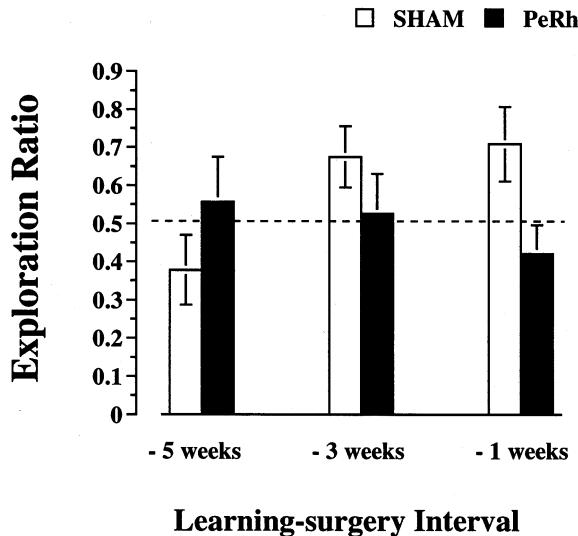


Fig. 5. Mean exploration ratios on the retrograde recognition tests. Error bars represent SEMs.

chance for any of the learning-surgery intervals, suggesting that PeRh rats did not recognize the sample object in any of the conditions. Planned comparisons revealed that the exploration ratios of SHAM and PeRh rats were significantly different in the -1 week condition, $t(8) 2.39$, $P < 0.02$, but not in the -3 or -5 week conditions.

4.2.6. Anterograde object recognition

The rats with perirhinal cortex lesions were significantly impaired on the anterograde object-recognition test. Fig. 6 shows the mean exploration ratios. The SHAM rats' ratios were significantly above chance ($P < 0.05$), indicating an exploratory preference for the novel object. The mean ratio for the PeRh rats, however, was significantly below chance ($P < 0.05$), indicating a preference for the sample object.

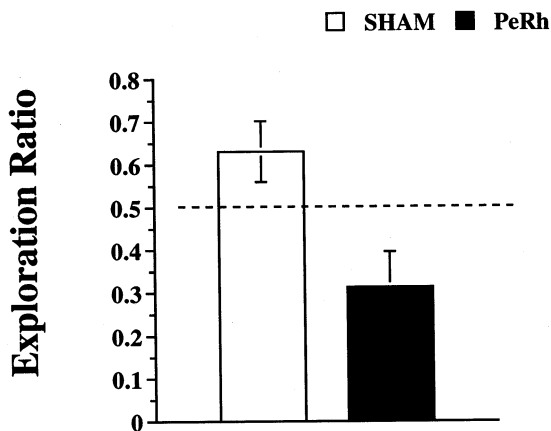


Fig. 6. Mean exploration ratios on the anterograde object-recognition test. Error bars represent SEMs.

Five of the 14 PeRh rats and four of the 18 SHAM rats directed all of their object exploration during the 2 min retention test toward only one of the objects, thus yielding ratios of either 1.0 (explored only the novel object) or 0.0 (explored only the sample object). For all five of the PeRh rats that explored only one object, that object was the sample, whereas for three of the four SHAM rats that explored only one object, it was the novel object. Even if the rats with ratios of 1.0 or 0.0 are removed from the analysis, the ratios of the remaining 9 PeRh rats were not significantly different from chance ($M = 0.488$), whereas the ratios of the remaining 14 SHAM rats were still significantly above chance ($M = 0.597$).

5. Discussion

The rats with perirhinal cortex lesions displayed no evidence of retrograde amnesia for the object-discrimination problems learned at either the remote time period (4 weeks before surgery) or the recent time period (1 week before surgery). The same rats displayed retrograde amnesia on the object-recognition task, without a temporal gradient. They showed no evidence of recognizing an object they were familiarized with either 5, 3, or 1 week before surgery, whereas the sham-lesioned rats recognized an object from 3 or 1 week before surgery. Similarly, rats with perirhinal cortex lesions performed like control rats on the anterograde tests of object-discrimination learning and retention, but not on the anterograde object-recognition test.

On the retention trials for the object-discrimination problems learned before surgery, the rats with perirhinal lesions performed like those with sham lesions in displaying a strong tendency to select S+. This was true whether the rats learned a single discrimination problem at both presurgery time periods, or five problems concurrently at both time periods. Performance on retention trials was unaffected by anterograde learning of object-reward associations, because correct responses were not rewarded on those trials.

The perirhinal lesions also failed to disrupt acquisition of the NEW discrimination problem learned after surgery. Overall, perirhinal rats and control rats did not differ significantly in the number of trials required to master this problem. These results are consistent with those of previous studies in rats [4,21] and monkeys [14], and they suggest that the perirhinal cortex plays a noncritical role in encoding information that underlies accurate object-discrimination performance. Some studies have found impaired acquisition of object-discriminations after perirhinal cortex lesions [16,28], but the lesions in those studies included a much larger portion of the entorhinal cortex than the lesions in the present study. Our PeRh rats sustained only relatively minor

damage to the lateral entorhinal cortex, but most of the perirhinal cortex was removed bilaterally in each of them. The perirhinal cortex lesions did not impair 24 h anterograde retention of the NEW discrimination problem. The latter result is somewhat inconsistent with a previous report of slight, but statistically significant, 24 h anterograde retention deficits for an object-discrimination problem in rats with PeRh damage [21].

The extinction procedure was used on retention trials for the remote and recent discrimination problems to facilitate interpretation of the results. Most previous studies of retrograde memory for object discriminations have assessed retention using trials identical to the original learning trials, and experimental and control animals have typically been compared in terms of percentage correct on postsurgery trials or the number of trials required to relearn the problems to some criterion. Both of these methods assess performance across multiple retention trials, and because the subject receives a reward for selecting S+ on those trials, it is possible that normal anterograde learning of object–reward associations could obscure a retrograde memory deficit. One solution is to compare groups on only the first retention trial because performance on that trial cannot be influenced by anterograde learning. But this solution may not be ideal because animals are likely to experience some degree of arousal, stress, or disorientation upon returning to a test situation for the first time following a long postsurgery recovery. Such responses would likely be greatest on the first few trials, before the subject habituates to the test situation. Moreover, these responses could interact with lesion effects, thereby obscuring the true status of retrograde memory on initial retention trials. An ideal solution would be to assess retention over several trials while minimizing anterograde learning confounds. The extinction procedure accomplished these objectives, because retention was assessed over several trials, and there was nothing the rats could have learned on those trials that would strengthen any existing object–reward associations. In fact, nonreward should have the opposite effect, so a persistent bias toward selecting the S+ can be attributed to retention of the positive associations acquired during the original presurgery training.

For most rats, the tendency to select the previously rewarded S+ on the REMOTE and RECENT problems was stable throughout retention testing, consistent with similar observations in a previous study that employed the same extinction procedure [21]. Presumably, rats continued to select objects on the extinction trials because of the intermittent availability of rewards on trials with the NEW problem that recurred throughout the retention sessions.

The rats were not overtrained on the object-discrimination problems, so the failure to observe retention deficits after perirhinal cortex damage was not due to a

ceiling effect. The groups' mean scores on the final presurgery training session with the REMOTE and RECENT problems ranged from 66.75 to 83.75% (Fig. 2). The SHAM rats' retention scores were also within this range (Fig. 4), and scores within this range on a percentage scale should be optimally sensitive to disruption by effective treatments. This reinforces the conclusion taken from the nonsignificant group differences in retention that retrograde memory for the discrimination problems was intact in the PeRh rats.

It has been proposed that the perirhinal cortex has a critical role in visual-stimulus identification [5–7,11]. Some of the evidence for this view is that deficits in visual-discrimination performance after perirhinal cortex damage can be accentuated by increasing the number of stimuli that must be distinguished from each other—for example, by increasing the number of problems that are learned concurrently [5]. In studies reporting severe retrograde amnesia for visual discrimination problems after rhinal cortex damage, the subjects learned several discrimination problems concurrently [5,14,26,28,34], whereas rats with perirhinal cortex lesions that learned only one discrimination problem at a time were only mildly impaired [21], or not at all [4]. We made a direct test of this hypothesis in the present experiment by requiring the rats to learn either a single discrimination problem or five problems at both presurgery time periods. The 5OD rats had to discriminate 20 objects, whereas the 1OD rats had to discriminate only four objects. Increasing the number of problems to be remembered did not reveal an impairment, but the possibility remains that increasing this requirement even more would have done so. This possibility awaits further investigation, but overall, the present results suggest that perirhinal cortex does not play a major role in the consolidation, long-term storage, or retrieval of object–reward associations, or any other information that is critical for object-discrimination performance.

The object-discrimination task was one of the first tasks used to model retrograde amnesia in monkeys with large medial temporal lesions [26,34], but the lesions in those early studies included several structures, and therefore, it is not clear what damage was responsible for the retrograde amnesia. Recent findings of intact retrograde memory for object discriminations in rats with selective hippocampal lesions [20] suggest that extrahippocampal damage was responsible for producing the retrograde amnesia in the previous monkey studies. The present findings suggest that damage to the perirhinal cortex was not a critical factor, unless it was a combination of damage to the perirhinal cortex and some other area.

Perirhinal cortex lesions probably spared object discrimination because accurate performance is supported by object- or response-reward associations, which are

thought to be mediated by cortical–amygdala and cortical–striatal systems, respectively [18,30]. In a previous study, rats with amygdala lesions were impaired at learning several object-discrimination problems concurrently, although they displayed normal acquisition of a single discrimination problem [23].

Despite showing no evidence of retrograde amnesia for the object-discrimination problems, the rats with perirhinal cortex lesions were impaired on the retrograde object-recognition test. They showed no evidence of recognizing the sample objects that were encountered at any of the presurgery time periods; their exploration ratios were not significantly different from chance regardless of whether the sample object had been encountered 5, 3, or 1 week before surgery. Sham-lesioned rats showed an exploratory bias toward a novel object when it was paired with a sample they had encountered either 1 week before surgery or 3 weeks before surgery, indicating that they remembered those sample objects.

This is the first report of retrograde amnesia in nonhuman animals on a test of object recognition following damage to one of the areas frequently damaged in patients with temporal lobe amnesia. This finding is important because recognition deficits may model the types of memory impairments displayed by amnesic patients more closely than the retrograde amnesia for object-discriminations that has sometimes been reported in monkeys with temporal-lobe damage. Laboratory animals learn discrimination problems over several trials, while selection of the S+ is reinforced by a contingent food reward. Accurate performance is likely to be maintained by processes similar to those underlying certain types of stimulus-response learning or habit formation, which are typically spared in amnesic patients. Indeed, the rats' persistent responding throughout the extinction trials of the retrograde object-discrimination tests is consistent with the notion that habit-formation played a major role in their discrimination performance, even after a retention delay of several weeks and even though the discrimination problems were not overlearned. This may be the exclusive basis for performance when discrimination problems are learned gradually over several trials, as they were in this experiment and in many previous studies in rats or monkeys, but stimulus recognition may also contribute to performance when easy discrimination problems are learned in only a few trials [33].

It is widely believed that temporal-gradients in retrograde amnesia reflect disruption of memory consolidation processes that would normally produce lasting neocortical representations of an event. We observed no evidence of a temporal gradient in the PeRh rats' retrograde recognition deficits, and therefore, no evidence that the perirhinal cortex is involved in the consolidation of neural representations of an object that would eventually be stored in neocortex. Instead

the findings suggest that object representations may be consolidated and stored in perirhinal cortex. If this is true, an explanation is needed for why the PeRh rats could still indicate which of two objects was previously associated with food reward on the object-discrimination task. Perhaps they had acquired an association between the food reward and some elementary stimulus feature of the object that is represented in circuitry outside of perirhinal cortex. It is also possible that rats experience a sense of familiarity akin to that which accompanies object recognition in humans, and this capacity depends on the perirhinal cortex and is necessary for normal performance on the spontaneous recognition task but not the object-discrimination task [1]. There is no reason why experiencing a sensation of familiarity should aid performance of the object-discrimination task—both stimuli involved in the discrimination have been encountered on many previous trials, and therefore both should be very familiar.

The results of the anterograde recognition test are less straightforward. As expected, the sham-lesioned rats displayed a strong exploratory bias toward the novel object. If the data are excluded for rats that explored only one of the two objects during the retention test, the perirhinal rats showed no evidence of discriminating between the sample and novel objects, as their mean exploration ratio was not significantly different from chance, whereas the mean ratio for the sham-lesioned rats remained significantly above chance. However, if data from all rats are included, the mean exploration ratio for the perirhinal group was significantly *below* chance. This result is puzzling, because it suggests that the rats with perirhinal cortex lesions recognized the sample object, but they now had a preference for familiar objects instead of the normal preference for novel objects. Such a scenario is not entirely implausible, and neither is one where an animal explores a sample and novel object equally, not because it has forgotten the sample, but because its natural bias has been altered or eliminated. There clearly are interpretational difficulties with the novelty-preference paradigm, and these have not been discussed very much in the literature despite a recent proliferation of studies that have used it to index memory in mice, rats, and monkeys [27]. Nevertheless, until we replicate the present finding that rats with perirhinal cortex lesions spent significantly more time exploring the sample object, we believe the emphasis should be on the fact that they did not show a preference for the novel object, and by this conventional criterion their anterograde object-recognition abilities were impaired.

The findings of impaired object-recognition memory and intact memory for object-discriminations in rats with perirhinal cortex lesions suggest this brain area plays a significant but limited role in memory for information about objects. The perirhinal cortex ap-

pears to be important for processing information needed to discriminate a familiarity difference between previously encountered and unencountered objects. This conclusion is consistent with several previous studies in rats or monkeys with perirhinal cortex lesions, using either the DNMS task [14,15,19,24,31,35], or the spontaneous novelty-preference paradigm [9,12], and with recent electrophysiological studies (reviewed in [1]) and Fos-imaging studies [29]. The present findings extend previous evidence for impaired object-recognition following perirhinal cortex damage to include impairments in retrograde recognition memory. The loss of this ability does not impair memory for object-discrimination problems, at least not under the conditions examined in this experiment.

Acknowledgements

This research was funded by NSERC of Canada, and FCAR, Que. Requests for reprints should be addressed to Dave G. Mumby, Centre for Studies in Behavioural Neurobiology, Department of Psychology, DS-413, Concordia University, 7141-Sherbrooke St. W., Montreal, Que., Canada H4B 1R6; electronic mail may be sent to mumby@vax2.concordia.ca.

References

- [1] Aggleton JP, Brown MW. Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. *Behav Brain Sci* 1999;22:425–44.
- [2] Aggleton JP, Keen S, Warburton EC, Bussey TJ. Extensive cytotoxic lesions involving both the rhinal cortices and area TE impair recognition but spare spatial alternation in the rat. *Brain Res Bull* 1997;43:279–87.
- [3] Aggleton JP, Shaw C. Amnesia and recognition memory: a reanalysis of psychometric data. *Neuropsychology* 1996;34:51–62.
- [4] Astur RS, Mumby DG, Sutherland RJ. Perirhinal cortex damage: effects on acquisition and retention of object and place discriminations in rats. *Soc Neurosci Abstr* 1995;21:1935.
- [5] Buckley MJ, Gaffan D. Impairment of visual object-discrimination learning after perirhinal cortex ablation. *Behav Neurosci* 1997;111:467–75.
- [6] Buckley MJ, Gaffan D. Perirhinal cortex ablation impairs visual object identification. *J Neurosci* 1998a;18:2268–75.
- [7] Buckley MJ, Gaffan D. Learning and transfer of object–reward associations and the role of the perirhinal cortex. *Behav Neurosci* 1998b;112:15–23.
- [8] Burwell RD, Witter MP, Amaral DG. Perirhinal and postrhinal cortices of the rat: a review of the neuroanatomical literature and comparison with findings from the monkey brain. *Hippocampus* 1995;5:390–408.
- [9] Bussey TJ, Muir JL, Aggleton JP. Functionally dissociating aspects of event memory: the effects of combined perirhinal and postrhinal cortex lesions on object and place memory in the rat. *J Neurosci* 1999;19:495–502.
- [10] Dix SL, Aggleton JP. Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behav Brain Res* 1999;99:191–200.
- [11] Eacott MJ, Gaffan D, Murray EA. Preserved recognition memory for small sets, and impaired stimulus identification for large sets following rhinal cortex ablation in monkeys. *Eur J Neurosci* 1994;6:1466–78.
- [12] Ennaceur A, Aggleton JP. The effects of neurotoxic lesions of the perirhinal cortex combined to fornix transection on object recognition memory in the rat. *Behav Brain Res* 1997;88:181–93.
- [13] Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats: I. Behavioural data. *Behav Brain Res* 1988;31:47–59.
- [14] Gaffan D, Murray EA. Monkeys with rhinal cortex lesions succeed in object discrimination learning despite 24-h intertrial intervals and fail at match to sample despite double sample presentations. *Behav Neurosci* 1992;106:30–8.
- [15] Glenn MJ, Mumby DG. Place- and object-recognition deficits following lesions of the hippocampus or perirhinal cortex in rats: a double dissociation. *Soc Neurosci Abstr* 1996;22:1120.
- [16] Kornecook TJ, Anzarut A, Pinel JPJ. Rhinal cortex, but not medial thalamic, lesions cause retrograde amnesia for objects in rats. *Neuroreport* 1999;10:2853–8.
- [17] Mayes AR. Memory and amnesia. *Behav Brain Res* 1995;66:29–36.
- [18] McDonald RJ, White NM. A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. *Behav Neurosci* 1993;107:3–22.
- [19] Meunier M, Bachevalier J, Mishkin M, Murray EA. Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *J Neurosci* 1993;13:5418–32.
- [20] Mumby DG, Astur RS, Sutherland RJ, Weisend MP. Retrograde amnesia and selective damage to the hippocampal formation: memory for places and object discriminations. *Behav Brain Res* 1999;106:97–107.
- [21] Mumby DG, Glenn MJ. Anterograde and retrograde memory for object discriminations and places in rats with perirhinal cortex lesions. *Behav Brain Res* 2000;114:119–34.
- [22] Mumby DG, Glenn MJ, Nesbitt CE, Kyriazis DA. Dissociation of object recognition and object discrimination in retrograde memory following lesions of perirhinal cortex in rats. *Soc Neurosci Abstr* 2000;26:469.
- [23] Mumby DG, Pinel JPJ, Kornecook TJ, Shen MJ, Redila VA. Memory deficits following lesions of hippocampus or amygdala in rats: assessment by an object-memory test battery. *Psychobiology* 1995;23:26–36.
- [24] Mumby DG, Pinel JPJ. Rhinal cortex lesions and object recognition in rats. *Behav Neurosci* 1994;108:11–8.
- [25] Mumby DG, Pinel JPJ, Wood ER. Nonrecurring-items delayed nonmatching-to-sample in rats: a new paradigm for testing nonspatial working memory. *Psychobiology* 1990;18:321–6.
- [26] Salmon DP, Zola-Morgan S, Squire LR. Retrograde amnesia following combined hippocampus–amygdala lesions in monkeys. *Psychobiology* 1987;15:37–47.
- [27] Steckler T, Drinkenburg WHIM, Sahgal A, Aggleton JP. Recognition memory in rats: I. Concepts and classification. *Prog Neurobiol* 1998;54:289–311.
- [28] Thornton JA, Rothblat LA, Murray EA. Rhinal cortex removal produces amnesia for preoperatively learned discrimination problems but fails to disrupt postoperative acquisition and retention in rhesus monkeys. *J Neurosci* 1997;17:8536–49.
- [29] Wan H, Aggleton JP, Brown MW. Different contributions of the hippocampus and perirhinal cortex to recognition memory. *J Neurosci* 1999;19:1142–8.
- [30] White NM. Mnemonic functions of the basal ganglia. *Curr Opin Neurobiol* 1997;7:164–9.
- [31] Wiig KA, Bilkey DK. Lesions of rat perirhinal cortex exacerbate the memory deficit observed following damage to the fimbria-fornix. *Behav Neurosci* 1995;109:620–30.

- [32] Wiig KA, Cooper LN, Bear MF. Temporally graded retrograde amnesia following separate and combined lesions of the perirhinal cortex and fornix in the rat. *Learn Mem* 1996;3: 313–25.
- [33] Zola-Morgan S, Squire LR. Medial temporal lesions in monkeys impair memory on a variety of tasks sensitive to human amnesia. *Behav Neurosci* 1985;99:22–34.
- [34] Zola-Morgan S, Squire LR. The primate hippocampal formation: evidence for a time-limited role in memory storage. *Science* 1990;250:288–90.
- [35] Zola-Morgan S, Squire LR, Amaral DG, Suzuki WA. Lesions of perirhinal and parahippocampal cortex that spare the amygdala and hippocampal formation produce severe memory impairment. *J Neurosci* 1989;9:4355–70.