

Intact Performance on Feature-Ambiguous Discriminations in Rats with Lesions of the Perirhinal Cortex

Robert E. Clark,^{1,2,*} Pamela Reinagel,⁵ Nicola J. Broadbent,² Erik D. Flister,⁵ and Larry R. Squire^{1,2,3,4}

¹Veterans Affairs Medical Center, San Diego, CA 92161, USA

²Department of Psychiatry

³Department of Neurosciences

⁴Department of Psychology

⁵Section of Neurobiology, Division of Biological Sciences
University of California, San Diego, La Jolla, CA 92093, USA

*Correspondence: reclark@ucsd.edu

DOI 10.1016/j.neuron.2011.03.007

SUMMARY

We developed a behavioral paradigm for the rat that made it possible to separate the evaluation of memory functions from the evaluation of perceptual functions. Animals were given extensive training on an automated two-choice discrimination task and then maintained their memory performance at a high level while interpolated probe trials tested visual perceptual ability. The probe trials systematically varied the degree of feature ambiguity between the stimuli, such that perceptual functions could be tested across 14 different levels of difficulty. As feature ambiguity increased, performance declined in an orderly, monotonic manner (from 87% correct to chance, 50% correct). Bilateral lesions of the perirhinal cortex fully spared the capacity to make feature-ambiguous discriminations and the performance of lesioned and intact animals was indistinguishable at every difficulty level. In contrast, the perirhinal lesions did impair recognition memory. The findings suggest that the perirhinal cortex is important for memory and not for perceptual functions.

INTRODUCTION

Structures within the medial temporal lobe (the hippocampus proper, dentate gyrus, and subicular complex and the perirhinal, entorhinal, and parahippocampal cortices) are critically important for memory (Squire and Zola-Morgan, 1991). Across several decades, behavioral studies of memory-impaired patients, monkeys, and rodents with bilateral damage to these structures have documented a striking impairment in memory, which occurs against a background of apparently preserved intellectual and perceptual functions (Milner et al., 1968; Squire and Zola-Morgan, 1991; Mishkin, 1982).

More recently, this view has been challenged by a growing literature suggesting that the perirhinal cortex, within the medial

temporal lobe, might also have a fundamental role in certain types of high-level visual perception in addition to its accepted role in memory (e.g., Bussey andaksida, 2005; Lee et al., 2005). Specifically, it has been proposed that the perirhinal cortex is required to resolve visual object discriminations when these discriminations contain a high degree of feature overlap or feature ambiguity (Bussey et al., 2002; Barense et al., 2005).

This perspective developed initially from work in the monkey (Eacott et al., 1994). Here, monkeys with bilateral lesions of the entorhinal and perirhinal cortex were impaired on both a 0 s delay and in a simultaneous matching condition. Because the stimuli used in this study shared many overlapping features, the authors suggested that these findings might reflect the requirement of the perirhinal cortex to identify stimuli when the stimuli are perceptually similar. Subsequent work in the monkey was specifically designed to examine the possible contribution of the perirhinal cortex to visual perception. These studies used visual discrimination learning paradigms to assess the performance of monkeys with perirhinal cortex lesions when various attributes of the stimuli were systematically manipulated. Impairments were observed when visual discriminations involved stimuli with high-feature overlap and where good performance appeared to require relatively complex object-level perception (Buckley and Gaffan, 1998; Buckley et al., 2001; Bussey et al., 2002, 2003).

Studies in humans with medial temporal lobe lesions have also addressed this issue, sometimes finding intact performance and sometimes finding an impairment (Shrager et al., 2006; Lee et al., 2005; Kim et al., 2011). A comprehensive review (Suzuki, 2009) suggests that one reason the matter has been difficult to settle in patient studies is that the locus and extent of damage varies among studies and patients with perceptual impairments might have damage to lateral temporal cortex in addition to medial temporal lobe damage. These difficulties need not apply to studies with animals where behavior can be tested after circumscribed lesions of perirhinal cortex or other structures. However, studies in animals encounter another difficulty. Specifically, in order to evaluate perceptual function in these studies, animals must typically be trained and must acquire new information. Accordingly, it is difficult to disambiguate impaired learning

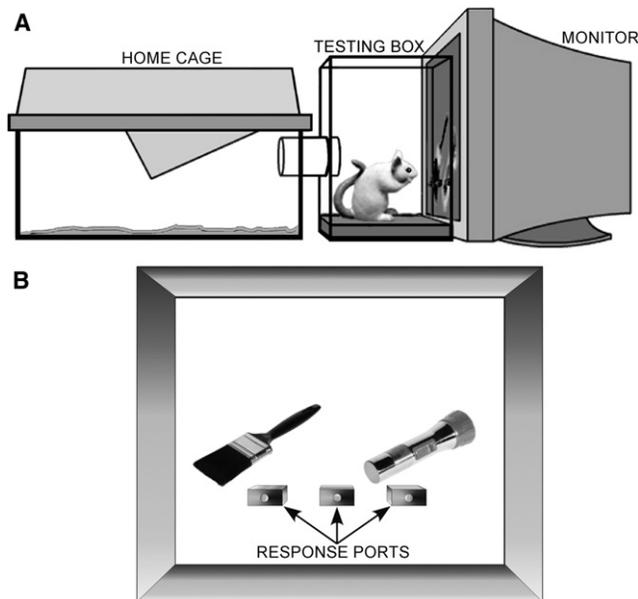


Figure 1. Illustration of the Automated Testing Apparatus

(A) The home cage was taken from a rack in the colony room and connected to a testing box via a circular tube for 2 hr each day, 7 days a week. During this time the Long-Evans rat was free to move between the testing box and the home cage. Food was available ad libitum in the home cage. Water was delivered only when the rat requested it and successfully completed trials within the testing box. Three response ports were mounted on a wall of the testing box immediately in front of the computer monitor.

(B) A cartoon of the rat's view of the CRT. The response ports detected licks and water could be delivered directly to the left and right ports. The rat requested a trial by licking the center port. When a trial was requested, S+ and S- appeared directly behind the left and right response ports. If the rat licked the port in front of the S+ a tone sounded and 16 ml of water was delivered to that port. If the rat licked the port in front of the S- a timeout period occurred during which the rat could not initiate another trial.

and memory from impaired perception (see Hampton 2005; Suzuki, 2009).

Here we report findings from a behavioral paradigm for the rat that made it possible to separate the evaluation of memory functions from the evaluation of perceptual functions (Figures 1 and 2). Animals were given extensive training and then maintained their memory performance at a high level while interpolated probe trials tested perceptual ability. The probe trials systematically tested perceptual functions across 14 different levels of feature ambiguity.

RESULTS

Neurohistological Findings Perirhinal Damage

Figure 3 shows photographs at three AP levels of coronal sections through the perirhinal cortex in an animal with a perirhinal lesion and comparable photographs of a control animal. Figure 4 illustrates the smallest (black) and largest (stippled) extent of the perirhinal cortex lesion. All rats sustained extensive bilateral damage to the perirhinal cortex (average damage

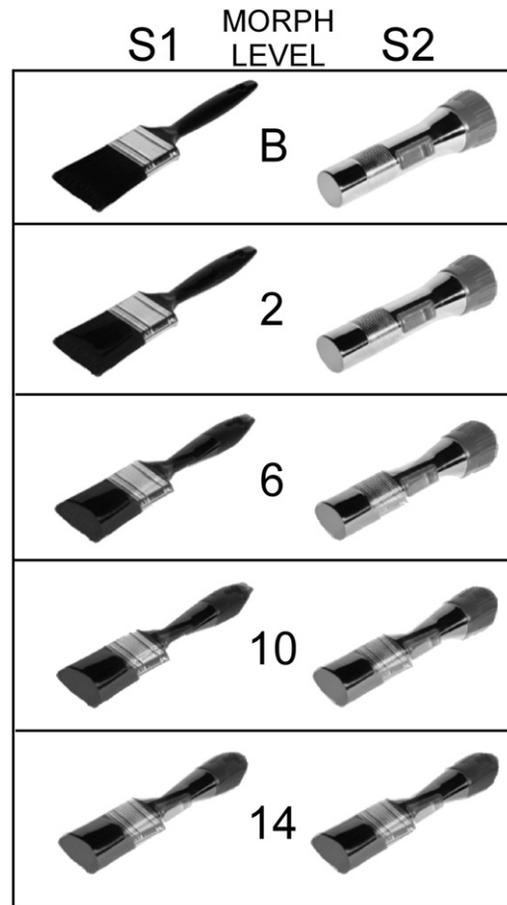


Figure 2. The Two Stimuli Used in the Basic Discrimination Task and the Same Two Stimuli as They Appeared in Four of the Fourteen Different Morph Levels Used during Testing

Morph levels 2, 6, 10, and 14 and the original (basic) discrimination task (B) are shown. The two stimuli are labeled S1 and S2.

91.7% \pm 2.3%; range 84.6%–100%). There was minor sparing of perirhinal tissue in most animals at the most extreme anterior and posterior levels.

Extraperirhinal Damage

All rats sustained limited bilateral damage (i.e., less than 10% of the structure's total volume) to ventral temporal association areas, lateral entorhinal cortex, and postrhinal cortex. Four rats had unilateral damage to the left piriform area. Two rats had unilateral damage to the ventral subiculum and three rats had unilateral damage to the ventral aspects of CA1 immediately adjacent to the rhinal sulcus. Two rats had unilateral damage to superficial layers of the parietal region and posterior association areas.

Behavioral Findings

Automated Discrimination Testing

Preoperative performance: discrimination acquisition. Animals successfully acquired the discrimination acquisition task in 11 to 67 days. The control (CON) group and the to-be-lesioned perirhinal (PR) group performed equally on the trials to criterion measure (CON: 13,369 \pm 3,742; PR: 12,772 \pm 2,700; $t[10] = 0.13$, $p > 0.1$).

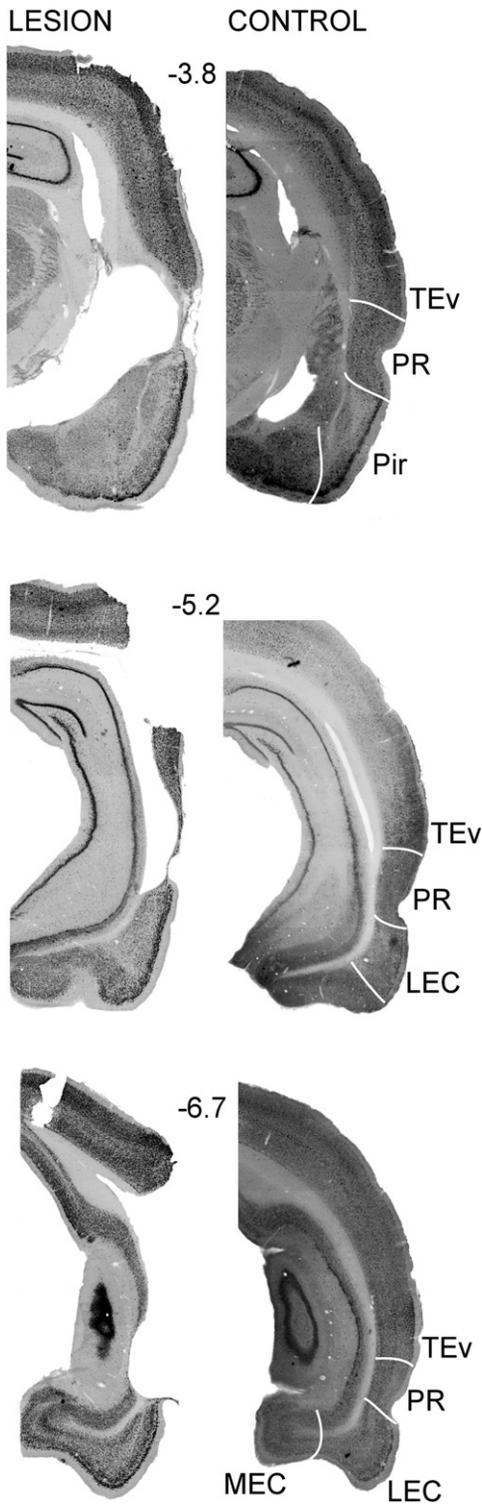


Figure 3. Photographs at Three AP Levels of Coronal Sections through the Perirhinal Cortex in an Animal with a Perirhinal Lesion and Comparable Photographs of a Control Animal
Perirhinal lesion (left) and comparable photographs of a control animal (right). Numbers (center) represent the distance (mm) posterior to bregma. TEv, ventral TE; PR, perirhinal cortex; LEC, lateral entorhinal cortex; MEC, medial

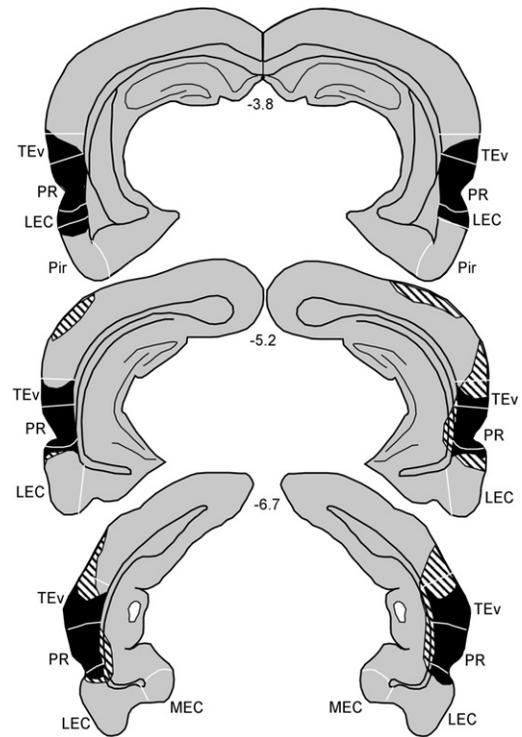


Figure 4. Reconstructions of Coronal Sections through the Perirhinal Cortex Showing the Smallest and Largest Lesion
Smallest (black) and largest (stippled) lesion. Numbers (center) represent the distance (mm) posterior to bregma. TEv, ventral TE; PR, perirhinal cortex; LEC, lateral entorhinal cortex; MEC, medial entorhinal cortex; Pir, piriform area. White lines indicate approximate borders between these structures.

Preoperative performance: morph probe trials. Figure 5 shows the preoperative performance of the CON group and the to-be-lesioned PR group during the morph probe trial phase of training. The two groups performed similarly on the basic discrimination trials (CON: 83.1% ± 2.6% correct; PR: 86.1% ± 1.0%; $t[10] = 1.1, p > 0.1$). The groups also performed similarly across the 14 morph levels (repeated-measures ANOVA for morph level: $F[1,10] = 0.4, p > 0.1$). Both groups exhibited a gradual decline in performance as the morphed stimuli became more similar (repeated-measures ANOVA for morph level: $F[1,13] = 67.0, p < 0.001$). Nonetheless, both groups performed above chance across each of the 14 different morph levels (all $t > 2.5$, all $p < .05$). For the most difficult stimulus pair, control animals performed at 55.3% ± 1.3% and the to-be-lesioned group performed at 53.1% ± 1.2%.

Postoperative Performance

Postoperative performance: postoperative discrimination reacquisition. The CON and PR groups reacquired discrimination after surgery in a similar number of trials (CON: 458 ± 268; PR: 491 ± 173; $t[10] = 0.10, p > 0.1$). We calculated a savings score

entorhinal cortex; Pir, piriform area. White lines indicate the approximate borders between these structures.

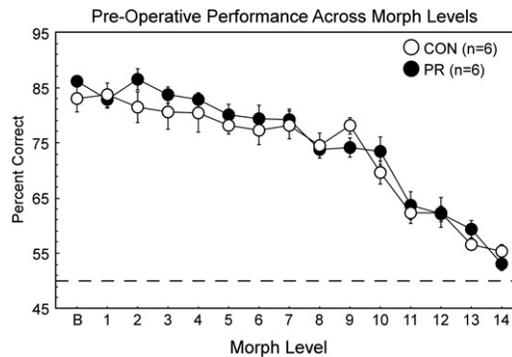


Figure 5. Preoperative Performance for Six Control Animals and Six to-Be-Lesioned Animals during Testing

Preoperative performance is measured by percent correct. The different morph levels were presented randomly during testing but here are arranged by difficulty level from easiest (level 1) to most difficult (level 14). Animals received 150 trials at each morph level and concurrently an additional 10,500 trials on the original (basic) discrimination (morph level B). Dashed line = chance performance (50%). CON, control animals; PR, to-be-lesioned animals. Error bars = SEM.

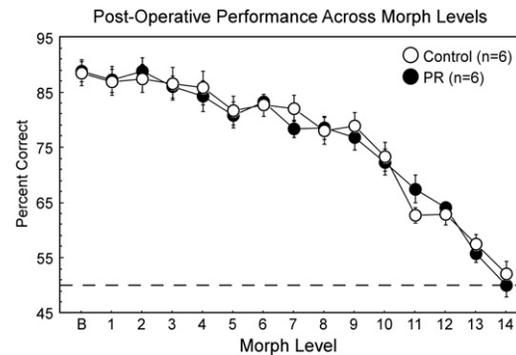


Figure 6. Postoperative Performance for Six Control Animals and Six Lesioned Animals during Testing

Postoperative performance is measured by percent correct. The different morph levels were presented randomly during testing but here are arranged by difficulty level from easiest (level 1) to most difficult (level 14). Animals received 150 trials at each morph level and concurrently received an additional 10,500 trials on the original (basic) discrimination (morph level B). Dashed line = chance performance (50%). CON, control animals; PR, lesioned animals. Error bars = SEM.

for the reacquisition of discrimination ($1 - [\text{postoperative trials-to-criterion/preoperative trials-to-criterion}]$). The two groups exhibited similar and substantial savings scores for discrimination (CON: $97\% \pm 1.0\%$; PR: $96\% \pm 1.0\%$; $t[10] = 0.7$, $p > 0.1$).

Postoperative performance: morph probe trials. Figure 6 shows the postoperative performance of the CON group and the PR group during the morph probe trial phase of training. The two groups performed similarly on the basic discrimination trials (CON: $88.5\% \pm 2.3\%$; PR: $88.7\% \pm 1.9\%$; $t[10] = 0.8$, $p > 0.1$). The groups also performed similarly across the 14 morph levels (repeated-measures ANOVA for group: $F[1,10] = 0.02$, $p > 0.1$). Specifically, the two groups exhibited a similar decline in performance as the morphed stimuli became more similar to each other (repeated-measures ANOVA for morph level: $F[1,13] = 102.0$, $p < 0.0001$) and there was no group-by-morph-level interaction ($F[1,13] = 0.80$, $p > 0.1$). The mean difference between groups across the 14 morph levels was 0.3% (range: -3.6% to $+4.7\%$). Both groups performed above chance at every morph level except the most difficult morph level, morph level 14 (morph levels 1–13: all $t > 2.5$, all $p < .05$). At level 14, both groups performed at the chance level (CON, $52.1\% \pm 5.5\%$; PR $50.0\% \pm 5.0\%$). These data indicate that PR lesions did not affect performance at any morph difficulty level, including the most difficult levels that had the highest amount of feature ambiguity.

Postoperative performance: partially occluded probe trials. Figure 7A shows the postoperative performance of the CON group and the PR group during the partially occluded probe trial phase of testing. The two groups performed similarly on the basic discrimination trials (CON: $84.1\% \pm 2.9\%$; PR: $81.0\% \pm 2.3\%$; $t[9] = 0.9$, $p > 0.1$). The groups also performed similarly across the four different occluded quadrant probe trials (upper left, upper right, lower left, and lower right) (repeated-measures ANOVA for group: $F[1,9] = 0.9$, $p > 0.1$). For both groups, some occluded conditions affected performance more than others (repeated-measures ANOVA for occluded quadrant:

$F[1,3] = 28.0$, $p < 0.0001$). Specifically, for both groups, when the lower left quadrant was occluded, performance was worse than on the basic discrimination trials (see Figure 7A; paired t test; CON: $t[4] = 2.7$, $p = 0.053$; PR: $t[5] = 3.6$, $p < 0.05$). However, even in this condition, both groups performed well above the level of chance (CON: 71.5% , $t[4] = 3.7$, $p < 0.05$; PR: 73.3% , $t[5] = 12.4$, $p < 0.001$).

We next considered the possibility that, despite the data in Figure 7A, the rats might have used local cues to solve the discrimination problem but different rats might have used different local cues in different quadrants. Accordingly, for each rat, we ordered the scores for each of the four conditions from best performance to poorest performance and asked whether performance was still above chance in all conditions. Figure 7B shows that, for the CON group, the worst quadrant probe condition yielded a score of $71.3\% \pm 5.7\%$, a value well above chance ($t[4] = 3.7$, $p < 0.05$). For the PR group, the worst quadrant probe condition yielded a similar score of $72.9\% \pm 2.1\%$, also well above chance ($t[5] = 10.9$, $p < 0.0001$) and not different from the CON group ($t[9] = 0.3$, $p > 0.1$). Because the four different probe conditions together occluded 100% of each stimulus, performance could not have been sustained on all of the occluded trials if a rat were solving the discrimination by using a local cue. Accordingly, these data indicate that rats in both groups were solving the discrimination problem by evaluating the stimuli as wholes.

Recognition Memory Testing

Figure 8 shows the recognition memory performance of the CON group and the PR group across the 3 hr, 24 hr, and 1 month delays. A repeated-measures ANOVA revealed a marginally significant effect for group ($F[1,9] = 3.8$, $p = 0.08$) and an effect of delay ($F[1,2] = 17.2$, $p < 0.001$), but no group-by-delay interaction either with or without the 1 month delay included ($F[1,1-2]$, both $F < 2.0$, $p > 0.1$). Both groups performed above the level of chance on the 3 hr and 24 hr delays (all $t > 4.1$, all $p < 0.01$).

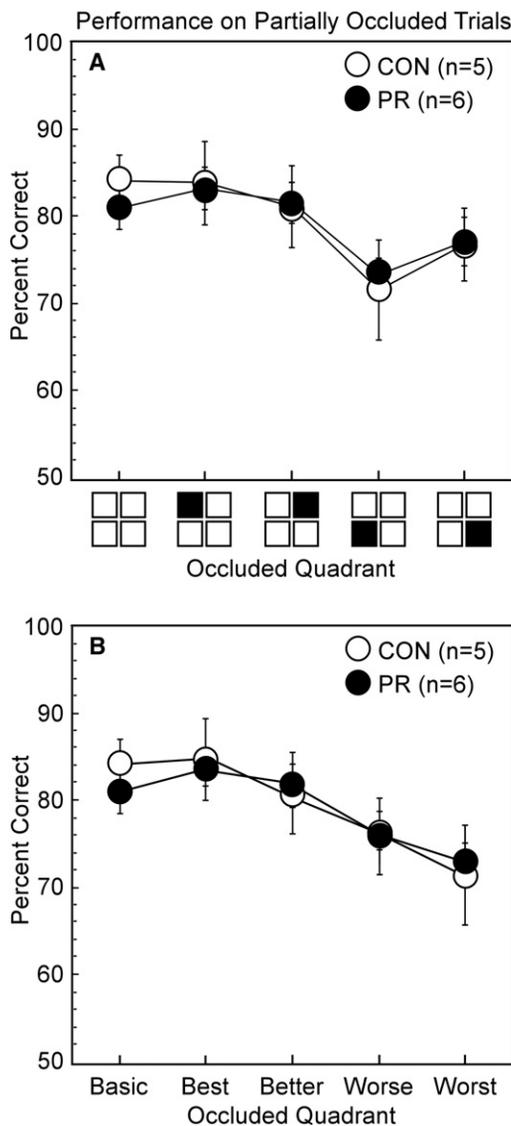


Figure 7. Postoperative Performance for Five Control Animals and Six Lesioned Animals during Occlusion Trials

Postoperative performance is measured by percent correct. Animals received 150 trials with each occluded quadrant and currently an additional 3000 trials on the original (basic) discrimination task (no quadrant occluded).

(A) On each trial, occlusion occupied 25% of each stimulus (upper left, upper right, lower left, or lower right). The four-box icons indicate the quadrant that was occluded (black).

(B) The same data as in (A) but now the occlusion probe trials are ordered from each rat's best condition to each rat's worst condition. The two groups performed similarly and above chance irrespective of how the trials were arranged, i.e., by occluded quadrant (A) or from best to worst (B). The data indicate that neither group used local cues to solve the discrimination problem. CON, control animals; PR, lesioned animals. Error bars = SEM.

Both groups failed to perform above chance on the longest delay (1 month: both $t < 0.6$, both $p > 0.1$).

Between group comparisons. At the 24 hr delay the CON group performed better than the PR group ($t[9] = 2.11$, $p = 0.06$;

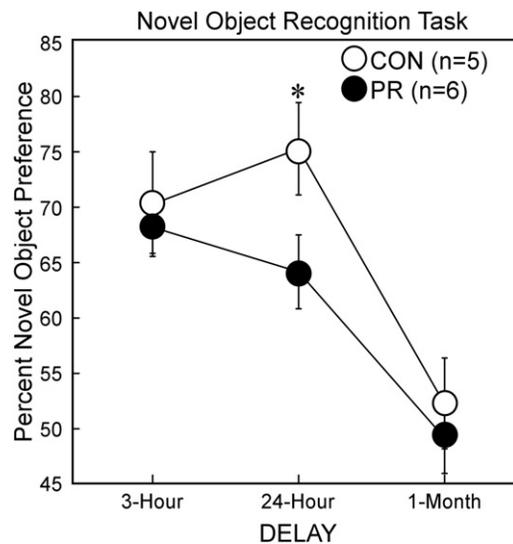


Figure 8. Postoperative Performance for Five Control Animals and Six Animals with Lesions of the Perirhinal Cortex on the NOR Task across 3 Hr, 24 Hr, and 1 Month Delays

Postoperative performance is measured by percent preference for the novel object. The asterisk indicates differences between groups ($p < 0.05$). CON, control animals; PR, animals with lesions of the perirhinal cortex. Error bars = SEM.

Mann-Whitney U test = 4.0, $p < 0.05$). There were no group differences on the other delays. We also applied the Mann-Whitney U test to all of the other between-group comparisons. The findings were the same as for the t tests in all cases. The results from the novel object recognition (NOR) test indicate that PR lesions produced detectable recognition memory impairment.

DISCUSSION

We determined whether the perirhinal cortex is critical for making perceptual judgments between stimuli that contain high degrees of feature ambiguity. The critical data appear in Figure 6. Probe trials were given intermittently while discrimination performance between the two stimuli was maintained at a high level. The probe trials varied the difficulty of the discrimination task by varying the similarity of the two stimuli across 14 steps (see Figure 2). If the perirhinal cortex were critical for feature-ambiguous discriminations, then performance should have been intact at the lower morph levels and progressively impaired as the stimuli began to share more features and become more difficult. This was not the finding. Both groups exhibited a high level of performance at lower morph levels (from 87.0% to 80.2% at levels 1–7) and worse performance at the higher morph levels (from 78.3% to 51.1% at levels 8–14). Importantly, performance of the two groups was indistinguishable across every morph level up to and including the point where performance degraded to chance (at level 14). As the probe trials covered the full range of performance (from 87% correct to chance performance of 50% correct) it cannot be the case that an impairment was missed

because the degree of feature ambiguity was insufficient. The results suggest that the perirhinal cortex is not critical for resolving feature-ambiguous discriminations. While this conclusion entails accepting a null hypothesis, the test was designed to be especially sensitive, with 14 separate morphed tests, and there was considerable opportunity for differences between normal animals and lesioned animals to emerge.

The question arises whether animals might have solved the discrimination by using a single local cue or some punctate feature of the S+ or S− rather than by treating the stimuli as whole objects. If performance had been based on local cues performance would have declined as the morph levels increased, not because the two stimuli became more feature-ambiguous and difficult but rather, because the local cue became more distorted (and less identifiable) as it was morphed into a feature of the other stimulus. To test for this possibility, we gave animals four types of probe trials, each of which occluded one of the four quadrants of each stimulus. If an animal were using a local cue to solve the discrimination then occluding the portion of the stimulus that contained the local cue would adversely affect performance, whereas occluding the other areas of the stimulus would have little or no effect. [Figure 7A](#) shows the performance of each group on the original discrimination and on the four probe trial types. There were no group differences and performance remained well above chance for all four probe trial types. Interestingly, however, performance of both groups tended to decline slightly when the lower quadrants were occluded. This finding suggests that the rats tended to focus on the lower portions of the stimuli, as reported previously (e.g., [Lashley, 1938](#); [Furtak et al., 2009](#)). Importantly, even on these occlusion trials (lower quadrants), both groups performed well above the level of chance (greater than 70% correct).

Another possibility is that the rats did use local cues to solve the discrimination problem but that different rats used local cues in different quadrants. To test for this possibility, we arranged each rat's scores on the four probe trials from best to worst (i.e., highest to lowest) irrespective of which quadrant was occluded. In this way the worst category included every rat's lowest quadrant score. If each rat used a different local cue, the removal of that cue should have disrupted performance and scores should have been poor in the worst category. However, [Figure 7B](#) indicates that performance for both groups was well above chance in the worst category and greater than 70% correct ([Figure 7B](#), rightmost data). These findings provide compelling evidence that the rats did not use local cues to solve the discrimination problem but rather solved the problem by making an object-level discrimination.

We also tested the same rats on the NOR task, a standard task of recognition memory in the rodent ([Clark and Squire, 2010](#); [Winters et al., 2008](#)). [Figure 8](#) shows the performance of both groups. The perirhinal lesion group was impaired on a 24 hr delay. Thus, while the discrimination task and the associated perceptual probe trials did not reveal any hint of impairment, recognition memory was impaired. Impaired recognition memory is the expected result in animals with perirhinal damage (e.g., [Prusky et al., 2004](#); [Kornecook et al., 1999](#); [Mumby and Pinel, 1994](#); [Buffalo et al., 1999](#); [Nemanic et al., 2004](#); [Bussey et al., 1999, 2000](#); [Ennaceur et al., 1996](#); [Winters and Bussey,](#)

[2005](#)). Note though that the recognition memory impairment observed here was milder than has been typically reported. For example, rats with perirhinal lesions are typically impaired on delays as short as 15 min (e.g., [Ennaceur et al., 1996](#); [Winters and Bussey, 2005](#)), whereas our animals were intact on a delay of 3 hr and impaired only on the 24 hr delay. Differences in lesion size between studies are unlikely to account for the different findings because our lesions were as large as, or larger, than those in previous studies ([Ennaceur et al., 1996](#); [Winters and Bussey, 2005](#)). It may be significant that the rats in our study had far more testing experience (i.e., thousands of training trials over several months in the discrimination task) and were tested for recognition memory much longer after perirhinal lesions (i.e., 6–9 months rather than a few weeks) than in any previous study of perirhinal lesions in rats. Perhaps one of these factors (or a combination of these factors) might be important. In any case, the main finding was that our lesions were sufficient to impair recognition memory.

Our finding of intact performance on feature-ambiguous discriminations after perirhinal lesions contrasts with prior work in the monkey. In monkeys, impairments were observed on discriminations that involved stimuli with high-feature overlap and that required complex object-level perception ([Buckley and Gaffan, 1998](#); [Buckley et al., 2001](#); [Bussey et al., 2002, 2003](#)). Yet it has been suggested that these and other impairments, which are ostensibly perceptual, could have also been due to impaired learning and memory ([Hampton, 2005](#); [Suzuki, 2009](#); see also [Baxter, 2009](#)). Importantly, when perception was studied with a protocol designed to minimize the influence of learning and memory, monkeys with perirhinal lesions performed normally, even on very difficult discriminations where the stimuli were rotated, enlarged, shrunk, desaturated, or degraded by masks ([Hampton and Murray, 2002](#)).

In the present study we developed a tactic to reduce the possible influence of learning and memory impairment on perceptual performance. Rather than train animals to learn many discriminations and then present single probe trials for each discrimination ([Hampton and Murray, 2002](#)), we trained animals to learn a single discrimination and then, while maintaining a high level of performance, presented 150 probe trials at each of 14 different levels of feature ambiguity. We suggest that rats with perirhinal cortex lesions exhibited intact performance on every probe trial level because performance did not require any new learning. The basic discrimination was very well learned and performance remained high throughout testing.

One study with rats deserves mention ([Bartko et al., 2007](#)). Lego blocks were used to construct sets of objects with different levels of feature overlap (four levels were used). By using an exploratory task in which rats prefer to explore the odd object in a group of three (with all objects available at the same time), rats with perirhinal cortex lesions performed normally when the objects were most distinct but were impaired when the objects had high degrees of feature overlap. Yet as noted previously ([Suzuki, 2009](#)), it is possible that rats must hold objects in memory as they move back and forth examining the different objects. In support of this idea, a related study found that rats with perirhinal lesions did exhibit impaired performance on this task but that rats with hippocampal lesions exhibited the same

pattern of impairment (N.J. Broadbent et al., 2009, Soc. Neurosci., abstract). These findings raise the possibility that impaired performance on this task might reflect impaired learning and memory rather than impaired perception.

Studies with feature-ambiguous stimuli have also been carried out with patients who have medial temporal lobe damage that includes the perirhinal cortex (Lee et al., 2005; Barense et al., 2007; Lee and Rudebeck, 2010). Yet attempts to replicate some of this work and to find impairments with new tests were not successful (Kim et al., 2011; Shrager et al., 2006). We (Squire and Wixted, 2011) and others (Suzuki, 2009, 2010) have suggested that patients with perirhinal damage who exhibit impaired performance on tasks of visual perception may have significant additional damage to the adjacent lateral temporal cortex.

In summary, we have demonstrated that the capacity to resolve feature ambiguity can be systematically studied in the rat with considerable rigor. When feature ambiguity was increased the discrimination became more difficult and performance declined in an orderly, monotonic manner. Yet performance of the control and perirhinal lesion groups was indistinguishable across every level of difficulty. Further probe testing ruled out the possibility that animals were using local cues to solve the discrimination problem. Lastly, the lesion group exhibited impaired recognition memory. These data support the view that the perirhinal cortex is important for memory and not for perceptual functions.

EXPERIMENTAL PROCEDURES

Subjects

The subjects were 12 female Long-Evans rats that were 5 weeks old at the beginning of the study. Rats were pair-housed and maintained on a 12:12 hr light:dark cycle with training and testing occurring in the dark cycle. Food was freely available. One control rat died before completing behavioral testing and a reduction in the size of the control group is reflected in Figures 7 and 8. All procedures were in accordance with animal protocols that were approved by the University of California, San Diego IACUC.

Behavioral Training and Testing Protocol (Overview)

Automated Discrimination Testing

Shaping. All discrimination training occurred in a specially designed apparatus (Figure 1A). Initial training began with a series of shaping steps that culminated in the acquisition of a preliminary two-choice visual discrimination problem (two distinctive black and white photographs).

Discrimination acquisition. A new discrimination problem was then introduced (S+ versus S−; Figure 1B). Once each rat successfully acquired the two-choice discrimination problem, a morph probe trial phase was begun.

Morph probe trials. During this phase, rats continued testing on the discrimination task. However, probe trials were intermittently presented (on 20% of the trials). Each probe trial involved two stimuli that were morphs of the S+ and S− stimuli. Fourteen pairs of morphed stimuli were used, such that from pair 1 to pair 14 each stimulus was increasingly endowed with the features of the other (i.e., the stimuli became increasingly similar; Figure 2). This phase continued until each subject completed 150 morphed probe trials at each of the 14 steps.

Surgery. After the completion of the morph probe trial phase, half of the rats underwent surgery (bilateral perirhinal lesions) and the other half served as controls.

Postoperative discrimination reacquisition. Rats were retrained to criterion on the same discrimination problem.

Postoperative morph probe trials. This phase was the same as the preoperative morph testing phase.

Partially occluded probe trials. After 2–3 months of testing on other automated tasks, rats were retrained to criterion on the original discrimination.

After reacquisition, rats continued testing on the discrimination task. However, probe trials (20% of total trials) were intermittently presented in which the S+ and S− stimuli were partially occluded.

Recognition Memory Testing

Rats were given the NOR task (Clark et al., 2000) with retention delays of 3 hr (four trials), 24 hr (two trials), and 1 month (four trials). This NOR task was given because it is known to be sensitive to perirhinal lesions (e.g., Ennaceur et al., 1996; Bussey et al., 2000; Winters and Bussey, 2005) and thereby would provide an independent test of the effects of our lesions on behavior.

Detailed Methods

Automated Discrimination Testing

Apparatus. The testing box included a CRT computer monitor immediately adjacent to a transparent enclosure that was integrated with a standard vivarium rat cage (Figure 1). During testing, the rat's home cage was attached to the testing box permitting the rat to enter the testing box to request and complete trials or to return to the home cage to sleep or eat. The rat could obtain water only by correctly responding on training trials. The testing box was fitted with three ports. Each port contained an integrated infrared beam-break detector. Behavior (licking a port) was detected by an infrared beam break and water reinforcement could be delivered directly to either the left or right port. The three ports were spaced equidistant from each other (9.4 cm) across the front of the transparent enclosure and immediately in front of the computer monitor (left-center-right). The rat initiated a trial by licking the center port. When a rat requested a trial a pure tone (500 ms, 750 Hz) was presented along with two visual stimuli (the S+ and S−). The two stimuli were presented directly behind the left and right response ports. The stimuli remained displayed until the left or right port was licked. Correct responses (i.e., licking a port in front of the S+) were rewarded with a pure tone (500 ms, 1.5 kHz) and delivery of approximately 16 μ l of water. Incorrect responses (i.e., licking the port in front of the S−) immediately blanked the monitor and initiated a brief timeout interval (range 2–6 s) such that licking the center port did not initiate a new trial. Rats were trained for 2 hr each day (7 day/week). Proprietary Matlab routines controlled all aspects of the training protocols, timing variables, stimulus and reward presentations, and collection of behavioral response data (Meier et al., 2011).

Training protocol. First, a series of shaping steps were presented so that the rats learned to retrieve water from the ports, request trials, and ultimately acquire a two-choice visual discrimination problem (two distinct black and white photographs). After this shaping phase, a new discrimination problem (black and white photographs of a paintbrush and a flashlight) was presented. The two images were scaled to equal size and matched for luminance and contrast (all pixel luminance distributions were matched). The two images were presented in grayscale against a black background on a linearized CRT monitor. This discrimination problem was used for the remainder of testing. The stimulus that served as the S+ was counterbalanced across rats. The S+ was equally likely to be presented on the left or right though this could be adjusted to overcome a response-side bias (for details see Meier et al., 2011). Each rat was trained to a learning criterion of 85% correct across 200 consecutive trials.

Morphed probe trials. After the acquisition of the discrimination problem, performance was evaluated by using feature-ambiguous probe trials. These probe trials increased the difficulty of the discrimination task by increasing the similarity of the S+ and S−. Probe trials were created by morphing the S+ and S− into one another in 14 steps (Morpheus Photo Animator; ACD Systems, Saanichton, Canada). Thus, one stimulus was gradually morphed into the other, physically changing each stimulus from one step to the next (Figure 2). This morphing procedure is similar to procedures used in previous work with monkeys (Bussey et al., 2003) and humans (Lee et al., 2005; Shrager et al., 2006). Note that one stimulus was not blended into the other. Rather, the entire stimuli were gradually altered so that they became more alike. Probe level 1 consisted of the least amount of feature overlap (i.e., the two stimuli were quite distinct and most similar to the training stimuli). At level 14 the two stimuli contained substantial feature overlap and appeared quite similar (Figure 2). During this phase of testing, 80% of the trials were standard trials (training stimuli). The remaining 20% of the trials were rewarded morphed probe trials. The order of the probe trials (levels 1–14) was pseudorandom

with the constraint that each of the 14 difficulty levels had to be presented once before any one difficulty level could be repeated. This procedure ensured that data for probe trials accrued at the same rate for every difficulty level.

This phase of testing continued until 150 probe trials were completed at each difficulty level. Thus, across this phase of testing each animal received 2,100 probe trials across the 14 different difficulty levels (150 × 14) and an additional 10,500 trials with the training stimuli.

Surgery. Animals were assigned to a perirhinal lesion group or a normal control group based upon their trials-to-criterion score for the discrimination task (to create two equal groups). The intention was to remove the entire perirhinal cortex bilaterally. For surgery, the rat was placed in a Kopf stereotaxic instrument and the incisor bar was adjusted until bregma was level with lambda. Bilateral excitotoxic perirhinal lesions were produced by local micro-injections of ibotenate acid (IBO; Bioscience Technologies, San Rafael, CA). IBO was dissolved in 0.1 M phosphate-buffered saline to provide a solution with a concentration of 10 mg/ml, pH 7.4. IBO was injected at a rate of 0.1 μ l/min with a 10 μ l Hamilton syringe mounted on a stereotaxic frame and held with a Kopf microinjector (model 5000). The syringe needle was lowered to the target coordinate and left in place for 1 min before beginning the injection. After the injection, the syringe needle was left in place for a further 5 min to reduce the spread of IBO up the needle tract. A total of 0.13 μ l of IBO per hemisphere was injected into the perirhinal cortex at the following AP, ML, and DV coordinates: $-3.0, \pm 6.4, -7.7$; $-4.0, \pm 6.5, -7.7$; $-5.0, \pm 6.8, -7.5$; $-6.6, \pm 6.8, -7.0$; and $-7.68, \pm 6.3, -6.7$. Once awake and responsive, each rat was returned to its home cage in the colony room for a 14 day recovery period. Control rats received a 14 day rest from testing.

Postoperative Testing

After recovery, each rat was retrained on the two-choice discrimination task to a criterion of 85% correct across 200 trials. They then moved on to the morphed probe trial phase by using the same procedures as were used preoperatively. Finally, a partially occluded probe trial phase was presented. This phase was intended to evaluate the possibility that rats might have solved the discrimination task by attending to a local cue of the stimulus rather than attending to the stimulus as a whole. If a local cue had been used, then masking that portion of the stimulus should markedly reduce performance. On each probe trial, occlusion occupied 25% of each stimulus (upper left, upper right, lower left, or lower right, along the long axis of each stimulus). The same quadrant was always occluded on both stimuli. During this phase of testing, 80% of the trials were standard trials (training stimuli). The remaining 20% of the trials were occlusion trials. The order of the occlusion probe types was pseudorandom with the constraint that each of the four probe types had to be presented before any one probe type was repeated. This procedure ensured that data for all probe types accrued at the same rate.

This phase of testing continued until 150 probe trials were completed for each of the four types of occlusion trials. Thus, across this phase of testing, each animal received a total of 600 occlusion probe trials and an additional 3000 trials with the training stimuli.

Recognition Memory Testing

Recognition memory was tested by using the NOR task (Clark et al., 2000). This task has been shown to be sensitive to bilateral perirhinal damage (Bussey et al., 1999, 2000; Ennaceur et al., 1996; Winters and Bussey, 2005).

Apparatus. The NOR task was conducted in an opaque plastic box measuring 35 cm × 41.5 cm × 50 cm high. Stimuli consisted of ceramic or plastic objects that varied in color and size (width = 7.6–8.9 cm; height = 7.5–12.7 cm). Three identical copies of each object were available. The objects were secured to the floor of the box with Velcro strips situated approximately 9 cm apart. A video camera mounted on the wall directly above the box was used to record the testing session for offline analysis. Overhead fluorescent lighting illuminated the box.

Procedure: habituation. Rats were acclimated to the testing room and chamber for 2 consecutive days prior to testing (45 min in the testing room and 5 min to explore the empty box).

Procedure: object familiarization. On each day of the 6 days of testing, rats were acclimated to the testing room for 45 min and then placed in the empty box for 1 min. Then the rat was removed and two identical objects were placed centrally 9 cm apart. The rat was then placed back in the box and allowed to explore for 15 min.

NOR Test

Each rat was first rehabilitated to the testing area by being placed in the empty box for 1 min. The rat was then removed, two objects (one novel object and a copy of the object from the familiarization phase) were placed in the box, and the rat was allowed to explore the objects for 15 min. Object exploration was later scored from video recordings of each trial by an experimenter who was blind to the group membership of the rats. Scoring continued until the rat had accumulated 15 s of object exploration. Object exploration was scored when the rat's nose was within 1 cm of the object and the vibrissae were moving (see Clark et al., 2000; Broadbent et al., 2004). Preference for the novel object was expressed as the percent time (out of 15 s of actual object exploration) that a rat spent exploring the novel object. The object that served as the novel object and the left and right positions of the novel object were counterbalanced within each group.

Delays

Three retention delays were tested (3 hr, 24 hr, and 1 month). First, rats were presented with four tests by using the 3 hr delay (a unique test on each of 4 days). They then received two tests by using the 24 hr delay with entirely new objects (a unique test on each of 2 days). Finally, they received four tests after a 1 month delay. For these tests, animals saw the same objects that had been used as the familiar objects during the 3 hr delay tests. The already-familiarized objects from the 3 hr delay test were paired with different novel objects (one unique test on each of 4 days).

Histology

At completion of testing, the rats were administered an overdose of sodium pentobarbital and perfused transcardially with buffered 0.9% NaCl solution followed by 10% formaldehyde solution (in 0.1 M phosphate buffer). The brains were then removed and cryoprotected in 20% glycerol and 10% formaldehyde. Coronal sections (50 μ m) were cut with a freezing microtome. Every fifth section was mounted and stained with thionin to assess the extent of the lesions. An additional series was prepared for immunolocalization of neuron-specific nuclear protein (NeuN) by using an anti-NeuN (1:500, Chemicon) monoclonal mouse antibody. A fluorescent donkey anti-mouse antibody (DYLIGHT 594, 1:250, Jackson ImmunoResearch) was used as the secondary antibody. NeuN-positive cells were assessed by using a Leica fluorescent microscope. Quantification of the perirhinal lesion was based on previous work showing that the extent of damage along the anterior/posterior axis is a good predictor of the lesion's efficacy (Bucci and Burwell, 2004; Burwell et al., 2004). Accordingly, we quantified the proportion of 14 sections along the anterior/posterior extent of the perirhinal cortex (AP range: -2.45 to -6.65 from bregma) that contained damaged tissue (Burwell et al., 2004).

ACKNOWLEDGMENTS

This work was supported by the Medical Research Service of the Department of Veterans Affairs, the National Institute of Mental Health, the National Science Foundation, and the James S. McDonnell Foundation. We thank Priya Velu, Laura Johnson Susan Davis, Brittany Masatsugu, Danielle Dickson, and Fan Li for their assistance. The rodent-shaping methods and training technology were developed by Philip Meier, E.D.F., and P.R.

Accepted: February 3, 2011

Published: April 13, 2011

REFERENCES

- Barense, M.D., Bussey, T.J., Lee, A.C., Rogers, T.T., Davies, R.R., Saksida, L.M., Murray, E.A., and Graham, K.S. (2005). Functional specialization in the human medial temporal lobe. *J. Neurosci.* 25, 10239–10246.
- Barense, M.D., Gaffan, D., and Graham, K.S. (2007). The human medial temporal lobe processes online representations of complex objects. *Neuropsychologia* 45, 2963–2974.
- Bartko, S.J., Winters, B.D., Cowell, R.A., Saksida, L.M., and Bussey, T.J. (2007). Perceptual functions of perirhinal cortex in rats: Zero-delay object recognition and simultaneous oddity discriminations. *J. Neurosci.* 27, 2548–2559.

- Baxter, M.G. (2009). Involvement of medial temporal lobe structures in memory and perception. *Neuron* 61, 667–677.
- Broadbent, N.J., Squire, L.R., and Clark, R.E. (2004). Spatial memory, recognition memory, and the hippocampus. *Proc. Natl. Acad. Sci. USA* 101, 14515–14520.
- Bucci, D.J., and Burwell, R.D. (2004). Deficits in attentional orienting following damage to the perirhinal or postrhinal cortices. *Behav. Neurosci.* 118, 1117–1122.
- Buckley, M.J., and Gaffan, D. (1998). Perirhinal cortex ablation impairs visual object identification. *J. Neurosci.* 18, 2268–2275.
- Buckley, M.J., Booth, M.C., Rolls, E.T., and Gaffan, D. (2001). Selective perceptual impairments after perirhinal cortex ablation. *J. Neurosci.* 21, 9824–9836.
- Buffalo, E.A., Ramus, S.J., Clark, R.E., Teng, E., Squire, L.R., and Zola, S.M. (1999). Dissociation between the effects of damage to perirhinal cortex and area TE. *Learn. Mem.* 6, 572–599.
- Burwell, R.D., Bucci, D.J., Sanborn, M.R., and Jutras, M.J. (2004). Perirhinal and postrhinal contributions to remote memory for context. *J. Neurosci.* 24, 11023–11028.
- Bussey, T.J., and Saksida, L.M. (2005). Object memory and perception in the medial temporal lobe: An alternative approach. *Curr. Opin. Neurobiol.* 15, 730–737.
- Bussey, T.J., Muir, J.L., and Aggleton, J.P. (1999). 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.* 19, 495–502.
- Bussey, T.J., Duck, J., Muir, J.L., and Aggleton, J.P. (2000). Distinct patterns of behavioural impairments resulting from fornix transection or neurotoxic lesions of the perirhinal and postrhinal cortices in the rat. *Behav. Brain Res.* 111, 187–202.
- Bussey, T.J., Saksida, L.M., and Murray, E.A. (2002). Perirhinal cortex resolves feature ambiguity in complex visual discriminations. *Eur. J. Neurosci.* 15, 365–374.
- Bussey, T.J., Saksida, L.M., and Murray, E.A. (2003). Impairments in visual discrimination after perirhinal cortex lesions: Testing ‘declarative’ vs. ‘perceptual-mnemonic’ views of perirhinal cortex function. *Eur. J. Neurosci.* 17, 649–660.
- Clark, R.E., and Squire, L.R. (2010). An animal model of recognition memory and medial temporal lobe amnesia: History and current issues. *Neuropsychologia* 48, 2234–2244.
- Clark, R.E., Zola, S.M., and Squire, L.R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *J. Neurosci.* 20, 8853–8860.
- Eacott, M.J., Gaffan, D., and Murray, E.A. (1994). Preserved recognition memory for small sets, and impaired stimulus identification for large sets, following rhinal cortex ablations in monkeys. *Eur. J. Neurosci.* 6, 1466–1478.
- Ennaceur, A., Neave, N., and Aggleton, J.P. (1996). Neurotoxic lesions of the perirhinal cortex do not mimic the behavioural effects of fornix transection in the rat. *Behav. Brain Res.* 80, 9–25.
- Furtak, S.C., Cho, C.E., Kerr, K.M., Barredo, J.L., Alleyne, J.E., Patterson, Y.R., and Burwell, R.D. (2009). The floor projection maze: A novel behavioral apparatus for presenting visual stimuli to rats. *J. Neurosci. Methods* 181, 82–88.
- Hampton, R.R. (2005). Monkey perirhinal cortex is critical for visual memory, but not for visual perception: Reexamination of the behavioural evidence from monkeys. *Q. J. Exp. Psychol. B* 58, 283–299.
- Hampton, R.R., and Murray, E.A. (2002). Learning of discriminations is impaired, but generalization to altered views is intact, in monkeys (*Macaca mulatta*) with perirhinal cortex removal. *Behav. Neurosci.* 116, 363–377.
- Kim, S., Jeneson, A., van der Horst, A., Frascino, J., Hopkins, R.O., and Squire, L.R. (2011). Memory, visual discrimination performance, and the human hippocampus. *J. Neurosci.* 31, 2624–2629.
- Kornecook, T.J., Anzarut, A., and Pinel, J.P. (1999). Rhinal cortex, but not medial thalamic, lesions cause retrograde amnesia for objects in rats. *Neuroreport* 10, 2853–2858.
- Lashley, K.S. (1938). The mechanism of vision: XV. Preliminary studies of the rat’s capacity for detail vision. *J. Gen. Psychol.* 18, 123–193.
- Lee, A.C., and Rudebeck, S.R. (2010). Human medial temporal lobe damage can disrupt the perception of single objects. *J. Neurosci.* 30, 6588–6594.
- Lee, A.C.H., Barense, M.D., and Graham, K.S. (2005). The contribution of the human medial temporal lobe to perception: Bridging the gap between animal and human studies. *Q. J. Exp. Psychol. B* 58, 300–325.
- Meier, P.M., Flister, E.D., and Reinagel, P. (2011). Collinear features impair visual detection by rats. *J. Vis.* 11, 22.
- Milner, B., Corkin, S., and Teuber, H.-L. (1968). Further analysis of the hippocampal amnesic syndrome: 14-Year followup study of H.M. *Neuropsychologica* 6, 215–234.
- Mishkin, M. (1982). A memory system in the monkey. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 298, 83–95.
- Mumby, D.G., and Pinel, J.P. (1994). Rhinal cortex lesions and object recognition in rats. *Behav. Neurosci.* 108, 11–18.
- Nemanic, S., Alvarado, M.C., and Bachevalier, J. (2004). The hippocampal/parahippocampal regions and recognition memory: Insights from visual paired comparison versus object-delayed nonmatching in monkeys. *J. Neurosci.* 24, 2013–2026.
- Prusky, G.T., Douglas, R.M., Nelson, L., Shabanpoor, A., and Sutherland, R.J. (2004). Visual memory task for rats reveals an essential role for hippocampus and perirhinal cortex. *Proc. Natl. Acad. Sci. USA* 101, 5064–5068.
- Shrager, Y., Gold, J.J., Hopkins, R.O., and Squire, L.R. (2006). Intact visual perception in memory-impaired patients with medial temporal lobe lesions. *J. Neurosci.* 26, 2235–2240.
- Squire, L.R., and Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science* 253, 1380–1386.
- Suzuki, W.A. (2009). Perception and the medial temporal lobe: Evaluating the current evidence. *Neuron* 61, 657–666.
- Suzuki, W.A. (2010). Untangling memory from perception in the medial temporal lobe. *Trends Cogn. Sci.* 14, 195–200.
- Winters, B.D., and Bussey, T.J. (2005). Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. *J. Neurosci.* 25, 52–61.
- Winters, B.D., Saksida, L.M., and Bussey, T.J. (2008). Object recognition memory: Neurobiological mechanisms of encoding, consolidation and retrieval. *Neurosci. Biobehav. Rev.* 32, 1055–1070.