Magnitude of the Object Recognition Deficit Associated With Perirhinal Cortex Damage in Rats: Effects of Varying the Lesion Extent and the Duration of the Sample Period

M. M. Albasser, M. Davies, J. E. Futter, and J. P. Aggleton
Cardiff University

The present study examines 2 factors that might moderate the object-recognition deficit seen after perirhinal cortex damage. Object recognition by normal rats was improved by extending (from 4 to 8 min) the sample period during which an object was first explored. Furthermore, there was a significant positive correlation between time spent in close exploration of the sample object and degree of successful novelty discrimination. In contrast, rats with perirhinal cortex lesions failed to benefit from increased close exploration and did not discriminate the novel object after even the longest sample period. Nevertheless, the lesions did not disrupt habituation across repeated exposure to the same object. The second factor was extent of perirhinal cortex damage. A significant correlation was found between total perirhinal cortex loss and degree of recognition impairment. Within the perirhinal cortex, only damage to the caudal perirhinal cortex correlated significantly with recognition memory deficits. This study highlights the critical importance of the perirhinal cortex within the temporal lobe for recognition memory and shows that the lesion-induced deficit occurs despite seemingly normal levels of close object exploration.

Keywords: temporal lobe, recognition, rat, perirhinal cortex, memory

The perirhinal cortex (areas 35 and 36) is thought to be necessary for normal recognition memory. While the first evidence came from electrophysiological (Brown & Aggleton, 2001; Brown, Wilson, & Riches, 1987) and lesion (Meunier, Bachevalier, Mishkin, & Murray, 1993; Murray & Mishkin, 1986; Zola-Morgan, Squire, & Amaral, 1989) studies of recognition by monkeys, subsequent studies of the rat have repeatedly shown that the perirhinal cortex appears to fulfill a very similar role (Aggleton, Keen, Warburton, & Bussey, 1997; Mumbey & Pinel, 1994; Zhu, Brown, & Aggleton, 1995, Zhu, Brown, McCabe, & Aggleton, 1995, Zhu, McCabe, Aggleton, & Brown, 1996). As a consequence, information about the ways in which the rat perirhinal cortex supports recognition memory is likely to generalize across species. The present study addresses two variables (lesion extent and stimulus sample time) likely to alter the severity of the object-recognition deficit typically seen after perirhinal cortex loss. Understanding the impact of these variables will help to explain inconsistencies across studies and inform theories concerning the precise role of the perirhinal cortex in recognition memory.

The first variable is the extent of perirhinal damage. Comparing this variable across studies is, however, difficult given that quantitative pathological information is typically not provided. Furthermore, studies have used different terminologies for the perirhinal region (e.g., Burwell, 2001; Swanson, 1992), allied to quite major differences in the placement of the perirhinal border with its adjacent cortical regions (e.g., Burwell, 2001; Burwell & Amaral, 1998, compared with Shi & Cassell, 1997, 1999). Related concerns include the possible contribution from damage to adjacent cortical areas (Nemanic, Alvarado, & Bachevalier, 2004). For these reasons the present study sought to relate the degree of perirhinal cortex damage with object recognition. This analysis included additional comparisons for tissue loss in the rostral, mid, and caudal perirhinal cortices, as these regions have different connectional properties (Furtak, Wei, Agster, & Burwell, 2007).

A second potentially important variable is the length of time the rat has with the sample object, prior to discriminating that object from a novel alternative. Apart from the first lesion study to show the importance of the rat perirhinal cortex for object recognition (Mumbey & Pinel, 1994), almost all studies have used spontaneous tests of object recognition (Aggleton et al., 1997; Barker et al., 2001, 2006; Bussey, Muir, & Aggleton, 1999; Davies, Machin, Sanderson, Pearce, & Aggleton, 2006; Ennaceur, Neave, & Aggleton, 1996; Mumbey, Glenn, Nesbitt, & Kyriazis, 2002; Mumbey, Piterkin, Lecluse, & Lehman, 2007; Norman & Eacott, 2004, 2005; Winters & Bussey, 2005; Winters, Forwood, Cowell, Saksida, & Bussey, 2004). These studies all comprise two phases (Ennaceur & Delacour, 1988). First, the rat is exposed to a novel object in a test arena and permitted to explore that object freely. This sample phase is followed by a test phase in which the rat is put back in the arena after a specified delay, but the arena now contains a novel object along with an identical copy of the sample object. Normal rats spend more time exploring the novel object, and this difference provides a measure of novelty discrimination.
It is likely that the duration of time spent initially exploring the sample object will affect the discrimination of novelty, though this has rarely been examined. Some studies use a fixed sample period from 3 to 5 min (Aggleton et al., 1997; Ennaceur et al., 1996; Mumbey et al., 2002), while others require the rats to explore the objects actively for a preset period that is typically from 25 to 30 s (Davies et al., 2006; Norman & Eacott, 2004, 2005; Winters & Bussey, 2005; Winters et al., 2004). These latter studies also use a maximum sample session duration if the rat does not reach the preset sample time (typically 4 or 5 min). The only study that appears to have examined whether the perirhinal lesion recognition deficit can be partially overcome by increasing the exploration of the sample object (Mumbey et al., 2007) found that when perirhinal cortex lesioned rats are given five sample periods with the same object, each of 5 min, they are able to show relatively good novelty discrimination after 24 hr retention but not after 3 weeks. With a single 5-min sample period, the same rats showed the expected perirhinal lesion deficit for the same 24-hr retention period (Mumbey et al., 2007).

The present study sought to examine the robustness of this perirhinal sparing associated with increased sample exploration (Mumbey et al., 2007). This effect is of interest as it is predicted by theories of perirhinal function that assume that this region supports object identification, a process likely to be aided by extended experience (Buckley, 2005; Eacott & Gaffan, 2005). This effect was examined by increasing the length of single sample periods, rather than by increasing the number of sample periods. Consequently, rats were tested on spontaneous object recognition using three different sample durations (4, 6, and 8 min). In all cases the retention delay was 24 hr, chosen because it is consistently sensitive to perirhinal damage after a single sample session (Mumbey et al., 2007; Norman & Eacott, 2004; Winters et al., 2004). A related issue is whether rats with perirhinal lesions show abnormal rates of habituation of exploration to a repeatedly presented object. This process was also examined as abnormalities in rates of habituation could confound the validity of the spontaneous recognition test.

Materials and Method

Subjects

Forty male rats of the pigmented DA (Dark Agouti) strain (Bantin and Kingman, Hull) were used in this study. All subjects were housed in pairs under diurnal conditions (14 hr light and 10 hr dark), and food and water were provided ad lib during testing. At the start of testing the animals were 4 months of age and weighed 220–250 g. All experiments were performed in accordance with the U.K. Animals (Scientific Procedures) Act (1986) and associated guidelines, thereby complying with American Psychological Association ethical standards for the treatment and care of animals.

Surgical Procedures

Animals were deeply anesthetized by intraperitoneal injection (60 mg/kg) of sodium pentobarbital (Sagatal, Rhone Merieux). The 20 rats receiving perirhinal cortex lesions (Perirhinal) were then each placed in a stereotaxic headholder (David Kopf Instruments, Tujunga, CA) with the nose bar at +5.0. A sagittal incision was made along the scalp and the temporal muscles retracted. An area of skull was then removed over the parietal cortex in each hemisphere, approximately 4–7 mm posterior to bregma. The perirhinal cortex lesions were made by injecting a solution of 0.09 M N-methyl-D-aspartic acid (NMDA; Sigma Chemical Company, Ltd., U.K.) dissolved in phosphate buffer (pH 7.2) in three sites per hemisphere using a 1-μl Hamilton syringe (Bonaduz, Switzerland). The stereotaxic coordinates of the lesion placements relative to ear-bar zero were (AP) 4.0, lateral (L) ± 5.7; AP 2.5, L ± 6.1; and AP 0.9, L ± 6.2. The depth (in millimeters), from bregma at the three sites was −9.2 (most rostral), −9.5, and −8.9 (most caudal). Bilateral injections of 0.20 μl were made for all three sites. The 20 animals acting as surgical controls (sham) received the same procedure and drugs as did the animals receiving lesions. This involved the removal of a bone flap and the needle being lowered but without the injection of NMDA. At the completion of all surgeries the skin was sutured and an antibiotic powder (Acramide, Dales Pharmaceuticals, Skipton, U.K.) applied. All rats also received a 5-ml subcutaneous injection of glucose saline.

Apparatus

All testing was conducted in a square arena that had a wooden floor 1 m by 1 m. The floor was covered by sawdust that was agitated between trials and regularly replaced. The solid walls were 43 cm high and painted matte gray. An overhead camera was used to record animals’ behavior for subsequent analysis.

The stimuli consisted of triplicate copies of objects made of glass or plastic that varied in shape, color, and size (9 × 8 × 5 cm to 17 × 13 × 5 cm), and were too heavy for the animal to displace. Pairs of test objects were placed in the arena 23 cm from the corner, and hence 54 cm apart. Objects used included cans, bottles, tins, glasses, and pots. The objects differed markedly and did not appear to share many common features (see Bartko, Winters, Cowell, Saksida, & Bussey, 2007). Every test session was video-recorded. The particular pairs of objects used were counterbalanced across the three sample conditions. Time spent exploring each object was defined as having the rat’s head within 1 cm of the object. This measure was recorded automatically using Ethovision (Noldus, The Netherlands) to ensure consistency.

Behavioral Testing

Pretraining. Prior to the start of testing, animals received two habituation sessions. During the first session, 2 rats were placed in the empty arena for a period of 5 min. During the second habituation period, rats were placed individually into the arena.

Object recognition. Testing for the three different sample times (4, 6, and 8 min) was counterbalanced in sequence. Testing was also counterbalanced with a parallel series of experiments that used a Y-shaped maze (after Winters et al., 2004) to assess object recognition (results not reported). Animals were taken into the experimental room and tested individually. During these sample periods the rats were allowed to explore two identical copies of the sample object. The total time spent exploring the two identical objects was recorded for all trials, and no criterion was set for a minimum amount of exploration during this period. A different pair of identical objects was used for each sample phase. The actual objects used and whether any given object was used as the sample (familiar) were counterbalanced.
After a delay of 24 hr, which was spent in the home cage with a cage mate, each rat was returned to the arena, which now contained a novel object and an identical copy of the object previously used during the familiarization phase. Again, these objects were placed equidistant from the sides of the arena wall and the placement of both the novel and familiar objects (left or right) was counterbalanced between animals. Each rat was tested once at each of the three sample durations. The test period lasted for 5 min, and times spent exploring both novel and familiar objects were recorded. For every rat there was a gap of 3 days after the test session before the next trial.

Object habituation. After completion of object recognition, we tested all rats for their rates of habituation to the same, repeated object (a golden globe 7 cm in diameter). For this test, four identical globes were placed 7 inches from each corner of the square arena. The arena was the same as that used for object recognition, but it was now surrounded by a circular black curtain, so limiting extramaze cues. The total amount of exploration of the objects was measured over four successive 5-min sessions. Each session was 3 days apart, with the same objects for all four sessions. Exploration was measured in the same way as for object recognition.

Histology. All animals were anesthetized with sodium pentobarbital (1 mg/kg) and perfused intracardially with a 0.9% saline solution, followed by a 5% formal saline solution. Their brains were removed and postfixed in a 5% formal saline solution; prior to cutting, they were transferred to a 25% sucrose solution overnight. The brains were placed on a freezing microtome and 40-μm coronal sections were cut. Every third section was kept and mounted onto gelatin-coated slides. The sections were then stained with cresyl violet, a Nissl stain.

Volumetric analyses. Estimates were made of the extent of the lesions in all 20 perirhinal animals. Coronal sections were viewed on a Leica DMRB microscope, photographed using an Olympus DP70 camera and the images transferred to a computer. Lesion measurements were carried out using the program analySISD (Soft-Imaging Systems). A set of three bilateral, standard coronal sections (rostral, mid, and caudal) were first constructed according to the cytoarchitectonic divisions of Burwell (2001). Lesion borders were then drawn in a frame area including the perirhinal cortex (areas 35 and 36), area TE, and the piriform cortex or the entorhinal cortex (depending on AP level).

The perirhinal cortex was subdivided into three subregions: rostral (posterior to AP -2.80 in relation to bregma; Paxinos & Watson, 1997), mid (posterior to AP -3.80), and caudal (posterior to AP -4.80). It should be noted that our rostral perirhinal measurements would include much of the caudal parietal insular cortex as described by Shi and Cassell (1999), who proposed a much more restricted perirhinal region than did Burwell (2001). For all lesion areas analyzed, measurements were taken from four consecutive sections from each hemisphere immediately caudal to each of these AP levels. Consequently, the extent of the lesion was mapped out on 12 coronal sections along the anterior–posterior (AP) extent of the perirhinal cortex. The tissue loss from all 24 hemispheres was summed to produce a total lesion size.

Analysis of behavior. Measurements were taken of the total exploration time for all the identical objects in the sample phase, as well as the time spent exploring the individual objects in the test phase. Recognition is typically assessed from two measures. D1 corresponds to the total time (in seconds) exploring the novel object minus the total time exploring the familiar object (i.e., the sample object). The discrimination ratio, D2 (Ennaceur & Delacour, 1988) is the difference in time spent exploring the novel and familiar objects divided by the total time spent exploring objects in the test phase (i.e., D1 divided by total exploration).

These two measures of discrimination were calculated across the entire test phase (5 min) and after the first 2 min. The latter interval was included in light of previous evidence (Dix & Aggleton, 1999) that it may provide the most sensitive measure of discrimination. Only the results for the D1 scores are presented, as it was found that some rats from both groups showed unusually low levels of exploration. A consequence was the generation of extreme D2 scores (both positive and negative). The occasional presence of these very high or very low scores led to increased variance and so increased the likelihood of null results in an experimental design that required multiple individual comparisons across different conditions. While the profile of results for D2 mirrored those for D1, they are not reported.

The behavioral data for D1 (2 min and 5 min) were analyzed in separate analyses of variance. When appropriate, the simple effects for each brain region were analyzed as recommended by Winer (1971). The probability level of 0.05 was taken as being statistically significant. In order to see whether the animals were performing above chance, one-sample t tests (two-tailed) were conducted. Associations between sample time and performance were examined using Pearson correlations. Correlating extent of perirhinal and extraperirhinal damage with recognition performance has the challenge that the variables that one wishes to compare are not themselves independent. Indeed, the cause of the perirhinal lesion is the same cause of the extraperirhinal damage, and so it is to be expected that the extent of perirhinal damage will correlate positively with extent of extraperirhinal damage (as was the case; see the Results section). For this reason we used partial regressions to examine the relationships between brain damage and performance.

Results

After first describing the location and extent of the perirhinal cortex lesions, the Results section considers whether increased sampling affected the extent of novelty discrimination by both the perirhinal lesioned rats and their controls, and then considers the relationships between amount of cortex loss and recognition performance.

Histological Findings

The extent and borders of the perirhinal and postrhinal cortices were taken from Burwell (2001). All 20 rats suffered bilateral loss of the perirhinal cortex, though there was some variability. Five animals were, however, excluded from the perirhinal lesion group on the basis of the location, extent, and symmetry of their lesions (“excluded cases”).

All rats in the perirhinal lesion group (n = 15; Figure 1A) had lesions centered in the perirhinal cortex that produced substantial bilateral damage to both areas 35 and 36. The measured damage involved between 32.9% and 79.2% of the total perirhinal cortex. It should be noted that these percentages are almost certainly underestimates of functional damage, as in all cases some of the
Did Perirhinal Cortex Lesions Affect the Total Amount of Active Object Sampling?

The first analyses considered the total times spent exploring the sample objects for the 4-, 6-, and 8-min sample conditions (see Figure 2). As was expected, there was a highly significant increase in object exploration as the sample period was made longer, $F(2, 66) = 12.3, p < .0001$. The perirhinal group did not, however, differ from the controls in the total amount of active sampling, $F(1, 33) = 2.17, p = .15$, nor was there an interaction between surgery and sampling ($F < 1$; see Figure 2).

Did the Duration of Active Sampling Predict the Extent of Novelty Discrimination?

For the sham animals, a clear, positive relationship (see Figure 3) emerged between the length of time spent exploring the sample objects and the subsequent degree of novelty discrimination. Correlations (Pearson $r$) between the total amount of sample exploration (combined across all the three time conditions) and the extent to which the novel object was discriminated (D1 from all 5 min combined across the three time conditions) showed a highly significant positive correlation: Pearson $r = .674$, $p = .001$ (see Figure 3). Unlike the sham animals, the perirhinal group ($n = 15$) showed no relationship between sample time and recognition in the square arena ($r = -0.188$, $p = .50$). The same pattern of results was found for the D1 measures taken after 2 min of each test session as the correlation for the sham animals was again significant ($r = .443$, $p = .05$), but this was not found for the perirhinal group ($r = .290$, $p = .29$).

Did the Length of the Sample Period (4, 6, or 8 min) Alter the Perirhinal Lesion Effect?

Figure 4A shows the degree of object-recognition performance (D1, 5 min) for the three different sample conditions. As was expected, the perirhinal group was impaired in relation to the sham

Figure 1. Diagrammatic reconstructions of the perirhinal lesions showing the cases with the largest (gray) and smallest (black) lesions. The numbers refer to the distance (in millimeters) from bregma according to the atlas of Paxinos and Watson (1997). (A) Perirhinal lesion group (15 animals); (B) excluded cases (5 animals). Adapted from The Rat Brain in Stereotaxic Coordinates (3rd ed.), G. Paxinos and C. Watson, 1997. Copyright 1997, with permission from Elsevier.

Figure 2. Bar charts showing the mean sample exploration times (plus or minus the standard error of the mean) of the sham and perirhinal groups during the three different sample-phase conditions (4, 6, and 8 min).
group, $F(1, 33) = 10.67, p = .003$. While simple effects showed that this D1 difference was significant only for the 6-min sample period, $F(1, 99) = 7.20, p = .009$, the perirhinal group had lower D1 scores for all three sample conditions. Furthermore, their D1 scores consistently failed to differ from chance (one-sample $t$ tests, two-tailed; 4 min, $p = .381$; 6 min, $p = .901$; 8 min, $p = .168$), that is, the perirhinal group failed to select the novel object. In contrast, the sham group had mean D1 scores significantly higher than chance for the two longer sample conditions (4 min, $p = .055$; 6 min, $p = .013$; 8 min, $p = .001$), that is, they successfully distinguished the novel object from the familiar object.

The same comparisons were made for the just the first 2 min of each test session (Figure 4B). The pattern of results was similar to that after 5 min. Although there was now no overall lesion effect for D1, $F(1, 33) = 2.74$, $p = .108$, one-sample $t$ tests again showed that only the sham animals discriminated the novel object with the longer sample periods (4 min, $p = .129$; 6 min, $p = .007$; 8 min, $p = .003$). Once again, the perirhinal group failed to discriminate the novel object in any of the three conditions (one-sample $t$ tests; 4 min, $p = .116$; 6 min, $p = .886$; 8 min, $p = .297$).

Figure 3. Correlations (Pearson $r$) between total time spent in close exploration of the sample object (4, 6, and 8 min conditions combined) and the overall D1 (novel minus familiar) measure of discrimination. While the sham animals ($n = 20$) benefit from more exploration, the perirhinal group ($n = 15$) show no improvement.

Figure 4. Mean D1 scores (plus or minus the standard error of the mean) for the three sample durations (4, 6, and 8 min). Upper graph (A) shows the D1 data from all 5 min of the test phase, while the lower graph (B) shows the D1 data from the first 2 min of each test session. A score significantly above 0 (one-sample $t$ test) indicates discrimination of the novel from the familiar object. * $p < .05$. ** $p < .01$. *** $p < .001$. 

Did Lesion Placement and Extent Correlate With the Ability to Discriminate Novel From Familiar Objects?

A second goal was to determine whether the extent of perirhinal damage ($n = 20$) predicted the degree of any recognition deficit. Partial regressions were used to examine these relationships in two stages. First, the extent of damage in the entire perirhinal cortex was considered, along with the total damage dorsal to perirhinal cortex (area TE), and the total damage ventral to perirhinal cortex (piriform cortex plus lateral entorhinal cortex). Next, a separate partial regression considered the extent of damage in the rostral, mid, and caudal perirhinal cortices. Analyses of postrhinal cortex damage could not meaningfully be conducted, because so many cases had no cell loss in this region, and only the small minority suffered any bilateral damage.
The first partial regression used the cumulative D1 results (all three sample conditions combined, 5 min) and revealed a significant negative correlation between recognition memory and overall perirhinal damage ($r = -0.47$, $p = .047$; Figure 5, left), that is, the greater the degree of damage, the poorer the recognition performance. A similar result was found when the D1 scores from just the first 2 min of each test phase were considered (Figure 5, right), as again there was a significant negative correlation with total perirhinal damage ($r = -0.54$, $p = .021$). Correlations were also calculated for the total amount of TE damage (5 min, $r = -0.10$, $p = .68$; 2 min, $r = -0.051$, $p = .84$) and of piriform plus entorhinal cortex (5 min, $r = .33$, $p = .18$; 2 min, $r = .22$, $p = .37$), but there was no evidence of a significant relationship with D1 (see Figure 5).

The second analysis just considered the perirhinal subregions (rostal, mid, and caudal). Partial regression with the D1 scores showed that there was always a negative slope, that is, more damage, worse performance (see Figure 6). The results from the first 2 min provided the only significant correlation (Figure 6, right), because the degree of caudal perirhinal damage was related to D1 performance ($r = -0.47$, $p = .04$).

Finally, we examined the correlations between the degrees of damage in the various regions of interest. There was a positive correlation between the extent of perirhinal damage and extent of damage in area TE ($r = -0.89$, $p < .0001$), as well as between perirhinal cortex and piriform plus entorhinal cortices ($r = .78$, $p < .0001$). Likewise, there was a positive correlation between the tissue loss in the mid and caudal levels of the perirhinal cortex ($r = .77$, $p = .0007$). No other correlations were significant.

**Did the Perirhinal Lesions Affect Rate of Habituation to a Repeated Object?**

There was no evidence that the perirhinal group displayed unusual levels of exploration or abnormal rates of habituation (see Figure 7). An analysis of variance using the total exploration times for each of the four sessions found a highly significant effect of session as exploration times fell, $F(3, 99) = 92.5$, $p < .0001$, but no evidence of a lesion difference, $F(1, 33) = 1.33$, or a lesion by session interaction ($F < 1$). The simple effects showed that both groups displayed a marked reduction in exploration across repeated sessions (both $ps < .001$).

**Discussion**

In keeping with numerous previous studies, perirhinal cortex lesions disrupted spontaneous object recognition. A significant recognition deficit was found when the results from the three different sample phase conditions (4, 6, and 8 min) were combined. Increasing the sample period (from 4 to 8 min) not only increased the amount of object exploration but also improved recognition 24 hr later by the sham controls. One consequence was
that the perirhinal lesion deficit became more evident with the longer sample periods as the use of a sample period that was too short (4 min, 24-hr retention) produced floor effects that obscured any lesion effect. Correlations based on the extent of tissue damage showed that perirhinal cortex damage correlated significantly with object recognition, with greater damage associated with poorer recognition. Within the perirhinal cortex, significant correlations were found between the loss of recognition memory and the extent of damage to the caudal perirhinal cortex. Damage to other temporal lobe regions did not correlate with recognition performance.

The first goal was to look at the impact of increasing the sample time on the effect of perirhinal cortex lesions. The rationale for this goal derives from the repeated finding that perirhinal cortex lesions do not always impair object recognition. Performance at a normal level has been reported in rats when there is essentially no delay (Bartko et al., 2007; Winters et al., 2004) or a short retention interval of less than 10 min (Ennaceur et al., 1996; Norman & Eacott, 2004, 2005). The implication is that other brain regions are better able to support this function if the retention interval is short and the objects easy to discriminate (Bartko et al., 2007). In contrast, studies with monkeys imply a delay-independent deficit after perirhinal lesions (Baxter & Murray, 2001; Ringo, 1991). At the same time, the effects of rhinal cortex lesions in monkeys seemingly disappear if object recognition is repeatedly tested with a small set size (Eacott, Gaffan, & Murray, 1994), that is, repeated exposure to the same objects disproportionately aids discrimination by those animals lacking the perirhinal cortex. These findings suggest an encoding deficit that can be compensated for if the animal spends more time exploring the object in the sample period, thus increasing its discriminability (Bartko et al., 2007; Eacott et al., 1994). Support for this suggestion comes from the report that rats with perirhinal lesions are able to discriminate the novel object from a familiar object after a 24-hr retention interval when the familiar object had been presented to the rat for a total of 25 min over 5 days (Mumby et al., 2007). The present study used sample times of 4 to 8 min, which includes times longer than those used as standard but less than the 25 min used by Mumby et al. (2007). The increase in sample periods in the present study (4 to 8 min) led to an almost doubling of close-proximity exploration of the sample object (see Figure 2), but did not aid subsequent discrimination of novelty after 24 hr by rats with perirhinal cortex lesions.

The failure of increased sampling, within the limits used in the present study, to ameliorate the perirhinal lesion deficit is reflected in the correlations (see Figure 3). No relationship was found in the perirhinal group between the degree of sampling and subsequent discrimination, and the slightly negative slope of the line of best fit indicates that the lack of an effect was not due to a failure to use slightly longer sample periods. While it is possible that the lack of

**Figure 6.** Correlations (n = 20) between extent of tissue loss in the rostral, mid, and caudal perirhinal cortex with recognition performance (cumulative D1 scores). Data are presented for the D1 scores from the entire session (5 min, on the left) and for the first 2 min (on the right) of each session. The best fit slopes correspond to the Pearson correlations, while the partial regression results are given in the boxes.
an improvement by the perirhinal lesion group reflects a floor effect, this explanation seems less likely given the fourfold increase in sample time by individual rats (Figure 3B) that was not reflected by any discrimination improvement. Similar sampling increases by the control group led to marked improvements in familiarity discrimination (Figure 3A). The lack of an improvement in the perirhinal lesion group might be seen as contradictory to the predictions of perceptual models of perirhinal cortex function (e.g., Bussey, Saksida, & Murray, 2005) for which factors that aid the discriminability of visual stimuli should ameliorate the impact of the surgery. There is, however, a circular problem unless you have independent measures of (a) what makes stimuli easier to discriminate and (b) how easy the object is to discriminate initially. In contrast, the sham control rats showed a significant positive correlation between sampling time and novelty discrimination. While this latter relationship is to be expected, the present study is one of the few occasions for which it has been formally demonstrated.

A further finding was that habitation across sessions to the same object appeared normal. This finding is informative, because any marked deficit in rates of habitation could confound the spontaneous recognition test. Although habitation within a session was not assessed, the consistent finding that rates of overall exploration for each sample session did not differ from that exhibited by the control animals would strongly suggest that this was again normal. Normal levels of habitation after perirhinal damage might seem surprising, but this would be predicted if the object was sufficiently salient and could be readily discriminated (Bussey & Saksida, 2002; Eacott & Gaffan, 2005). A possible shortcoming is that object recognition for the particular object used in the habitation study was not subsequently tested for recognition, though recent findings (Mumby et al., 2007) indicate that the perirhinal group should have been able to demonstrate clear recognition of the now-familiar object. That same study also found that the amount of sample explorations did not differ between the sham and perirhinal lesion groups across sessions (Mumby et al., 2007), though surprisingly in that study neither group showed a clear reduction in exploration across sessions that is, habitation.

The ability of rats with perirhinal lesions to discriminate novel objects after short intervals (Ennaceur et al., 1996; Norman & Eacott, 2004, 2005) or to benefit from very extensive object sampling (Mumby et al., 2007) raises questions about the involvement of this area for object-recognition memory. One potential contributing factor to these examples of spared performance is that the lesions were incomplete, that is, behavior was supported by remaining perirhinal cortex. The correlations in the present study show that sparing is an important element. Another likely factor is the extent to which other temporal lobe areas can support object recognition in the absence of the perirhinal cortex. The implication is that these other areas require more time to compile an object for recognition, but if so, it is beyond the time scale of the sample periods used in the present study. One candidate region is the postrhinal cortex (Nemanic et al., 2004), because it has many similar connections to the perirhinal cortex (Burwell & Amaral, 1998), although it has been found that rats with postrhinal cortex damage appear to perform normally on spontaneous object recognition with retention delays of up to 10 min (Norman & Eacott, 2005). A second candidate is area TE, because electrophysiological (Zhu, Brown, & Aggleton, 1995), immediate-early gene expression (Zhu, Brown, McCabe, et al., 1995; Zhu et al., 1996), and lesion correlation (Nemanic et al., 2004) studies have implicated this area in the visual recognition of novelty. A third candidate region is the entorhinal cortex, because lesions here can induce mild delayed nonmatching-to-sample deficits in monkeys (Leonard, Amaral, Squire, & Zola-Morgan, 1995; Meunier et al., 1993), though the few studies in rats suggest little or no effect on recognition memory (Yee & Rawlins, 1998). A notable feature of all three candidate regions is that they border the perirhinal cortex, and so partial damage can occur when attempting to make complete perirhinal cortex lesions.

Arguably the most important finding from the structural correlations was the significant relationship between extent of total perirhinal cortex damage and recognition memory performance. This correlation is consistent with that found in monkeys (Baxter & Murray, 2001) where, again, the larger the lesion, the poorer the recognition performance. While this relationship may seem unsurprising, it helps to cement the notion that the perirhinal cortex is of critical importance and links more closely findings from rodents and monkeys (macaques). Furthermore, the slope of this relationship shows that with incomplete damage, you would expect to find only partial effects, with the resultant need for larger group numbers to demonstrate any impairment. Consistent with this conclusion, an earlier study (Wiig & Bilkey, 1994) that examined rats with subtotal perirhinal cortex lesions (mean of 25% damage) found no deficit for the exploration of novel objects.

Within the perirhinal cortex the only significant correlation was with damage in the caudal perirhinal cortex. This result is intriguing, because studies that have disrupted object recognition by infusion into the perirhinal cortex have typically targeted the caudal perirhinal cortex (e.g., Barker et al., 2006; Griffiths et al., 2008; Warburton et al., 2005; Winters & Bussey, 2005). Furthermore, immediate-early gene expression studies have found that c-Fos activity in the caudal perirhinal cortex is raised after exposure to novel stimuli (Zhu, Brown, McCabe, et al., 1995; Zhu et al., 1996; Wan, Aggleton, & Brown, 1999). Caution is, however,
required in making a specific link with the caudal perirhinal cortex, because none of the above studies included direct comparisons with the rostral perirhinal cortex. Furthermore, in the present study, damage in the caudal perirhinal cortex correlated with other damage, for example, in the mid-perirhinal cortex. At the same time, there was no evidence that extraperirhinal cortex damage correlated with recognition. Although this null result does not provide definitive evidence, because none of the candidate areas was systematically targeted, the data clearly support the pre-eminence of the perirhinal cortex for object-recognition memory.

Finally, it is valuable to consider the implications of the positive correlation found in the control rats between the amount of object sampling and object recognition (D1). In both the sample and test phases, both objects are potentially visible throughout the entire test period. It is, however, assumed that only close active exploration (in the present study the criterion was <1 cm distance) provides the information that the animal requires to first learn about the object and then discriminate the novel object (Ennaceur & Delacour, 1988). This assumption is integral to the spontaneous object recognition test, yet it does not appear to have been formally examined. The present study provides clear support for the view that close exploration is a valid measure and that it represents activity that is qualitatively distinct from that which occurs during the remainder of the session.

References


Received May 13, 2008
Revision received July 10, 2008
Accepted July 28, 2008

E-Mail Notification of Your Latest Issue Online!

Would you like to know when the next issue of your favorite APA journal will be available online? This service is now available to you. Sign up at http://notify.apa.org/ and you will be notified by e-mail when issues of interest to you become available!