Memory Impairment on a Delayed Non-Matching-to-Position Task After Lesions of the Perirhinal Cortex in the Rat

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Previous research conducted in monkeys and rats has established that the perirhinal cortex is critically involved in object- or stimulus-recognition memory, whereas other research suggests that region may contribute to memory for object discriminations. These findings do not rule out the possibility that the perirhinal cortex plays a more general role in memory. The present experiment addressed whether selective lesions of the perirhinal cortex would result in a delay-dependent deficit on a test of memory that did not involve stimulus recognition or object memory. Rats with bilateral perirhinal lesions were tested on a delayed non-matching-to-position task. Lesions of the perirhinal cortex did not interfere with acquisition or performance at short (0–4 s)-delay intervals, but lesions did impair performance at longer delays. It is suggested that the perirhinal cortex is involved in maintaining representations of trial-specific information over time.

It is now well established that bilateral damage to the medial temporal lobe of the mammalian brain results in a profound and persistent amnesia. Lesion studies conducted in primates and rodents over the last 2 decades have identified a number of structures within the medial temporal region that contribute to the formation and storage of new memories (reviewed in Eichenbaum, Otto, & Cohen, 1994; Murray, 1996; Squire, Knowlton, & Musen, 1993). The identified structures include the hippocampal formation (entorhinal cortex, dentate gyrus, Ammon's horn, and the subicular complex) and the surrounding perirhinal and parahippocampal cortices. The severity of amnesia following lesions of the medial temporal region is commonly assessed using the trial-unique delayed non-matching-to-sample (DNMS) task. This task requires the animal to retain information about trial-specific stimuli over variable delay intervals and is thought to tax recognition memory. Lesion studies using this testing procedure have demonstrated severe impairments with combined damage to either the perirhinal and parahippocampal cortices (Suzuki et al., 1993) or the perirhinal and entorhinal cortices (Gaffan & Murray, 1992; Mumby & Pinel, 1994; Murray, Bachevalier, & Mishkin, 1989). Moreover, studies using more selective lesions indicate that damage limited to the perirhinal cortex is sufficient to cause recognition memory deficits (Ennaceur & Aggleton, 1997; Meunier, Bachevalier, Mishkin & Murray, 1993; Ramus, Zola-Morgan, & Squire, 1994; Wiig & Bilkey, 1995; Wiig, Cooper, & Bear, 1996). Thus, the evidence suggests that the perirhinal cortex plays an integral role in object- or pattern-recognition memory.

The perirhinal cortex in the rat receives unimodal association input from all sensory modalities. Its visual and visuospatial input arises in the postrhinal cortex, a region that is similar to the primate parahippocampal cortex and is predominantly innervated by occipital, posterior parietal, and retrosplenial cortices (Burwell, Witter, & Amaral, 1998). On the output side, the perirhinal cortex provides a substantial input to the hippocampal formation through its projection to the entorhinal cortex (Burwell & Amaral, 1998; Naber, Caballero-Bleda, Jorritsma-Byham, & Witter, 1997). Given this profile of higher order association input and connectivity with the hippocampal formation, it is not surprising that the perirhinal cortex is involved in mnemonic processes. This involvement, however, does not appear to be confined to just visual object or pattern recognition. The DNMS deficits observed following lesions of the perirhinal cortex are not limited to one sensory modality; impairments can be seen in the olfactory (Otto & Eichenbaum, 1992) and tactual (Suzuki et al., 1993) as well as in the visual (Murray & Mishkin, 1986; Wiig & Bilkey, 1995; Zola-Morgan et al., 1989) modalities. Other findings suggest that in addition to recognition memory, the perirhinal cortex contributes to object discrimination learning (Buckley & Gaffan, 1997; Myhrer & Wangen, 1996; Wiig et al., 1996) and the formation of stimulus–stimulus (paired) associations (Bunsey & Eichenbaum, 1993; Higuchi & Miyashita, 1996; Murray, Gaffan, & Mishkin, 1993). There is also evidence that the perirhinal cortex participates in information processing in the spatial domain (Otto, Wolf & Walsh, 1997; Wiig & Bilkey, 1994a, 1994b; but see Ennaceur, Nave, & Aggleton, 1996).

Because the perirhinal cortex is clearly a polymodal association region and because deficits resulting from perirhinal damage are not confined to any particular sensory modality, it is reasonable to suggest that this region may play a more general role in memory beyond the processing of...
stimulus properties of items to be recognized or remembered. One possibility suggested by Eichenbaum et al. (1994) is that the perirhinal cortex participates in the maintenance of representations across time, thereby permitting additional processing by other medial temporal lobe structures. This proposal is supported by the finding that animals with lesions of the perirhinal cortex are consistently impaired on tasks requiring the animal to retain information across relatively long delay intervals. Furthermore, studies of single-unit activity within the perirhinal cortex of monkeys and rats performing DNMS tasks have demonstrated delay-related neuronal activity (Fuster & Jervey, 1981; Miyashita & Chang, 1988; Young, Otto, Fox, & Eichenbaum, 1997).

Within this framework, an informative experiment would be to test animals with lesions of the perirhinal cortex on a task that requires storage of information over variable delay intervals but does not depend on the processing of the physical features of items to be recognized (e.g., comparing and processing stimulus properties of three-dimensional junk objects, complex visual stimuli, or specific odors). In the present experiment, rats with lesions of the perirhinal cortex were trained on a delayed non-matching-to-position (DNMTP) task using several delay protocols. This task assesses the rats' ability to remember, after a variable-delay interval, which one of two identical levers was pressed during the sample phase of the trial. The task procedures were designed to preclude the use of simple response mediation strategies, such as body position, by requiring nose-poking behavior on the opposite wall of the testing chamber between the sample and choice phases of the task. Thus, the DNMTP task, as used in the present study, requires the temporary storage of a representation of a single experience but does not require recognition of a trial-unique stimulus item. Indeed, the stimulus properties of the two levers are exactly the same. By requiring the retention of information across varying delay intervals, the DNMTP task permits a careful analysis of the effects of experimental manipulations on the persistence of stored information.

Method

Subjects

Ten male Sprague-Dawley (Charles River, Wilmington, MA) rats served as subjects in this experiment. The animals weighed between 150 and 180 g before surgery. They were individually housed in wire mesh cages and were maintained on a 12-hr light-dark cycle. Subjects had free access to water but were food deprived to 85% of their free-feeding body weight. At the start of the behavioral training, rats weighed between 240 and 310 g. All subjects were involved in a previous behavioral experiment that was aborted early during shaping procedures because of animal caretaker error. Six weeks transpired between surgery and the present experiment.

Surgery

All rats were anesthetized with sodium pentobarbital (65 mg/kg). Subjects were placed in a stereotaxic apparatus, where a midline incision was made and the scalp retracted to expose the skull. The perirhinal cortex lesions were made by drilling holes through the skull at the coordinates: 3.3/4.3/5.3/6.3/7.3 mm p to bregma and 4.8 mm L to the midline. Monopolar electrodes (Teflon-coated wire, 125 μm in diameter), oriented laterally at 10° from the vertical, were lowered at each site to a depth of 6.8 mm measured from the surface of the skull. For the lesion group (n = 5), direct current at 2 mA was passed through the electrodes for a duration of 10 s at each site. The electrodes were then removed, and the wound was sutured. Control rats (n = 5) received sham operations in which electrodes were lowered to the perirhinal cortex and then withdrawn without current being passed. Postoperatively, the animals were kept warm and monitored until spontaneous movement occurred. Once stabilized, they were returned to their home cages and left to recover for 10 days before behavioral testing.

Apparatus

All behavioral testing was conducted in an operant testing environment (MED Associates, East Fairfield, VT) interfaced with a 386 microcomputer and controlled by MED-PC V2.1 software package (Tatham, 1991). Custom software written in MED-PC notation controlled the behavioral tasks and recorded task events and responses. Experiments were conducted in 24 × 30.5 × 29 cm operant test chambers with modular component panels in the front and back, Plexiglas side walls and top, and a floor constructed of 4.8-mm rods placed 1.6 cm apart. The testing chamber was enclosed in a 62 × 56 × 56 cm sound-attenuating chamber fitted with an exhaust fan, which provided airflow to the test chamber and background white noise. On the front wall of the test chamber were two retractable levers spaced 12 cm apart and 6.5 cm above the floor. A food pellet receptacle was located halfway between the two retractable levers. On the back wall were five evenly distributed 2 × 2 × 2 cm nose-poke holes equipped with photobeams and stimulus lights. Only the central nose-poke hole was used for the present task. One 28-V, 100-mA, partially shaded houselight illuminated the chamber during the testing session, except during the 5-s time-out intervals imposed on error trials.

Behavioral Procedures

Pretraining on the DNMTP task consisted of four stages. In Stage 1, rats were trained to press either the left or the right lever on successive trials. Each leverpress was rewarded with a food pellet (45 mg; Noyes, Lancaster, NH). In this and all successive stages of shaping and training, the intertrial interval was always 5 s. In Stage 2, rats were trained to nose poke at the back of the chamber when the cue light was on in the center nose-poke hole, to gain access to the levers. Both levers were extended immediately after the nose-poke response. A response on either lever was reinforced. In Stage 3, either the left or the right lever was presented and was retracted when pressed. The leverpress resulted in the illumination of the nose-poke hole. A nose-poke then produced extension of both levers, and a press on either lever was reinforced.

The final stage of pretraining began once all rats had learned the complete chain of responses. In this stage, rats acquired the nonmatching rule. The extension of the single lever at the beginning of a trial constituted the sample phase, and the extension of both levers after the intervening nose poke constituted the choice phase. The choice was presented immediately after the nose poke without delay. Rats were rewarded for choosing the lever that was
Histology

Following completion of behavioral training, subjects were deeply anesthetized with sodium pentobarbital and transcardially perfused using an automatic pump with the flow rate set at 35–40 ml/min. Normal saline at room temperature was perfused for 2 min, to clear the blood. This was followed by a solution of 4% paraformaldehyde in 0.1 M sodium phosphate (pH 7.4) at 4°C for a duration of 15 min. The brains were then removed from the skull, postfixed for 6 hr at 4°C in paraformaldehyde solution, and cryoprotected for at least 24 hr at 4°C in a solution of 20% glycerol in 0.1 M phosphate (pH 7.4).

The brains were coronally sectioned at 40 μm on a freezing microtome. Sections were collected in four series for processing and storage. One 1:4 series was collected in a 10% formalin solution in preparation for cell staining. That series was subsequently mounted and stained for Nissl using thionin. The remaining three 1:5 series were collected and stored at −20°C in cryoprotectant-tissue-collecting solution consisting of 30% ethylene glycol and 20% glycerol in sodium phosphate buffer (pH 7.4).

Coronal sections at 320-μm intervals were used to assess the amount of tissue damage. Using camera lucida techniques, section contours were added, regional borders added and the location of tissue damage circumscribed. Tissue damage was identified by missing tissue, obvious necrosis, or marked thinning of the cortex (approximately 50%). The resulting drawings were digitized, and a computer program was used to obtain area measurements of perirhinal Areas 35 and 36 and of the tissue damage. The resulting measures for each coronal section included the total area of perirhinal Areas 35 and 36 and the total area of tissue damage. An estimate of the volume of the lesion was then computed for each subject. The proportion of Areas 35 and 36 that were damaged was calculated. The proportion of the lesion that involved adjacent cortices was also calculated.

Data Analysis

For pretrained, statistical analysis was employed to assess the effects of perirhinal lesions on acquisition of the nonmatching rule.
Table 1

Estimated Volume of Tissue Damage

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>PR</th>
<th>Area 36</th>
<th>Area 35</th>
<th>Tev</th>
<th>POR</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT7</td>
<td>62</td>
<td>44</td>
<td>69</td>
<td>3</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>DT8</td>
<td>63</td>
<td>42</td>
<td>80</td>
<td>0</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>DT9</td>
<td>55</td>
<td>41</td>
<td>59</td>
<td>1</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>DT11</td>
<td>15</td>
<td>18</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DT15</td>
<td>42</td>
<td>26</td>
<td>63</td>
<td>2</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

*Note.* PR = perirhinal cortex; Tev = ventral temporal association areas; POR = postrhinal cortex; EC = entorhinal cortex.

<table>
<thead>
<tr>
<th>% area damaged</th>
<th>% lesion outside PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject ID</td>
<td>PR</td>
</tr>
<tr>
<td>DT7</td>
<td>62</td>
</tr>
<tr>
<td>DT8</td>
<td>63</td>
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<td>DT9</td>
<td>55</td>
</tr>
<tr>
<td>DT11</td>
<td>15</td>
</tr>
<tr>
<td>DT15</td>
<td>42</td>
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</tbody>
</table>

Significance level was \( p < .05 \). Results with probabilities of less than .10 were discussed as trends toward significance. All statistical analyses were performed using SAS V.6.1 (SAS Institute, 1996).

Results

Histology

The perirhinal lesion included portions of Areas 35 and 36, bilaterally, in all lesioned subjects. The extent of damage to the perirhinal cortex ranged from 15% to 63% (see Table 1). Figure 1 shows the extent of the smallest and largest lesion (DT11 and DT8, respectively). As is apparent in this illustration, the location of the damage for the smallest lesion suggests that the empirical assessment of the lesion size for that case may not accurately represent the amount of disruption to the cortex. Although the tissue damage is small in area, there is damage to both hemispheres and both subdivisions. Moreover, some amount of damage was observed throughout the rostrocaudal extent of the perirhinal cortex for that case. Damage outside of the perirhinal cortex was minimal, ranging from 3% to 17% of the total lesion. The bulk of the extraperirhinal damage was to the rostral portion of the lateral entorhinal area, but a small amount of damage was also observed in the postrhinal cortex and Tev.

Figure 2 shows high-magnification photomicrographs of the lesion at three rostrocaudal levels of a representative lesioned brain (DT9).

Cell-stained sections were further assessed for more subtle damage to the entorhinal cortex, the hippocampus, or the external capsule. No additional necrosis or thinning of cortex was observed in the entorhinal cortex at higher magnification in any lesion case. The hippocampus appeared normal in every case. Minor involvement of the external capsule was observed bilaterally, but only at rostral levels in cases DT9 and DT15. Minor involvement of the external capsule was observed unilaterally, and only at rostral levels in cases DT7 and DT8. Nevertheless, in these cases, the bulk of the external capsule was entirely intact. No involvement...
of the external capsule at any level was observed in DT11. The amount of damage to the external capsule was not related to performance on the DNMTPT task.

**Behavior**

All subjects were trained on the nonmatching rule for 11 sessions. Control and lesioned rats learned the basic nonmatching-to-position task at the same rate, exhibiting similar choice accuracies when no delay was interposed between presentation of the sample and choice (see Figure 3A). A repeated measures ANOVA indicated that there was no significant group difference in percentage correct ($p > .58$), nor was there any Group × Session interaction ($p > .74$). Control and lesioned subjects also performed similarly at very short delays (0, 1, 2, and 4 s; see Figure 3B), where there was a main effect of delay on percentage correct, $F(3, 24) = 4.62$, $p < .023$, but neither a group difference ($p > .61$) nor a Group × Delay interaction ($p > .63$).

With the addition of longer delay intervals (8 s, 16 s, and 32 s) to the short-delay protocol, lesioned subjects tended to perform less accurately than controls. Repeated measures ANOVA revealed a significant main effect of delay, $F(6, 48) = 43.40$, $p < .0001$, but no main effect of group ($p > .108$) and no Group × Delay interaction ($p > .418$). The lesioned subjects performed less accurately, numerically, at delays of 8 and 16 s (see Figure 3C). When subjects were trained on a protocol in which more of the delays were distributed about those intervals (0, 5, 10, 15, 20, 25, and 30 s), lesioned subjects were clearly impaired relative to control subjects on the measure of percentage correct (Figure 4A). Statistical analysis revealed significant main effects of delay, $F(6, 48) = 14.25$, $p < .0001$, and group, $F(1, 8) = 6.63$, $p < .032$, and a Group × Delay interaction, $F(6, 48) = 2.56$, $p < .039$. Planned univariate contrasts revealed no difference at the zero delay ($p > .28$). There were trends toward group differences at delays of 5–15 s ($p$ values ranged from .062 to .064) and significant group differences at delays of 20–30 s ($p$ values ranged from .017 to .044).

Subjects were then trained on two additional protocols that included delays of 1 min or more. For delays ranging from 4 s to 128 s, the pattern of results was similar to those previously described (see Figure 4B). There was a main effect of delay, $F(6, 48) = 80.12$, $p < .0001$. There was no overall effect of group ($p > .25$), but there was a significant Group × Delay interaction, $F(6, 48) = 3.00$, $p < .041$. Lesioned subjects performed significantly less accurately than controls at the 16-s delay interval ($p < .01$). Similar results were obtained with the protocol in which delay intervals were 0, 10, 20, 30, 40, 50, and 60 s (see Figure 4C). Statistical analysis indicated a significant main effect of delay, $F(6, 48) = 33.32$, $p < .0001$. Additionally, there were

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1 On these long protocols, 1 control subject performed at 0% accuracy on the longest delay interval (60 or 128 s in the two protocols, respectively) in every session despite normal performance at all other delays. Individual trial data indicated that this subject completed only 50% of trials at the longer delay intervals while completing 100% of trials at all other delays. Values with extreme $z$ scores (outside ± 3.67) were determined to be outliers and were adjusted to reduce influence on analyses. Accordingly, the performance of this animal at these delays was determined to be an outlier. The outlying score was changed to 1 unit less extreme than the closest data point in the distribution of that particular variable, as recommended by Tabachnick and Fidell (1989).
Figure 3. A: Accuracy of performance during acquisition of the nonmatching rule during 11 sessions of training, 100 trials/session. B: Accuracy of performance on the delayed non-matching-to-position (DNMTP) task with short-delay intervals. Data represent the last five sessions of training on this delay protocol. There was no difference between lesion and control subjects in acquisition of the nonmatching rule or in performance at short delays. C: Accuracy of performance on the DNMTP task with the addition of three longer delay intervals. Data represent the last five sessions of training on this delay protocol. There was no difference between lesion and control subjects on performance with these delay intervals.

Figure 4. Percentage correct on the delayed non-matching-to-position (DNMTP) task on three different behavioral protocols. Data represent performance on the last five sessions of training for each. A: Accuracy of performance with delay intervals distributed from 5 to 35 s. Lesioned subjects exhibited a delay-dependent impairment with this protocol. B: Accuracy of performance with delay intervals ranging from 4 to 128 s. Again, lesioned subjects exhibited a delay-dependent impairment on this delay protocol. C: Accuracy of performance on the DNMTP task with delay intervals ranging from 10 to 60 s. Lesioned subjects exhibited a trend toward an overall group difference and a delay-dependent impairment. †p < .10. *p < .05.
trends toward a group effect ($p < .056$) and toward a Group × Delay interaction ($p < .08$).

For a final week of training, subjects were returned to the original protocol of delay intervals ranging from 5 s to 30 s, to assess the effects of continued training (see Figure 5A). Statistical analysis indicated main effects of delay, $F(6, 48) = 16.50, p < .0001$, and group, $F(1, 8) = 7.95, p < .023$. There was also a Group × Delay interaction, $F(6, 48) = 4.46, p < .039$. Planned contrasts revealed no group difference at the zero delay interval ($p > .29$), significant differences at delay intervals of 5–25 s ($p$ values ranged from .015 to .04), and a trend toward a difference at 30 s ($p < .058$).

Although the lesioned group was clearly impaired on the measure of percentage correct in a delay-dependent manner, percentage correct may not be a pure measure of accuracy. It has been suggested that percentage accuracy is subject to the influence of cognitive, perceptual, and response biases (Sahgal, 1987). Thus, impairment on percentage correct may not reflect a delay-dependent memory impairment but rather may reflect differences in mediation of performance by nonmnemonic response strategies. To address these issues, additional measures derived from signal detection were calculated and analyzed for the subset of the data shown in Figures 3C, 4C, and 5A. These measures included indices of choice accuracy, $A'$ and $SI$, and three measures of bias, $I_Y$, $B''$, and $RI$. The results are shown in Table 2. Signal-detection analysis confirmed the findings of the percentage correct measure, suggesting that the observed deficits associated with perirhinal lesions were attributable to group differences in accuracy of performance.

For the data shown in Figure 3C (delays of 0, 1, 2, 4, 8, 16, 32 s), in which there was no delay-dependent impairment associated with perirhinal lesions on the measure of percentage correct, there was also no delay-dependent impairment evident in the accuracy indices (Table 2). The analysis of the cognitive bias index, $I_Y$, indicated that there was a significant effect of delay ($p < .0005$) and a trend toward a group effect ($p < .060$). There was also a significant effect of delay ($p < .0001$) for the perceptual bias index, $B''$. For the responsivity index, $RI$, there was a trend toward a main effect of delay ($p < .066$) and a significant effect of group ($p < .012$). There was no Delay × Group interaction for any bias index.

For the data shown in Figure 4C (delays of 0, 10, 20, 30, 40, 50, and 60 s), the results of signal-detection analyses reflected those for percentage correct, in that evidence for a delay-dependent impairment was confirmed by the accuracy indices. For both $SI$ and $A'$, there were significant main effects of delay ($p < .0001$). Trends toward main effects of group were observed for both indices ($p < .069$ for $SI$ and $p < .083$ for $A'$). For $SI$, there was a significant Group × Delay interaction ($p < .039$). For $A'$, there was a trend toward a Group × Delay interaction ($p < .077$). Analysis of the cognitive bias index, $I_Y$, revealed a trend toward a main effect of delay ($p < .057$) and a significant main effect of group ($p < .002$) but, again, no interaction between the two terms. For the perceptual bias index, $B''$, there were trends toward a main effect of delay ($p < .082$) and toward a Group × Delay interaction ($p < .074$). No significant effects or interactions were observed for $RI$.

The results for the final week of training in which delay intervals were 0, 5, 10, 15, 20, 25, and 30 s provided further
Table 2
Results of Signal-Detection Analysis

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>df</th>
<th>Accuracy measures (F)</th>
<th>Bias measures (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% A'</td>
<td>SI</td>
</tr>
<tr>
<td>Delays: 0, 1, 2, 4, 8, 16, 32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>1, 8</td>
<td>3.58†</td>
<td>1.32</td>
</tr>
<tr>
<td>Delay</td>
<td>5, 40</td>
<td>42.80**</td>
<td>39.78**</td>
</tr>
<tr>
<td>Group × Delay</td>
<td>5, 40</td>
<td>0.78</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Delays: 0, 10, 20, 30, 40, 50, 60

| Group              | 1, 8 | 3.07† | 5.04† | 4.41† | 19.99** | 2.19 | 0.04 |
| Delay              | 5, 40 | 56.20** | 18.85** | 22.64** | 2.22† | 2.01† | 1.32 |
| Group × Delay      | 5, 40 | 2.13* | 2.26† | 2.44* | 0.96 | 2.07† | 1.31 |

Delays: 0, 5, 10, 15, 20, 25, 30

| Group              | 1, 8 | 3.13† | 8.87* | 8.55* | 6.06* | 0.90 | 0.47 |
| Delay              | 6, 48 | 29.59** | 17.77** | 19.09** | 2.08† | 1.01 | 0.62 |
| Group × Delay      | 6, 48 | 2.61* | 5.02** | 4.72** | 0.66 | 1.89 | 0.99 |

Note. Delays are in seconds. % A' = accuracy index; SI = sensitivity index; 1Y = Index Y; 1O = bias index; RI = responsivity index.
†p < .10. *p < .05. **p < .005.

Additional analyses were conducted to rule out other performance differences that might explain the delay-dependent impairment in percentage correct reflected deficits in accuracy and not other aspects of performance (Table 2 and Figure 5). We observed an effect of delay on accuracy, as measured by the A' index (p < .0001), a significant group effect (p < .022), and a significant Group × Delay interaction (p < .021). Similarly, analysis of SI indicated that there was a significant effect of delay (p < .0001), a group difference (p < .025), and a significant Group × Delay interaction (p < .002). Analysis of the measure of perceptual bias for the data shown in Figure 5 indicated that for B", there was no effect of delay (p > .37) or group (p > .35) and no Group × Delay interaction (p > .14). Analysis of the RI indicated that there was no effect of delay (p > .67) or group (p > .57) and no Group × Delay interaction (p > .44). Analysis of A' index, however, indicated that there was no effect of delay (p > .15), a significant effect of group (p < .02), and no Group × Delay interaction (p > .59).

Findings regarding nose-poking behavior indicated that body position mediation strategies were unlikely to have contributed to differences in accuracy of performance. Both groups exhibited a high rate of nose poking (Table 3) that was not delay dependent. However, lesioned subjects exhibited significantly faster latencies to respond to the choice levers on correct trials, F(1, 9) = 7.18, p < .028. Lesioned subjects were similar to controls on the percentage of trials that were not completed (p > .987).

To examine the effects of perirhinal damage on management of interference, post hoc analyses were conducted on the data collected in the final week of testing. Although there was no overt manipulation of interference demands within the DNMTP task, it was possible to examine the hypothesis that proactive interference accrued during the session and differentially affected the performance of lesioned subjects as compared with controls. If proactive interference builds.
up within a session, this would be manifested by a decrease in accuracy of performance over the course of the session. Performance at all delays was averaged across 10 blocks of 10 trials each, for each group (see Figure 6). A repeated measures ANOVA indicated that there were significant main effects of group, \( F(1, 8) = 9.32, p < .016 \), and block, \( F(1, 9) = 2.96, p < .015 \), but no Group × Block interaction (\( p < .57 \)). Post hoc contrasts to test the hypothesis that there was a linear trend in performance across blocks indicated that the slope of the line fitted to percentage correct was not different from zero (\( p > .647 \)) and the slope of the lines fitted for each group were not different from each other (\( p > .651 \)).

After addressing issues of response biases, mediation, and interference, a final post hoc analysis was conducted to further examine the effects of perirhinal lesions on performance as a function of delay interval. A final repeated measures ANOVA examined the performance of all animals across all delay protocols. Summary scores were computed for each animal by averaging percentage of correct performance at particular delay intervals across protocols (see Figure 7). Additionally, delays that were similar also were averaged. Thus, the repeated measure of delay included delays of 0, 1–4, 5–8, 10–15, 16–20, 25, 30–32, 35, 40, 50, 60–64, and 128 s. Statistical analysis indicated a main effect of delay, \( F(11, 88) = 40.21, p < .0001 \), and a trend toward a main effect of group (\( p < .068 \)). There was also a significant Group × Delay interaction, \( F(11, 88) = 3.63, p < .013 \). Contrasts at each delay revealed no group differences at the zero (\( p > .21 \)) and 1–4-s delay intervals (\( p > .35 \)), significant differences at delay intervals of 5–20 s (\( p \) values ranged from \( .026 \) to \( .045 \)), and a trend toward a difference at 25 s (\( p < .075 \)). There was also a difference at 60–64 s (\( p < .023 \)), but no other group differences were significant at delay intervals longer than 30 s (\( p \) values ranged from \( .12 \) to \( .41 \)).

Finally, to determine whether lesion size and location were associated with the poorer performance of animals in the lesion group, a correlation analysis was performed between a measure of performance accuracy and measures of the extent of tissue damage. Average performance at all delays in the last week of training was calculated for lesioned and control animals. Accuracy of performance for lesioned rats on this measure ranged from 53% to 89% (\( M = 69, SD = 17 \)). Accuracy of performance for control rats on this measure ranged from 86% to 98% (\( M = 91, SD = 5 \)). This measure of performance in the lesioned group was not significantly correlated with any of the estimates of tissue damage presented in Table 1, either within the perirhinal cortex (columns 1–3, \( p \) values ranged from \( .14 \) to \( .54 \)) or outside the perirhinal cortex (columns 4–6, \( p \) values ranged from \( .15 \) to \( .66 \)).

**Discussion**

Rats with bilateral lesions of the perirhinal cortex were tested on a spatially guided DNMTP task, which, at least superficially, requires the animal to actively maintain a representation of the position of a lever. Rats with lesions of the perirhinal cortex exhibited a clear delay-dependent memory impairment on this task. Although initial acquisition of the task and performance across short delay intervals were unaffected by perirhinal lesions, choice accuracy at longer intervals was significantly lower in the lesioned animals. Taken together with findings of previous studies that have reported a delay-dependent impairment in memory for stimulus properties of objects or cues (Mumby & Pinel, 1994; Murray & Mishkin, 1986; Otto & Eichenbaum, 1992; Suzuki et al., 1993), the present experiment provides evidence for a delay-dependent impairment in accuracy of performance. The present study extends previous findings in that the delay-dependent impairment was observed even when memory for the stimulus properties of particular cues was not involved, that is, the stimulus properties of both levers were the same. Thus, although the results obtained from the present experiment are consistent with a role for the perirhinal cortex in memory, the deficit reported here goes
beyond the interpretation that the perirhinal cortex is critical to stimulus or object memory.

The pattern of results suggests that the observed impairment is a mnemonic deficit that cannot be explained by nonmnemonic factors. One issue is whether the deficit in choice accuracy is secondary to a performance deficit. In the present experiment, lesioned and intact subjects exhibited similar latencies in all aspects of the tasks when delay intervals ranged from 10 to 60 s. In the last week of training, however, when delay intervals ranged from 5 to 30 s, lesioned subjects responded significantly more quickly during the choice phase of the task when making a correct choice (about 500 ms), but all other latencies were similar. This more rapid response of lesioned subjects on correct trials is difficult to interpret but might reflect differences in response mediation strategies, that is, perirhinal-lesioned subjects may be more prone to using response mediation strategies to achieve optimal performance, although not in a delay-dependent fashion. This is consistent with an interpretation that response mediation strategies may have ameliorated the deficit observed in lesioned subjects but not with an interpretation that differences in response mediation strategies underlie the impairment observed in lesioned subjects.

A number of recent studies have addressed response mediation issues in similar tasks (Gutnikov, Barnes, & Rawlins, 1994; Herremans & Hijzen, 1997). In many of these protocols, the subject is not required to leave the front of the cage between sample and choice phases of the task (Herremans, Hijzen, Welborn, Olivier, & Slangen, 1996; Stanhope, McLenachan, & Dourish, 1995). When this is the case, subjects can rely on body position to encode the location of the correct response across a delay. Even when nose poking is required at the central panel, there is some evidence that subjects may “look at” the position of the correct lever and subsequently respond on the last lever looked at (Herremans et al., 1996). Alternatively, it has been suggested that nose poking during the delay prevents the use of strategies that permit solution of the DNMT task without recourse to memory (Dunnett, 1992). In the present task, rats were required to respond with a nose poke at the back of the chamber to initiate presentation of the choice levers, so that a simple body position mediation strategy was not possible. All rats adopted a steady rate of nose poking across all delays. Although lesioned rats exhibited a nonsignificantly slower rate of nose poking (2.0/s as opposed to 3.0/s), the rate of nose poking would still preclude body position as a mediation strategy. Moreover, observations during task performance indicated that rats did not adopt a systematic turning strategy to mediate performance. Finally, the presence of the nose-poke hole on the back of the chamber precludes the use of looking as a response mediation strategy because the subjects must, at some point, look away from the panel on which the choice levers will subsequently appear.

Another possible explanation for the present results is that the perirhinal damage interrupted lesioned subjects’ ability to manage interference. With an intertrial interval of 5 s, one might expect proactive interference to build up over the session, resulting in poorer performance as the session progressed. In the present study, there was no evidence for a decrease in accuracy over the course of training sessions for either group. These findings are consistent with investigations of perirhinal function using other behavioral paradigms. Otto and Eichenbaum (1992) found that combined perirhinal–entorhinal lesions in rats resulted in a delay-dependent deficit in accuracy of performance on an odor-guided continuous non-matching to sample task but had no effect on intertrial interference. Thus, the weight of the evidence is against an interference hypothesis to explain the deficit observed in the present experiment.

It has been proposed that the functions of the perirhinal region and those of the hippocampus can be dissociated experimentally based on the spatial demands of a task, with the hippocampus being specialized for processing spatial information (Ennaceur et al., 1996). Superficially, the DNMT task is a spatial task in that the rat must remember something about position. Moreover, hippocampal neuronal activity is related to task-relevant events within the DNMT task, such as spatial position of a lever press (Deadwyler, Bunn, & Hampson, 1996). It is not clear, however, what strategy is used to perform the task, that is, the task may not be a place task or an allocentric spatial task. Nevertheless, the finding that lesions of the perirhinal cortex result in a deficit on this task may suggest to some that the encoding and storage of spatial information is, to some extent, dependent on the intact functioning of the perirhinal cortex. Based on our findings that acquisition of the DNMT task and performance at short-delay intervals were unimpaired in lesioned subjects, it appears that rats with lesions of the perirhinal cortex are able to learn and process task information despite its spatial nature. Accordingly, the deficit observed following perirhinal damage must be due to some factor other than any spatial processing requirements of the DNMT task.

It could be argued that the memory impairment observed in the present experiment was due to deafferentation of the hippocampus rather than to damage to the perirhinal cortex itself. Neuroanatomical evidence supports the interpretation that the impairment associated with perirhinal lesions on DNMT does not result from deafferentation of the hippocampus. The hippocampus receives a significant amount of cortical input apart from input received from the perirhinal cortex. A recent study of the cortical afferents of the entorhinal cortex, for example, revealed that the entorhinal cortex receives substantial cortical input aside from its perirhinal afferents and that this input includes, but is not limited to, the input from the postrhinal cortex (Burwell & Amaral, 1998). Even with complete ablation of the perirhinal cortex, the hippocampal formation would still receive input via the entorhinal cortex from other associational regions including the retrosplenial cortex, the postrhinal cortex, and the posterior parietal area.

A limitation of electrolytic lesion methods is that fibers of passage coursing through the targeted cortex can be damaged; however, it is likely that the large majority of the nonperirhinal input to the hippocampus arrives via the fornix and the external capsule/angular bundle. Indeed, the density of myelinated fibers in the perirhinal cortex is relatively low (Burwell & Amaral, 1996). In the present study, the external capsule appeared to be largely spared, and the angular
bundle was entirely spared. Notably, the behavioral profile of the perirhinal lesioned animals on the present task was considerably different from that of rats with hippocampal damage on the same task. Whereas hippocampal damage does produce an impairment on this task, the deficit is manifest both in reacquisition of the basic task and in performance once delays are introduced (Aggleton et al., 1992; Ennaceur et al., 1996). Additionally, the balance of the evidence suggests that rats with hippocampal or fornix lesions are impaired at delay intervals shorter than 5 s (Aggleton et al., 1992; Dunnett, 1985; Rawlins, Maxwell, & Sindem, 1988; Rawlins & Tsaltas, 1983) and even zero delay intervals (Aggleton, Keith, & Sahgal, 1991; Aggleton, Neave, Nagle, & Sahgal, 1995). In contrast, perirhinal lesions were found not to affect performance at delays of 4 s or less. Thus, the present findings extend evidence from other paradigms that there is a cortical contribution to memory separate from that of the hippocampus (Bunsey & Eichenbaum, 1993; Murray & Mishkin, 1986; Zola-Morgan et al., 1989). The neuroanatomy and behavioral background suggest that the contribution of the perirhinal cortex to the DNMTP task goes beyond the provision of spatial information to the hippocampal formation.

Although the evidence indicates that the perirhinal contribution to the DNMTP task is not limited to providing sensory information to the hippocampus, it may be that the perirhinal contribution to this task is dependent on the information received by means of its postrhinal input. The postrhinal cortex provides the predominant visuospatial input to the perirhinal cortex via its connections with occipital, posterior parietal, and retrosplenial cortices (Burwell & Amaral, in press). It would be interesting to compare the effects of perirhinal damage on the DNMTP task with the effects of postrhinal damage.

In the present experiment, rats with perirhinal cortex lesions performed as well as controls when the schedule of delay intervals included more short delays and fewer longer delays (e.g., delays of 0, 1, 2, 4, 8, 16, and 32 s). This finding is consistent with a previous study demonstrating that rats with lesions of the perirhinal cortex were unimpaired when delay intervals of 0, 2, 4, 8, 16, and 32 s were used (Ennaceur et al., 1996). The results of the present study also demonstrated, however, that rats with perirhinal cortex lesions exhibited a striking memory deficit when longer delay intervals were introduced to the training program. This finding suggests that the particular delay intervals used by Ennaceur et al. (1996) did not adequately sample the delays at which perirhinal lesioned subjects exhibit deficits on this task.

On the basis of the present results, it seems that the critical factor in producing a perirhinal-related memory deficit is the length of the delay interval. This is consistent with findings from numerous other paradigms in which subjects with ablations of the perirhinal cortex exhibit profound impairments on tasks that require the maintenance of information over longer delay intervals (Meunier et al., 1993; Murray & Mishkin, 1986; Otto & Eichenbaum, 1992; Wig & Bilkey, 1994a; Zola-Morgan et al., 1989), although the particular delay intervals affected vary with task demands. Taken together, these results suggest that the perirhinal cortex functions as a temporary memory store in which representations of trial-specific information are maintained for limited periods of time.

In conclusion, the results of the present experiment provide added support for the involvement of the perirhinal cortex in memory function. It is suggested that the perirhinal cortex, perhaps in conjunction with other parahippocampal cortical regions, is necessary for the temporary maintenance of information across delay intervals that exceed the capacity of a short-term memory store.

References


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