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Extensive Cytotoxic Lesions Involving Both the Rhinal Cortices and Area TE Impair Recognition But Spare Spatial Alternation in the Rat

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ABSTRACT: Rats with cytotoxic lesions of the perirhinal, postrhinal, and TE cortices (Rh+TE, *n* = 7) were compared with surgical control animals (*n* = 7) on a series of spontaneous object recognition tests. The Rh+TE group was associated with a failure to select the novel object. This recognition deficit contrasted with the apparently normal ability of the same animals to learn and perform a spatial working memory test (T-maze alternation). The animals were also tested on the acquisition of an automated visual discrimination task in which the stimuli were presented on a visual display unit (VDU) equipped with a touch screen. The animals with Rh+TE lesions showed only a borderline deficit on this task. These findings are consistent with other evidence implicating the rhinal region in recognition memory. More importantly, they also provide a dissociation between spatial working memory and object recognition and, hence, show that extensive rhinal lesions are not sufficient to disconnect the hippocampus functionally. © 1997 Elsevier Science Inc.

KEY WORDS: Perirhinal cortex, Spatial memory, Object recognition, Learning, Memory, Rat.

INTRODUCTION

A fundamental reevaluation of the relative contributions of the hippocampus and the adjacent temporal cortical regions to memory has occurred over the last few years. This has been prompted by lesion studies in monkeys that have demonstrated that cortex lateral to the hippocampus, most especially the perirhinal cortex, is vital for visual recognition [13,16,35]. Further support has come from electrophysiological studies, which have found cells in the same (perirhinal) region that consistently change their firing rate when visual stimuli are repeated, i.e., become familiar [3,22]. In the light of these findings and the plentiful connections between the perirhinal cortex and the hippocampus [6,25,31] it has been proposed that in the primate brain these two regions function conjointly to form a medial temporal lobe memory system [7,24]. Within this system, hippocampal function is thought to be heavily dependent upon inputs from the perirhinal cortex, while the perirhinal cortex retains some independent mnemonic functions [7,14,24].

It has more recently become evident that the perirhinal cortices of the rat are also involved in recognition memory. Lesions in this region can impair tests of object recognition [11,15,27] and olfactory recognition [19]. Furthermore, single-unit recordings have revealed the presence of cells in the perirhinal cortex and area TE that change their responses to familiar stimuli in a manner very similar to that found in monkeys [32]. Other evidence has come from the expression of the immediate early gene c-*fos*, a marker of neuronal activity. When rats are shown novel objects an increase in c-*fos* expression occurs in a number of sites, including the perirhinal cortex and area TE but not the hippocampus [33]. In a subsequent experiment, novel objects were placed in the visual field of one eye of the rat while only familiar objects were visible to the other eye [34]. This ''paired viewing'' procedure, which helped to ensure that any differential c-*fos* expression was not a result of changes in activity or arousal, led to a greater expression of c-*fos* in both the perirhinal cortex and area TE in the hemisphere receiving inputs from the eye exposed to novel stimuli [34].

The consistent involvement of area TE in electrophysiological and c-*fos* expression studies indicates that this region, along with the perirhinal cortex, might be involved in object recognition. This evidence led to the present study, which examined the effects of cytotoxic lesions that were intended to involve both the perirhinal cortex and area TE. The lesions were also intended to extend caudally so as to involve the recently described ''postrhinal'' region [4], which receives many visual inputs. It was predicted that this extensive lesion might produce more robust object recognition deficits than those observed after relatively selective cytotoxic lesions centred in the perirhinal cortex [11]. To examine recognition we measured the differential exploration of novel and familiar objects by rats, a procedure that takes advantage of their spontaneous preference for novelty [10].

This study also provided the opportunity to assess whether the loss of these cortical regions (rhinal plus TE) functionally disconnects the hippocampus. In a previous experiment it was found that neurotoxic lesions of the perirhinal cortex, which spared much of the postrhinal cortex, did not disrupt tests of spatial memory that are sensitive to hippocampal damage [9,11]. This dissociation was reexamined in the present study as the cortical lesions were more extensive and so might provide a more

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complete disconnection of entorhinal/hippocampal afferents. For this reason the rats were tested on a spatial alternation task, known to be sensitive to hippocampal dysfunction [1,21]. Finally, the rats were tested on a visual discrimination task in which the stimuli were displayed on a computer touch screen [5]. This arrangement made it possible to present stimuli of matched luminance, to better assess the nature of any visual deficits.

MATERIALS AND METHODS

Subjects

The study involved 14 male rats of the pigmented DA strain (Bantin and Kingman, Hull). Throughout the period of the experiment the animals were housed in pairs under diurnal conditions (14 h light/10 h dark). At the time of surgery they were aged 8 months and weighed between 252 g and 318 g. Three months prior to surgery all rats had been used in a study that assessed the effects of lorazepam (IM) on spontaneous object recognition.

Surgical and Histological Procedures

The rats were divided into two groups; rhinal and TE lesions (Rh + TE, $n = 7$), and surgical controls (CONT, $n = 7$). Prior to surgery all animals were deeply anaesthetized by intraperitoneal injection (60 mg/kg) of pentobarbitone sodium (Sagatal) and then placed in a stereotaxic headholder (David Kopf Instruments, Tujunga, CA) with the nose bar at +5.0. The scalp was then cut and retracted to expose the skull. Craniotomies were then made directly above the target regions, and the dura cut to expose the cortex.

For the cortical lesions (Rh+TE), injections of 0.09 M *N*-methyl-D-aspartic acid (NMDA) (Sigma Chemical Company Ltd., Poole, UK) dissolved in phosphate buffer (pH 7.2) were made through a 1 μl Hamilton syringe into four sites in each hemisphere. Each injection was made gradually over a 5-min period and the needle was left in situ for a further 5 min before being withdrawn. The stereotaxic coordinates relative to ear-bar zero were: AP +2.4, LAT ± 6.0, HT +1.6; AP +0.7, LAT ±6.2, HT +2.4; AP −0.8, LAT ± 6.2, HT +3.9; and AP −0.8, LAT ± 6.2, HT +5.5. A total of 0.25 μl NMDA was injected at each of the two rostral sites (AP +2.4 and +0.7), while 0.2 μl NMDA was injected at each of the two caudal sites (both AP −0.8). The skin was then sutured and an antibiotic powder (Acramide, Dales Pharmaceuticals, Skipton) applied. At the completion of all surgeries the animals received 5 ml glucose saline (SC) containing etamiphylline (Millophyline, Arnold's, Romford; 35 mg/kg, SC), a cardiac stimulator. Postoperative care also included systemic analgesia (temgesic, Reckett and Colman, UK). The control animals (CONT) received exactly the same surgery and dural opening but no injections of NMDA were made.

On completion of the experiment all animals were killed with an overdose of Euthatal and perfused intracardially with saline followed by 10% formol-saline. The brains were then removed and placed in 10% formol-saline for a minimum of 2 h. Following fixation the brain was transferred to 20% sucrose in 0.2 M phosphate buffer and left overnight. The brain was then cut on a freezing microtome into 60 μm coronal sections, and sections were mounted and then stained with cresyl violet, a Nissl stain.

Spontaneous Object Recognition Test

Apparatus. The apparatus consisted of an open box (100 × 100 × 50 cm) made of wood, the inside of which was painted gray. The floor was covered with sawdust. Triplicate copies were made of the objects to be discriminated, which were made either of glass, wood, plastic, or metal. Care was taken to ensure that the pairs of objects being tested were composed of the same material so that they could not readily be distinguished by olfactory cues. The stimuli included sets of objects assembled from the same plastic components (Duplo, Lego Group). This ensured that the stimuli provided the same olfactory cues but differed in visual appearance. The height of the objects ranged from 10 to 18 cm, while their weight ensured that they could not be displaced by the rats.

Procedure. Three weeks after surgery all rats were given three habituation sessions. For each of these sessions the rat was placed in the test box (empty) for 6 mins. Forty-eight hours later, testing began. Rats were then given a total of nine test sessions, with an interval of 3–5 days between each session. In none of these tests did the experimenter know the identity of the animals. Throughout these tests the rats were on ad lib food and water.

Each test session consisted of two phases. In the initial sample phase two identical objects (A1 and A2) were placed in the far corners of the box, each 10 cm from the side wall. A rat was then placed in the middle of the box and the total time spent exploring the two objects was determined from video taped recordings. Exploration of an object was defined as directing the nose to the object at a distance of less than 2 cm and/or touching it with the nose. Turning around or sitting on the object was not considered as exploratory behaviour. After a delay of 15 min the rat was reintroduced to the box (choice phase). The box now contained a third identical copy of the familiar object (A3) and a new object (B). These were placed in the same locations as the sample stimuli. The location of the two choice objects was counterbalanced between rats and across sessions. As far as could be ascertained, the objects had no natural significance for the rats and they had never been associated with a reinforcer.

For the first six sessions both the sample phase and the choice phase lasted for 3 min, with an interval of 15 min in between. The stimuli used in these sessions were grouped into pairs that used the same class of stimuli. Thus, Sessions 1 and 2 used plastic (Duplo) objects, Sessions 3 and 4 used painted wooden objects, and Sessions 5 and 6 used other plastic (Duplo) objects.

The procedure was modified slightly for the last three sessions. In order to more closely equate the exposure times to the sample stimulus between animals, the sample phase was ended as soon as the test animal had explored the sample objects for a total of 25 s. In practice, this meant that the sample phase was substantially reduced from 180 s to between 32 and 123 s (median 46 s).

Spatial Forced Alternation

Apparatus. The animals were tested in a T-maze. The floors of the T-maze were 10 cm wide and made of wood painted white. The stem was 70 cm long with a guillotine door located 25 cm from the beginning. The cross piece was 140 cm long and at each end there was a food well 2 cm in diameter and 0.75 cm deep. The walls of the maze were 17 cm high and made of clear Perspex. The maze was supported on two stands 94 cm high. Lighting was provided by a fluorescent light suspended 164 cm above the apparatus.

Procedure. Testing began 6 days after completion of the recognition tests. Each animal was given 1 or 2 days of pretraining in order to run reliably down the stem of the maze to find food pellets in the food wells in both arms. Following this, the experiment began. At the start of each trial, which consisted of two stages, three food pellets (45 mg Campden Instruments, Loughborough), were placed in each food well and a metal barrier was placed at the neck of the T-maze so closing off one arm. As a

consequence, the animal was forced to enter a preselected arm on each sample run and then allowed to eat the food there. The animal was then picked up and confined in the start box for a delay of 15 s, during which the metal barrier was removed. The door to the start box was then opened and the animal allowed a free choice between the two arms of the T-maze. On this choice run the criteria for selecting an arm consisted of the rat placing a back foot in one of the arms. No retracing was permitted. If the rat had alternated, i.e., had entered the arm not previously visited on the sample run, it was allowed to eat the food reward before being returned to its cage. If the other arm was chosen, i.e., the same arm as visited on the sample run, the rat was confined to that arm for approximately 10 s, and then returned to its cage.

The rats were tested in groups of three or four, with each rat having one trial in turn, so that the intertrial interval was about 4 min. The animals received six trials a day, for a total of six sessions. This was immediately followed by a further four sessions, each of 10 trials. For half of the trials within each session there was a delay of 10 s between the choice run and the test run, and for the remainder there was a delay of 60 s. The rest of the procedure remained unchanged. Throughout the test period the animals were food deprived, but their body weight did not fall below 85% of free feeding weight.

Pattern Discrimination

Apparatus. Testing was conducted in an automated apparatus in which a computer visual display unit (VDU) presented the stimuli. The VDU was attached to a touch screen so that the animals could select computer produced stimuli by directly responding on the VDU with their noses [5]. This apparatus was housed within a wooden sound-attenuating box. The inner chamber measured 48 × 30 × 30 cm, and consisted of a metal frame, clear Perspex walls, and an aluminium floor. Located centrally on the wall at the rear of the chamber was a food magazine attached to a pellet dispenser (Campden Inst., Loughborough), access to which was via a hinged Perspex panel that was monitored by a microswitch. A pressure-sensitive area of floor measuring 14 × 10 cm and located directly in front of the food magazine made it possible to detect the presence of the rat in this area. The VDU, on which the stimuli were presented, was located at the other end of the chamber. Surrounding the VDU was a touchscreen attachment (Microvitec Touch-tech 501) that monitored when the rats made contact with the screen or came very close. A black Perspex mask was attached to the face of the VDU, approximately 2 cm from the surface of the display. This mask served to block access to the VDU display except through response windows each measuring 6 cm high × 8 cm wide. These were positioned 13 cm apart (from centre to centre). A shelf extending 7 cm from the surface of the Perspex mask was positioned just beneath the response windows, approximately 15 cm above the floor of the chamber. The combined effect of the response windows and the shelf was to force the animals to stop, rear up, and stretch toward the stimuli with a head-on approach, thus facilitating the rats' attention to the stimuli. Two stimuli were used, a white cross (7 cm high, 5 cm wide) and a white rectangle (5 × 4 cm). These stimuli were matched for brightness by ensuring that an equivalent area of the screen was illuminated for each stimulus.

Procedure. The rats were initially trained to nose poke at the display panels and to take food (45 mg food pellet) from the magazine. By the end of pretraining (which took four sessions) the rats were able to nose poke at a large yellow square displayed on the touch sensitive panel. Responding was reinforced by a VI 40 s schedule. The square was randomly presented in one of the response windows and remained on the screen until the rat responded to it, after which the rat was rewarded with the magazine light, a tone, and a 45 mg pellet. Once a rat was able to obtain 50 reinforcements within 20 min, it was moved on to the simple discrimination task.

Each discrimination trial began with the simultaneous presentation of the two stimuli, contingent upon the animal being located on the rear floor panel following a 5-s intertrial interval. The rat was then required to approach the VDU display and select a stimulus by responding to it directly via a nose poke. Correct responses were followed by the disappearance of the stimuli and the presentation of a 1 s, 4 KHz tone, concomitant with illumination of the magazine light and delivery of a 45 mg food pellet into the magazine. Incorrect responses resulted in the disappearance of the stimuli and the houselight being extinguished for a ''time-out'' period of 5 s.

The same pair of stimuli (a cross and a rectangle) were presented on every trial. For half of the animals in a group the cross was correct (S+), for the other half the rectangle was correct (S+). A nose poke to the S+ was rewarded with a tone, the magazine light, and a food pellet. A nosepoke to the S− was followed by extinction of the houselight for a 5-s time-out period. A correction procedure was implemented such that following an incorrect response the trial was repeated, i.e., the S+ remained in the same location.

Performance Measures and Analysis. Four measures were recorded during acquisition of the discrimination: (1) the percent correct responses; (2) the percent bias, which corresponds to the number of responses either to the right or left response window, depending on a particular animal's bias during a session, expressed as a percent of total trials for that session; (3) the mean choice latency, which is the mean time from the onset of S+ and S− to the time the rat made a nose poke to one of the stimuli; (4) the mean magazine latency, which is the time from a correct nose poke to the time the rat entered the magazine to collect a reward. Three of the CONT animals failed to complete this task, as they would not perform the full number of trials per session. The results from these animals were discarded.

RESULTS

Histological Analysis

The Rh+TE lesions consistently removed all of the perirhinal tissue at the levels depicted in Figs. 1 and 2. As intended, the lesions extended caudally and involved virtually all of the postrhinal area and that portion of area TE dorsal and rostral to the rhinal region. Within the extent of the lesion there was little or no evidence of neuronal sparing. At more rostral levels the lesions consistently encroached ventrally to include the most lateral parts of the piriform cortex, while more caudally they involved the lateral portions of the lateral entorhinal cortex. The lesions were more variable in their dorsal extent, although they consistently included nearly all of the visual area TE. In some cases the lesions also involved more dorsal auditory regions, including primary auditory areas. The involvement of the latter region was always unilateral. The perirhinal damage extended to the depths the rhinal sulcus, and in all cases there was a restricted region of cellular loss in that portion of the CA1 hippocampal field immediately adjacent to the most caudal part of area TE. In four cases the neurotoxin resulted in an additional region of unilateral damage in parietal somatosensory cortex (Fig. 2). Although this extracortical damage sometimes extended into more dorsal and caudal visual areas, it was always confined to one hemisphere.

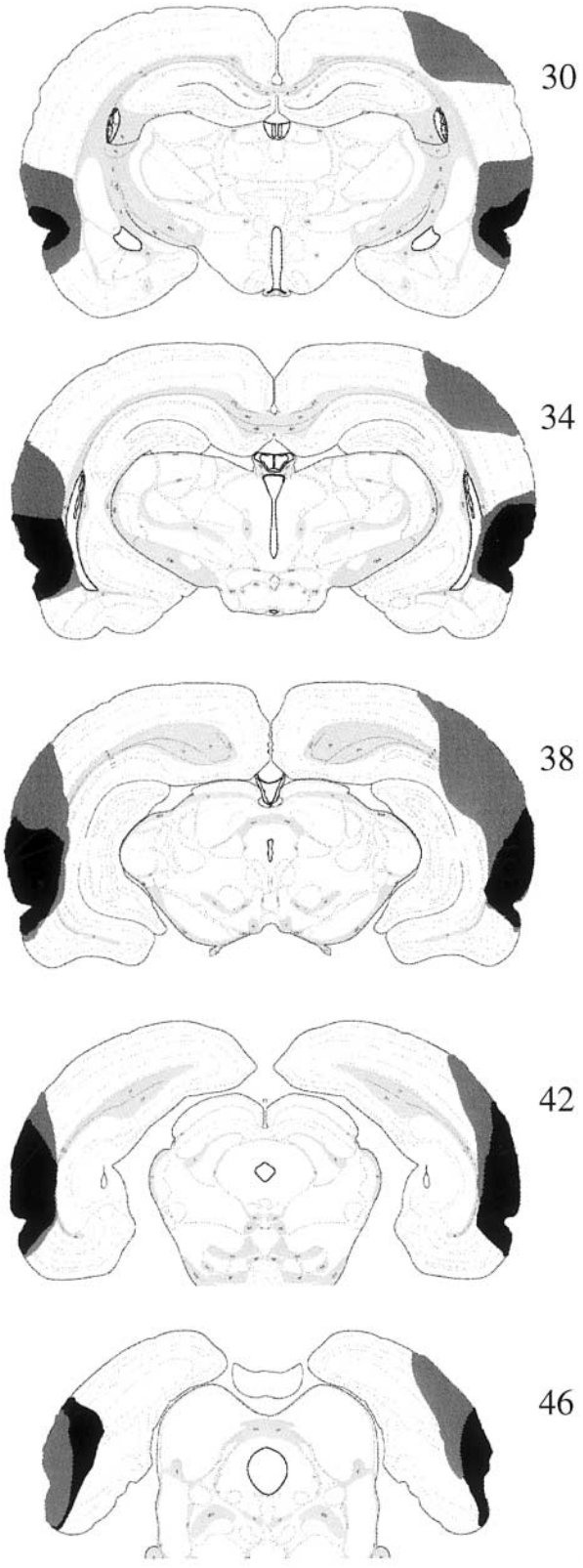

FIG. 1. Series of five coronal sections illustrating the extent of the largest (gray) and smallest (black) Rh+TE lesion. The figures, section numbers, and anatomical boundaries are taken from the brain atlas of Swanson [26].

Object Recognition

Exploration During Sample Period. We first considered the duration of exploration in the sample period as a group difference in this phase might confound all other comparisons. There was, however, no evidence that the groups differed in the total amount of exploration (sessions 1–6) or in the time required to explore the objects for 25s (sessions 7–9) in the sample phase (all $p > 0.2$, two tailed).

Recognition During Test Period—Between and Within Group Comparisons. Combined total exploration times for the novel objects and for the familiar objects were calculated for those sessions using matched objects and conditions (i.e., sessions 1 and 2, sessions 3 and 4, sessions 5 and 6, and sessions 7, 8, and 9). From these four sets of sessions, exploration times were calculated for both the first minute of each test session and for the entire 3-min session. From these results we calculated (1) the difference in time spent exploring the novel and familiar objects (d1) for each of the four sets of objects, and (2) the proportion of total exploration time (d2) spent exploring the novel objects for each set of objects (i.e., the difference in exploration times for the novel and familiar object, d1, was divided by the total time spent exploring the objects). This discrimination ratio (d2) is arguably more sensitive as it takes into account individual differences in the total amount of exploration time. Comparisons using these d1 and d2 indices were applied both for the entire 3 min of each test (choice) session and for just the first minute. The latter was included as all animals readily explored the objects in the test phase, and so the initial period of exploration could logically be regarded as the most sensitive measure of discrimination, i.e., when the familiarity difference is the greatest.

A two-way analysis of variance using the difference scores (d1) for the first minute of each session (Fig. 3) revealed evidence of group effect, $F(1, 12) = 4.32$, $p = 0.060$. A similar analysis using the discrimination index scores (d2) for the first minute (Fig. 3) indicated a more clear-cut group effect, $F(1, 12) = 6.85$, $p = .023$. Both of these differences reflected the poor performance of the Rh+TE animals. Similar comparisons using the data for all 3 min (Fig. 3) of each session did not support such a clear group difference for d1, $F(1, 12) = 2.75$, $p = .12$, but once again, the d2 index provided evidence of a group difference, $F(1, 12) = 4.49$, $p = 0.056$. There were no significant group by object set interactions in any of these analyses.

Further analyses then assessed whether both groups showed evidence of a significant preference for the novel object over the familiar object, i.e., whether they discriminated the novel object. A series of matched sample *t*-tests (all 6 df) compared the times spent exploring the novel and the familiar objects for sessions 1 and 2 combined, sessions 3 and 4, sessions 5 and 6, and sessions 7, 8, and 9 (Table 1). From Fig. 1 it can be seen that the CONT animals were able to discriminate the novel from the familiar object for sessions 1+2, 3+4, and 7–9. This was seen in the significant difference both for the first minute and for all 3 min (Table 1). In sessions 5+6, however, the CONT rats only displayed borderline levels of discrimination between the Duplo objects ($0.1 > p > .05$). This prompted a final comparison using the combined data from all four Duplo sessions (sessions 1, 2, 5, and 6). The CONT animals now showed convincing evidence that they were able to discriminate the novel from the familiar Duplo stimuli (3 min, $t = 3.96$, $p = 0.0075$; 1 min, $t = 2.81$, $p = 0.031$).

The same set of analyses for the Rh+TE group revealed a different pattern of results (Table 1). This group consistently failed to spend more time exploring the novel object for all but sessions 3+4. As with the control animals, we combined all four

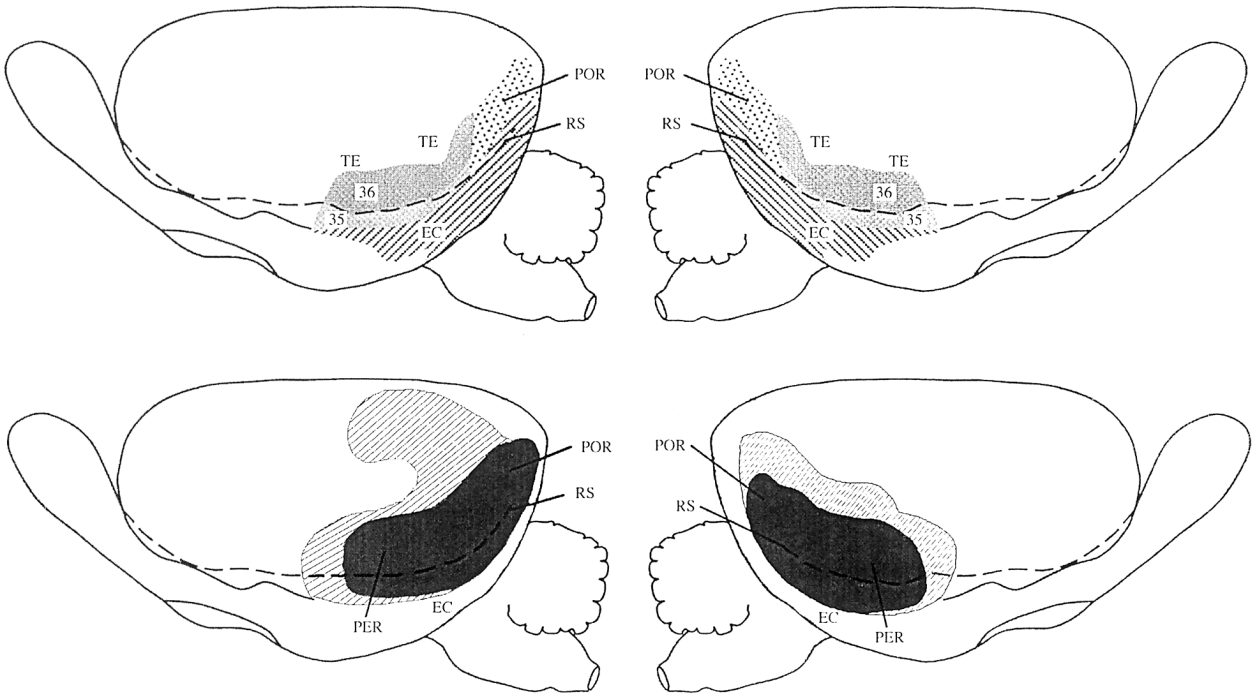

FIG. 2. Surface view of the left and right hemispheres. The upper figure shows the extent of the perirhinal, postrhinal, and TE cortices (TE2 and TE3) as described by Burwell et al. [4]. The lower figure shows the extent of the largest (striped) and smallest (gray) of the Rh+TE lesions. Abbreviations: EC, entorhinal cortex; PER, perirhinal cortex (areas 35 and 36), POR, postrhinal region, RS, rhinal sulcus.

Duplo sessions (1, 2, 5, and 6) but unlike the control group the Rh+TE animals still failed to show clear evidence of an overall preference for the novel stimuli (3 min, $t = 1.81$, $p > 0.1$; 1 min, $t = 1.91$, $p > 0.1$). The only evidence of a clear preference for the novel stimuli was found for the remaining pair of sessions (sessions 3+4, 3 min, $t = 4.81$, $p = 0.003$; 1 min, $t = 3.86$, $p = 0.008$).

Finally, we examined the confidence limits (95%) of the mean d2 indices for each of the four sets of objects. As a score of zero corresponds to a lack of discrimination between the novel and familiar objects we determined whether the lower margin of the 95% confidence limit for the d2 scores was at or below this level. Using this analysis the CONT animals discriminated at both 1 min and 3 min for all sets of objects except for sessions 5+6 (3 min). In contrast, the Rh+TE animals failed to discriminate in any of the sets of sessions with the exception of sessions 3+4 (where they discriminated at both 1 min and 3 mins).

T-Maze Alternation

The total percent correct scores over the six training sessions and the four delay sessions (20 trials 10 s delay, 20 trials 60 s delay) are shown in Fig. 4. It is immediately evident that the scores of the two groups are strikingly similar and did not differ. In fact, the overall mean score of the Rh+TE group was marginally better than that of the CONT animals over the first six sessions ($t < 1$), while comparisons of the delay data confirmed the lack of any group effect, $F(1, 12) = 0.17$, or any group by delay interaction ($F = 0$). There was, however, a clear effect of delay, $F(1, 12) = 10.15$, $p = 0.0078$.

Pattern Discrimination

Figure 5 shows the mean scores (percent correct excluding correction trials) of each group for each of the 12 sessions. It can be seen that both sets of animals rapidly acquired the task, although there was a slight difference in the level of the resultant plateau of performance. An analysis of variance was carried out in which all of the sessions were treated separately and an arcsine transformation carried out on the scores. This transform was used as the scores in the later sessions became close to ceiling level. There was some evidence of an impairment in the Rh+TE group, but the group effect did not reach significance, $F(1, 9) = 4.30$, $p = 0.068$. There was no group by session interaction, $F(11, 99) = 1.24$, but there was the expected improvement with session, $F(11, 99) = 17.6$, $p < 0.001$. Additional comparisons showed that there were no group effects as regards percent bias ($F < 1$), choice latency ($F < 1$), or magazine latency ($F < 1$).

Finally, we compared the percent correct scores for each session using the results from all trials (i.e., including all correction trials). This again revealed no significant group effect, $F(1, 9) = 3.24$, $p = 0.106$, and no significant group by session interaction, $F(11, 99) = 1.47$, $p = 0.153$.

DISCUSSION

The present study examined the behavioural effects of cytotoxic lesions of the rhinal cortices (perirhinal plus postrhinal) and the adjacent area TE. The principal goals were to determine if the loss of this tissue would produce a severe visual recognition deficit and whether a functional disconnection of the hippocampus would result. Previous studies have indicated that damage to perirhinal cortex can disrupt recognition in rats [11,15,27], but in those studies using spontaneous exploration the deficits appear relatively mild [11], while in those studies using a DNMS procedure [15,27] the deficits seem less severe than those observed after perirhinal lesions in monkeys [13,16,35]. For these reasons the current surgery was modified so that the lesions extended

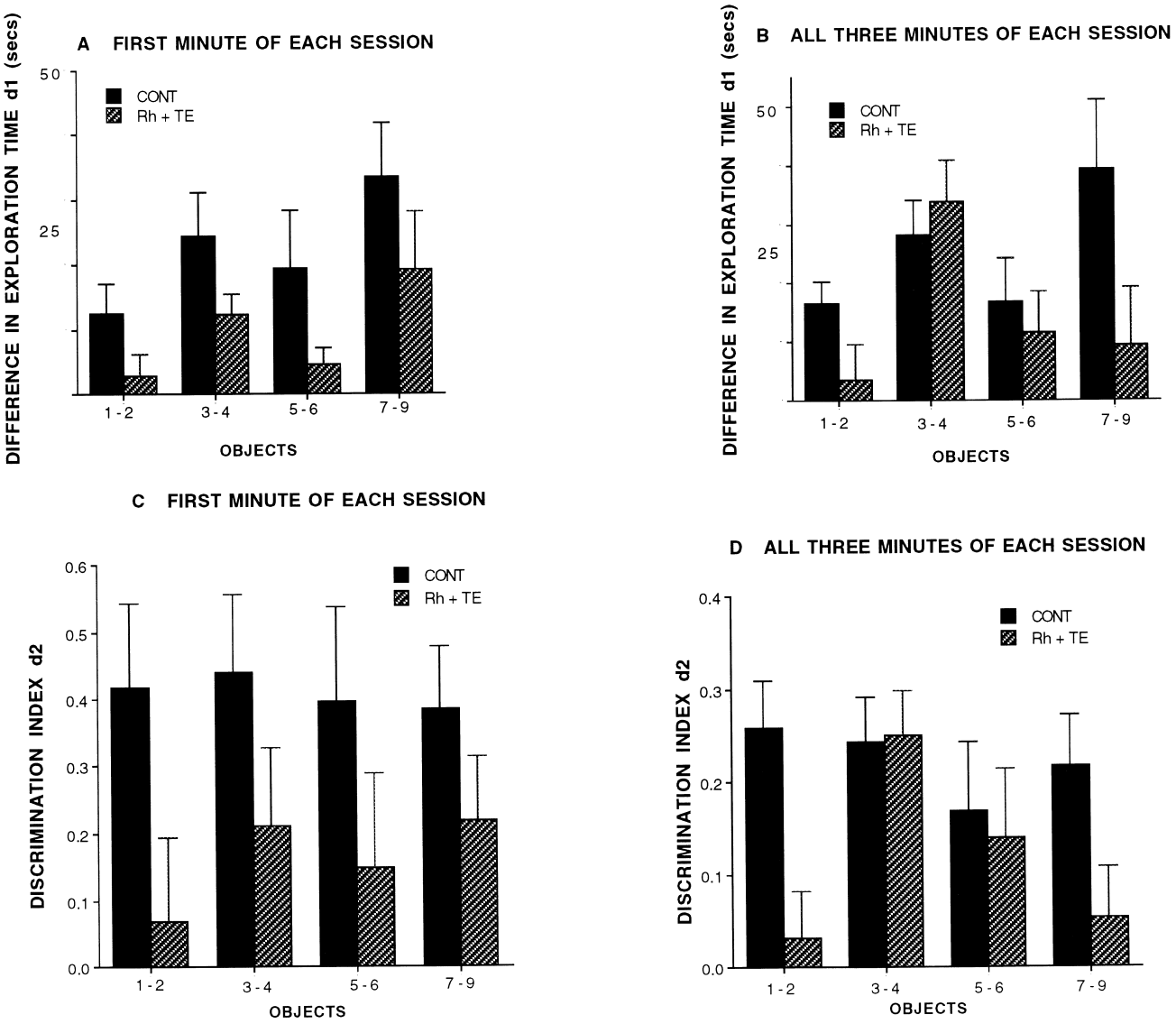

FIG. 3. Spontaneous object recognition: the top two graphs show the mean difference in exploration times (d1) between the novel and familiar stimuli for the first minute (A) and for all 3 min (B) of the four sets of test sessions. The bottom two graphs show the proportion of total exploration time spent exploring the novel object (d2) during the first minute (C) or for all 3 min (D) of the four sets of test sessions. The sessions have been grouped according to the nature of the stimuli and testing procedure. The vertical bars show the standard error of the means.

dorsally, to include area TE, and caudally to include the postrhinal area. The rationale for including area TE arose from electrophysiological and c-*fos* activation studies that have revealed that this region, like the perirhinal cortex, responds differentially to familiar visual stimuli [32,33]. The rationale for including the postrhinal cortex arose from new anatomical evidence showing that this region has numerous anatomical links with both the perirhinal and entorhinal cortices [4], and that it is a major recipient of visual inputs [4]. The use of a neurotoxin served to minimise the possible involvement of adjacent white matter. By producing a more complete loss of rhinal tissue than in previous experiments, the study also provided a more demanding test of whether this cortical region is necessary for the performance of spatial tasks known to be dependent on hippocampal function [11].

A series of experiments using a spontaneous test of recognition showed that the rhinal lesions markedly attenuated preference for novelty. Evidence for this deficit emerged both from comparisons with the control animals and from within group comparisons that showed the frequent failure of the Rh+TE animals to spend significantly more time with the novel objects. The between group differences were most evident for the index d2, which has the advantage of taking into account individual differences in the overall amount of exploration in the test phase. This was especially so for the first minute of the test session. The fact that these d2 scores were consistently higher for the first minute of each test session than for all 3 min (Fig. 3) supports its use as a more accurate recognition measure, and accords with the assumption that a rat that can readily identify the novel object will spend more of its initial time with that object, but as it does so the object rapidly loses its novelty. It is also the case that the lesion effects seemed particularly evident in those tests using Duplo objects (sessions 1, 2, 5, and 6). This may be pertinent

TABLE 1

WITHIN GROUP COMPARISONS OF THE TIMES SPENT EXPLORING THE NOVEL AND FAMILIAR OBJECTS (PROBABILITIES LESS THAN 0.1 ARE SPECIFIED, $*p < 0.05$, $\dagger p < 0.01$).

	Sessions			
	1 + 2	3 + 4	5 + 6	7–9
3 min	$t = 2.73$*	$t = 4.80\dagger$	$t = 2.33$	$t = 3.35$*
	$p = 0.034$	$p = 0.003$	$p = 0.067$	$p = 0.016$
CONT				
1 min	$t = 6.64\dagger$	$t = 3.73$*	$t = 2.22$	$t = 4.04\dagger$
	$p = 0.004$	$p = 0.01$	$p = 0.069$	$p = 0.007$
3 min	$t = 0.60$	$t = 4.81\dagger$	$t = 1.65$	$t = 0.93$
		$p = 0.003$		
Rh + TE				
1 min	$t = 0.82$	$t = 3.86\dagger$	$t = 1.61$	$t = 2.16$
		$p = 0.008$		$p = 0.074$

as these objects were all made of the same material and, hence, would not provide differential olfactory cues. They would also present very similar tactile cues. Thus, the familiarity discrimination almost certainly depended on visual cues. It should be added that the deficit in the Rh+TE group was not due to decreased exploration in the sample phase, as this was monitored for sessions 1–6, and held constant at 25 s for all animals in sessions 7–9.

The recognition deficit is consistent with a previous, similar study that looked at the effects of neurotoxic perirhinal damage in rats [11], except that the larger lesions in the present study appeared to produce a more robust deficit. The implication that it is necessary to produce an extensive rhinal lesion in order to disrupt recognition severely in rats, also accords with the failure of restricted, subtotal perirhinal lesions to disrupt a similar, spontaneous exploration test [29]. The Rh+TE animals were not, however, always indiscriminate in their choice of stimuli, and for the complex objects used in sessions 3 and 4 they displayed a clear preference for the novel stimulus. Further studies will be required to determine if this reflects the use of nonvisual cues to discriminate the stimuli, or whether it reflects the fact that these complex objects were much easier to discriminate visually. Clarification of this issue may have to wait for recognition tasks that use stimuli displayed on a VDU [5].

Other relevant lesion evidence has come from the delayed nonmatching-to-sample (DNMS) procedure, which can also be used to test object recognition. Monkeys with perirhinal lesions are extremely poor at learning and performing DNMS tasks [13,16,35]. Rats with perirhinal damage are also impaired but the deficit appears milder than that observed in monkeys. Thus, it has been found that rats with perirhinal lesions can acquire [27] or reacquire [15] a DNMS task using complex objects within normal limits, whereas monkeys show a clear acquisition deficit. Although perirhinal lesions in rats appear sufficient to impair DNMS when the retention delay is extended [15,27], the present results and those of other recent studies [32–34] suggest that the perirhinal cortex may function in parallel with area TE and/or the postrhinal cortex to signal familiarity. It now remains to be systematically tested as to whether damage to the postrhinal cortex or area TE can accentuate the DNMS recognition deficit associated with perirhinal damage.

The Rh+TE animals showed no evidence of a deficit on the T-maze alternation task. This was true even when the retention

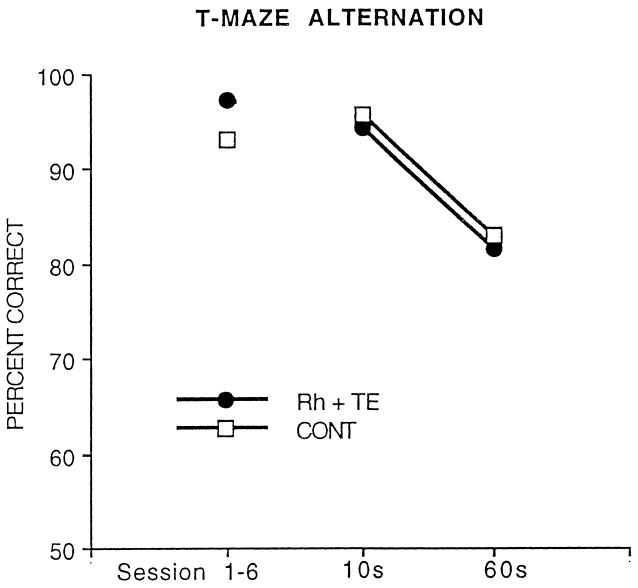

FIG. 4. Mean percent correct responses in the acquisition (sessions 1–6) and retention delay sessions (10 s and 60 s) for the spatial forced alternation task in a T-maze.

interval was increased to 60 s, so removing ceiling effects. This result is especially striking given that this same task is highly sensitive to hippocampal damage [1,21] and to lesions of the fornix [9,23]. It should be noted that these severe alternation deficits associated with hippocampal system damage are observed in rats of the same strain, tested in precisely the same manner [1,9,23] to that used in the present study. Thus, this spared ability not only shows that the limited hippocampal damage in the CA1 field was insufficient to induce a deficit, but also reveals more evidence of a dissociation between tasks dependent on the rhinal cortices and tasks dependent on the hippocampus [11,12]. Previous studies of rats with perirhinal damage have

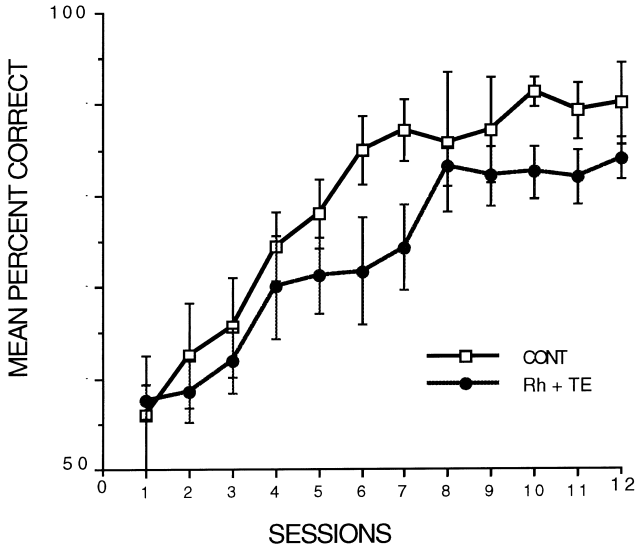

FIG. 5. Mean percent correct (and standard error) scores for the automated visual discrimination task over sessions 1–12.

also examined performance on spatial working memory, as well as spatial reference memory tasks. The findings have not, however, been consistent. Neurotoxic lesions of the perirhinal cortex have been found to spare T-maze alternation [11] and radial arm maze performance [9], while relatively posterior electrolytic lesions of the same region impaired T-maze alternation in a delay dependent manner [28]. The latter lesions also increased the latency to find a platform in the Morris water maze [30], while considerably smaller perirhinal lesions were found to leave Morris water maze performance intact [29]. It is, however, most unlikely that lesion size per se is a factor as the present Rh+TE lesions, which spared spatial alternation, were considerably more extensive than the conventional lesions that were associated with a deficit [28]. It seems, therefore, that the presence of a spatial deficit is more associated with the use of conventional lesion techniques (i.e., with electrolytic perirhinal lesions). This suggests that in these cases damage may have occurred to white matter at the level of the rhinal fissure, so disrupting connections between the temporal cortex, entorhinal cortex, and hippocampus [17,18].

In spite of the extent of their lesions, the Rh+TE animals performed at near normal levels on the pattern discrimination task. Although the number of control animals was fewer for this task, it is still evident that the Rh+TE group were able to acquire this task even though the discrimination could not be solved by simply using differences in luminance levels. There was, however, some indication of a mild impairment, and a very similar borderline deficit was found in the only other study to examine the effects of perirhinal lesions in rats on both recognition and visual discrimination [27]. The evident ability of the Rh+TE animals to perform this relatively difficult discrimination (the stimuli were two dimensional and of matched luminance) supports the notion that the deficits on the object exploration tasks reflect a relatively specific loss of recognition or familiarity information. This accords with recent electrophysiological [32] and neuronal activation [33,34] studies, and is further supported by the normal performance of the Rh+TE animals on the T-maze alternation task. This is because previous studies [2] using the same apparatus and protocol have demonstrated that rats standardly learn to alternate using allocentric cues, i.e., they rely on distal visual cues. This indicates that the Rh+TE animals were able to use distal visual cues to guide T-maze alternation even though they failed the recognition tests. There is clearly, however, a need to demonstrate unambiguously whether rhinal lesions in rats can disrupt visual recognition but not visual discrimination.

Probably the most striking result in the present study is the dissociation between the effects of the rhinal lesions on a test of object recognition and a test of spatial working memory. Although the recognition and spatial tasks were not carried out concurrently, the alternation task followed only a few days after the last recognition session. There was no suggestion that the Rh+TE animals were initially impaired on the spatial task, nor was there any evidence that the recognition impairment was attenuated with repeated testing. Thus, it seems unlikely that the normal alternation shown by the Rh+TE animals reflected a sudden recovery of function. The dissociation, therefore, indicates that the loss of this rhinal tissue is insufficient to disconnect the hippocampus from the sensory inputs that it requires for spatial processing. Furthermore, transection of the fornix produces precisely the opposite pattern of deficits on these two tasks to those shown by the Rh+TE animals. That is, fornix lesions severely impair T-maze alternation but spare tests of spontaneous object recognition [8,11,23]. As fornix transection serves to disconnect and so functionally lesion the hippocampus, this double dissociation runs counter to the influential view that the perirhinal cortex and the hippocampus function in an interdependent manner [7,24]. A similar double dissociation has been found in studies using monkeys [12]. Likewise, it has been reported that fornix lesions disrupt contextual fear conditioning by rats while lesions including the perirhinal cortex did not affect performance [20]. These findings do not show that the interconnections between the perirhinal cortex and hippocampus are of no functional significance; rather, they question some of current proposals concerning the ways in which they interact to aid the encoding and storage of information.

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